

Article

# Spatial and Temporal Characterization of *Escherichia coli*, Suspended Particulate Matter and Land Use Practice Relationships in a Mixed-Land Use Contemporary Watershed

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**Abstract:** Understanding land use practice induced increases in *Escherichia (E.) coli* and suspended particulate matter (SPM) concentrations is necessary to improve water quality. Weekly stream water samples were collected from 22 stream gauging sites with varying land use practices in a representative contemporary mixed-land use watershed of the eastern USA. Over the period of one annual year, *Escherichia (E.) coli* colony forming units (CFU per 100 mL) were compared to suspended particulate matter (SPM) concentrations (mg/L) and land use practices. Agricultural land use sub-catchments comprised elevated *E. coli* concentrations (avg. 560 CFU per 100 mL) compared to proximate mixed development (avg. 330 CFU per 100 mL) and forested (avg. 206 CFU per 100 mL) sub-catchments. Additionally, agricultural land use showed statistically significant relationships ( $p < 0.01$ ) between annual *E. coli* and SPM concentration data. Quarterly PCA biplots displayed temporal variability in land use impacts on *E. coli* and SPM concentrations, with agricultural land use being closely correlated with both pollutants during Quarters 2 and 3 but not Quarters 1 and 4. The data collected during this investigation advance the understanding of land use impacts on fecal contamination in receiving waters, thereby informing land use managers on the best management practices to reduce exposure risks.

**Keywords:** *Escherichia coli*; suspended particulate matter; water quality; land use practices; experimental watershed

## 1. Introduction

Fecal pollution is the greatest contributor to water borne disease human morbidity and mortality rates globally [1]. Freshwater fecal pollution and subsequent increases in pathogenic bacteria (e.g., *Escherichia (E) coli*), cause disease outbreaks, including diarrhea, urinary tract infections, respiratory illness and pneumonia [2,3]. The World Health Organization reported that 2.2 million deaths are caused by diarrhea annually, due to the consumption of fecal contaminated water [1]. An improved understanding of factors leading to increased fecal contamination in receiving waters will be useful in reducing outbreaks of waterborne disease and improving water quality. Furthermore, understanding the factors impacting the health and exposure risks of fecal pollution can be used to decrease the threat posed by fecal organisms. For example, the environmental persistence of fecal

microbes can be extended when occurring with suspended particulate matter (SPM) [4,5]. Therefore, monitoring SPM in conjunction with fecal pollution can provide greater insight into water quality, through more accurate assessments of the persistence of fecal microbes.

Suspended particulate matter (SPM), defined as heterogeneous aggregates of mineral fragments, organic matter and microbial fractions, comprises the greatest water pollutant by volume globally [6]. Excess SPM in freshwater can impact water quality by decreasing the amount of transmitted light, thereby restricting or eliminating the photosynthesis of aquatic plants and dramatically influencing the aquatic food chain [7,8]. Therefore, understanding the factors influencing fluxes in SPM (e.g., land use practices) is important from an ecosystem management perspective. Additionally, increases in SPM can clog the gills of fish, thus lowering resistance to disease and decreasing developmental growth rates [7] while also elevating water temperatures, thereby disrupting the metabolic processes of various aquatic biota [8]. Thus, changes in SPM concentrations in receiving waters can entail serious consequences for various aquatic organisms. SPM can also act as a conveyance system for other pollutants including heavy metals, chemicals and pathogens, including fecal microbes (as discussed above) [8–12]. Insofar as excess sediment can be harmful to aquatic ecosystems, too little sediment can also be harmful, leading to the scouring of river channels, erosion and reduced nutrient inputs [8,13]. Consequently, understanding the factors leading to increases or decreases of SPM in receiving waters is important from a water quality perspective [14].

Previous work investigating fecal contamination and SPM reported statically significant, Pearson's product moment, correlations ( $r > 0.9$ ) between the two pollutants [15]. Moreover, the strength of the relationships between fecal pollution and SPM reported in previous work [15,16] has led to speculation that SPM concentrations (and the turbidity caused by SPM) can potentially serve as a proxy for fecal contamination [16]; however, this is yet to be verified. The relationship between fecal contamination and SPM has been attributed to similar transport processes influencing both pollutants during run-off events [17], including similar in-stream transport physics [18], and the sorption of fecal microbes to SPM [4,5]. Additionally, certain land use practices can simultaneously elevate both SPM and fecal microbe concentrations in receiving waters [19,20]. For example, agricultural land use practices are commonly associated with increased fecal (e.g., *E. coli*) and SPM concentrations relative to other land use types [19–22]. This is often related to the presence of livestock [23], with livestock population density being correlated to fecal indicator organism concentration [24]. Manure application in agricultural areas has also been linked to increased concentrations of fecal microbes in receiving waters [4]. Conversely, agricultural practices such as soil tillage and soil exposure yield increased SPM concentrations in the receiving waters of agricultural areas [19,21]. Differing land use practices in a given area (watershed) can therefore account for differing *E. coli* and SPM concentrations in receiving waters and should thus be accounted for when monitoring these pollutants.

Despite the progress of previous research, knowledge gaps regarding the relationship between fecal contamination and SPM remain. For example, few studies investigated the relationships between SPM and fecal contamination in mixed land use settings. Furthermore, the majority of studies included limited sampling locations [20,25] and tended to occur in areas of similar land use types [16,26], or were controlled laboratory simulations [27]. Additionally, previous work on fecal contamination typically focused on storm events and therefore report disproportionately elevated fecal concentrations in receiving waters [28]. Clearly, knowledge regarding fecal concentrations outside of storm events is lacking, creating challenges for proper management practices. Similarly, previous work investigating fecal concentrations in receiving waters typically utilized shorter (weeks or months) sampling periods [20], which fail to account for the seasonal variability in land use practices. For example, seasonal variability in agricultural land use practices can lead to changes in fecal microbe concentrations in receiving water, particularly in areas where manure is applied [29]. Manure is typically only applied in specific seasons, thereby leading to corresponding increases in the fecal contamination of associated receiving waters during these seasons [29]. Therefore, a study design capable of distinguishing the effects of different land uses and seasonality would be useful

for investigating fecal contamination and SPM in receiving waters and for better informing water resource managers.

Previous studies have used many different study design methods, including different sampling regimes, to advance the understanding of *E. coli* regimes. Study designs have included laboratory based designs comprising simulations [30] and field based designs comprising event based sampling [31], periodic sampling [32], stochastic sampling [33] and nested-scale experimental watersheds [20]. The nested-scale and paired experimental watershed study design is a method that has been successfully used to quantify the effects of land use practices on receiving waters in mixed land use settings [34–40]. Nested watershed study designs divide a larger watershed into a series of sub-catchments to investigate the influence of land use practices on the environmental variables of interest [35,37,41–43]. Sub-catchment delineation isolates different land use practices and hydrologic characteristics [41]. Paired watersheds comprise at least two physiographically similar watersheds (control and treatment) from which data are collected [41]. The study design enables the identification of the influence and cumulative effect of various land use practices on the response variable of interest through the quantification of the influencing processes observed at the sub-catchment scale [44]. Therefore, the approach allows for the effective disentanglement of factors (e.g., land use practices and SPM) that influence a given response variable of interest (e.g., fecal microbe concentration), thus providing quantitative information regarding hydrologic and water quality regimes related to specific land-uses [41]. Given its proven application in over a century of studies, the nested-scale and paired experimental watershed study design is an accepted optimal study design for investigating current knowledge gaps regarding fecal contamination, SPM and land use practices.

The Appalachian region of the USA is well-suited for researching knowledge gaps concerning fecal contamination, SPM and land use practices. The region is representative of many locations globally given that it suffers from widespread, frequent, and problematic fecal pollution [45]. Additionally, Appalachia is physiographically diverse, encompassing distinct Northern, Central and Southern regions, consisting of dissimilar geographic, climatological, and ecological characteristics [46]. For example, the temperate climate and well-distributed year-round rainfall characteristics of Central Appalachia [47] are similar to those of areas such as Uruguay or Southern Brazil [48], and many other locations. Conceivably, other temperate areas comprising year-round precipitation (e.g., Uruguay) will benefit from research conducted in the Central Appalachian region as the results will be comparable and transferable. Furthermore, water quality is a primary concern in rural Appalachia as thousands of residents are exposed to water quality problems, specifically regarding microbial contamination [49]. Water quality problems in rural areas are exacerbated by inadequate wastewater treatment infrastructure, isolation due to geographically inaccessible terrain, and poverty [49]. Consequently, water quality is a primary concern, and insight into both SPM and fecal pollution (e.g., *E. coli* concentrations) is necessary to effectively inform policy makers and water resource managers regarding water quality to make the best management practice decisions in Appalachia and physiographically similar locations globally.

The overarching objective of the current investigation was to quantify fecal contamination (*E. coli* concentration) and SPM concentrations in receiving waters relative to differing land use practices from numerous sites in a mixed-land use contemporary watershed of Appalachia. Sub-objectives included (1) investigating the relationship between fecal microbe concentration (*E. coli* colony forming units) and SPM concentrations in receiving waters, and (2) determining the influence of quarterly (seasonal) changes on the relationship between *E. coli* and SPM. The study outcomes were to improve the understanding of the influence of land use practices on both fecal contamination and SPM pollution, providing land use managers with insight into factors influencing water quality in receiving waters.

## 2. Methods

### 2.1. Study Site Description

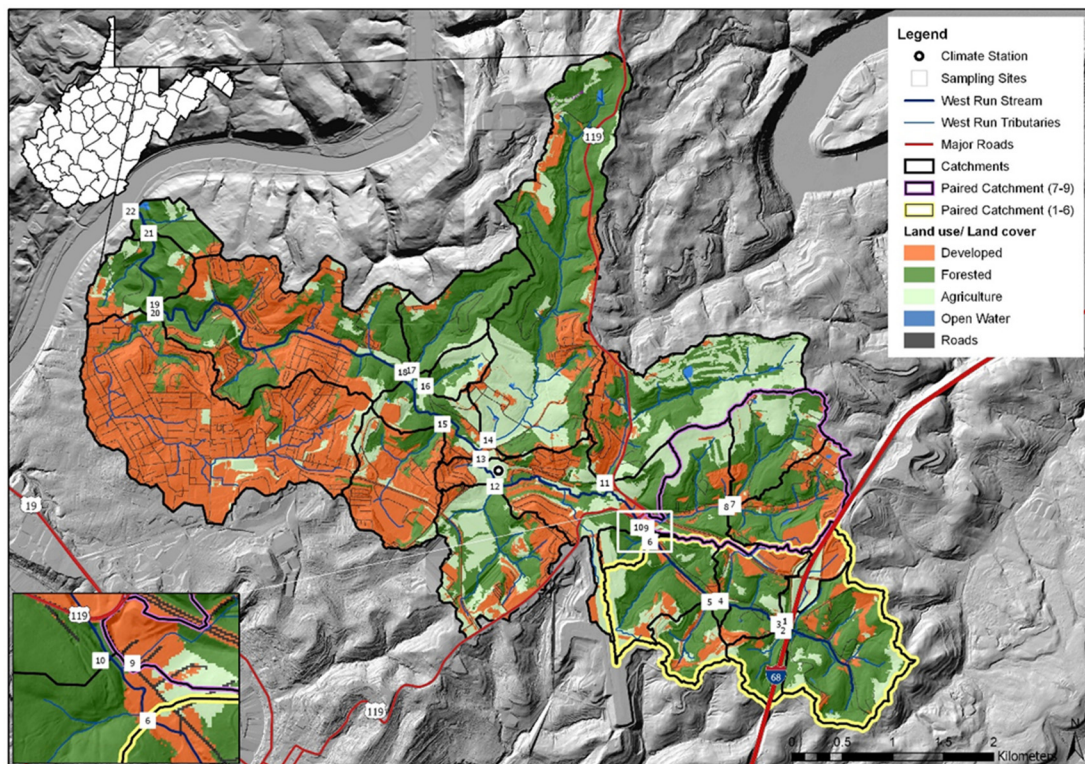
This research took place in West Run Watershed (WRW) a 23 km<sup>2</sup> mixed-land use urbanizing watershed located in Morgantown, West Virginia, USA. West Virginia's climate varies between cold and humid with warm summers, to temperate and humid with hot summers [50]. In Morgantown, WV, located in Monongalia County (and including the WRW), the climate is characterized by the lack of a dry season, cold winters (mean monthly temperature < 0 °C) and warm-to-hot summers (mean monthly temperature > 22 °C) [50]. Historically (1981–2010), Morgantown received approximately 1060 mm of average annual precipitation, with the coldest (January) and driest (February) months having an average daily temperature of −0.4 °C and an average monthly precipitation of 66 mm, respectively [51]. Conversely, the warmest and wettest month (July) comprised an average daily temperature of approximately 23 °C and an average monthly precipitation of 117 mm [51].

West Run Creek, the primary drainage of WRW, is a third order tributary of the Monongahela River, and includes many land use practices including agriculture, urban and forested areas [22]. Based on the 2016 National Agriculture Imagery Program (NAIP) land use and land cover data, WRW includes 42.7% forested land use, 37.7% mixed development (urban and commercial areas) land use and 19.4% agricultural land use practices. West Run Creek is a narrow, moderately entrenched stream with multiple small floodplains [20,52]. The elevation of the headwaters of WRW is 420 m above mean sea level [22]. Conversely, the elevation of the confluence of WRW with the Monongahela River is 240 m above mean sea level [20]. The watershed includes relatively rugged terrain, featuring numerous Paleozoic era rock outcroppings [20]. The headwaters of WRW contain the most recent geological formation (Monongahela series) [20]. Two coal formations are also present in the watershed, namely the Upper Kittanning coal and the Pittsburg coal seam [20]. Historic mining of the Pittsburg coal seam negatively impacted water quality in WRW, particularly in the headwaters [53].

A nested-scale and paired experimental watershed study design [35,44,54–56] comprising twenty-two study sites (i.e., gauge sites) was implemented in 2017. Sampling sites (numbered in downstream order) were located in West Run Creek (#3, #4, #6, #10, #13, #18, #19, #21 and #22) and its first and second order confluence tributaries (#1, #2, #5, #7, #8, #9, #11, #12, #14, #15, #16, #17 and #20) and included varying land use practices (Table 1; Figure 1). Both field surveys and GIS were used to identify the study sites and related sub-catchments. At the time of this investigation, forested land use was the predominant land use in WRW, accounting for 42.7% of the total land use practices in the watershed. Additionally, forested land use was the predominant land use type in all sub-catchments except #1, #11, #15, #16 and #20. Sub-catchments #1, #15 and #20 were primarily mixed development, whereas sub-catchments #11 and #16 were primarily agricultural (Table 1). Conversely, 85.84% of sub-catchment #17 was forested land use, the highest among the sub-catchments. This sub-catchment, which served as a reference sub-catchment (control) for the current work, also comprised 9.4% agricultural and 4.8% mixed development land use practices. Sub-catchment #17 is, therefore, considerably different to sub-catchment #12 (34.5% forested, 33.7% agriculture and 31.7% agriculture) despite both comprising predominantly forested land use practices. In general, at the time of the investigation, mixed development comprised the second largest percentage of land use practices (37.7%) and agricultural land use practices accounted for the lowest percentage of land use practices (19.4%) in WRW.

**Table 1.** Land use/land cover characteristics (% cover) and total drainage area (km<sup>2</sup>) at 22 monitoring sites in West Run Watershed (WRW), West Virginia, USA. Note: land use percentages may not sum to 100%, as not every category is included (i.e., wetland, open water, etc.) and some categories are combinations of others (e.g., mixed development = urban + residential). Final row (Site #22) indicates the total values for the entire watershed.

Site	Mixed Development (%)	Agriculture (%)	Forested (%)	Drainage Area (km <sup>2</sup> )
1	53.23%	38.70%	8.07%	0.30
2	13.58%	12.20%	74.21%	0.29
3	22.35%	16.17%	61.32%	1.87
4	25.88%	14.91%	59.00%	2.48
5	23.35%	25.51%	51.14%	0.38
6	23.91%	17.25%	58.70%	3.72
7	16.33%	28.60%	54.91%	0.78
8	30.78%	16.47%	52.35%	1.55
9	27.57%	19.33%	52.84%	2.29
10	24.92%	18.40%	56.49%	6.18
11	18.15%	41.87%	39.16%	1.75
12	31.77%	33.72%	34.51%	1.75
13	26.83%	25.77%	47.15%	10.53
14	16.19%	26.43%	56.92%	3.36
15	70.28%	10.31%	19.42%	0.98
16	5.38%	58.72%	35.16%	0.25
17	4.78%	9.38%	85.84%	0.75
18	25.98%	24.88%	48.86%	16.41
19	29.45%	22.45%	47.85%	18.88
20	89.16%	4.19%	6.61%	3.42
21	38.10%	19.46%	42.23%	22.93
22	37.71%	19.38%	42.66%	23.24



**Figure 1.** Monitoring/sampling locations for the current investigation, with land use/land cover, in West Run Watershed, Morgantown, West Virginia, USA.

## 2.2. Data Collection

Climate data collected for the current work included precipitation (Campbell Scientific TE525 Tipping Bucket Rain Gage), average air temperature, relative humidity (Campbell Scientific HC2S3 Temperature and Relative Humidity Probe), and average wind speed (Campbell Scientific Met One 034B Wind Set instrument). Data were recorded at a 3 m height during the study period (2 January 2018–1 January 2019) by a climate station located within approximately 30 m of Site #13 (Figure 1).

For the current work, weekly water grab-samples were collected as per Petersen et al. [20], Hubbard et al. [57], Kellner and Hubbard [43], and Zeiger and Hubbard [42,58] from each monitoring site (stream order  $\leq 3$ ). Water sample collection was initiated at 09:00 at Site #1 and continued in numerical order of sites. Sites #9 and #10 were exceptions, as they were sampled before Sites #7 and #8, due to their location relative to other sites (Figure 1). The proximity of Sites #9 and #10 to Site #6 meant that overall sampling time was reduced by sampling them after Site #6, increasing the comparability of the samples during sample processing. The sampling period for the study (2 January 2018–1 January 2019, thus 53 weeks) was one calendar year to account for seasonal variability in the *E. coli* concentration and SPM data. Notably, the sampling period was longer than in typical studies on fecal contamination [59,60], allowing for a comprehensive quantification of fecal contamination (*E. coli*) regimes at sub-catchment mixed-land-use scales. The high-resolution study design resulted in a total of 1166 spatio-temporally delineated fecal contamination (*E. coli*) concentration and SPM concentration values.

Following collection, the samples were transported to the Interdisciplinary Hydrology Laboratory, located in the Davis College of Agriculture, Natural Resources and Design at West Virginia University, for analyses. In the laboratory, water samples were refrigerated (at 3.3 °C), and gravimetric analyses (vacuum filtration) were conducted as per the American Society for Testing and Materials, test number D 3977-97, [61] within a few days of collection to determine the mass of suspended particulate matter (SPM). Additionally, fecal contamination was quantified immediately upon arrival at the laboratory using *Escherichia (E) coli* as an indicator organism, as per previous work [20,62]. *E. coli* coliform forming units (CFU) were enumerated using the U.S. Environmental Protection Agency (EPA) approved Colilert test [63], developed by IDEXX Laboratories Inc. The applied method used an MPN approach to estimate the *E. coli* CFU concentration; therefore, *E. coli* concentration data were referred to as CFU, not MPN, during the investigation. The test, included in Standard Methods for Examination of Water and Wastewater was developed to estimate fecal concentrations in water samples without requiring sample dilution [63,64]. A combination of Colilert's Defined Substrate Technology nutrient-indicator (ONPG), and a selectively suppressing formulated matrix created low chances of recording inaccurate results (chance of reporting false positives  $\pm 10\%$ ). With this test, most non-target organisms are unable to grow given that they lack the enzyme to metabolize the provided carbon source (ONPG) [63]. The formulated matrix selectively suppresses the few non-target organisms that can metabolize ONPG [63]. The number of *E. coli* colony-forming units (CFU) per 100 mL of sampled water was estimated using the Quanti-Tray system, comprising 96 total wells: 48 large wells (49, including the overflow well) and 48 small wells [63]. The Colilert (ONPG) substrate was added to 100 mL of sampled water, sealed in the Quanti-Tray, and incubated at 35 °C for 24 hours, as per Colilert's instructions [29]. Following incubation, fluorescing (positive for *E. coli*) wells were quantified using a UV light and converted, with a 95% confidence interval, into a concentration of *E. coli* (CFU per 100 mL) using the Quanti-Tray Most Probable Number (MPN) table. The *E. coli* concentration range resultant from the Quanti-Tray/MPN table method was  $<1$  to 1011.2 CFU. Therefore, *E. coli* concentrations in excess of 1011 CFU per 100 mL could not be accurately estimated. This limitation was an allowable shortcoming of the current work given the focus on the weekly detection of small *E. coli* concentrations occurring between storm events.

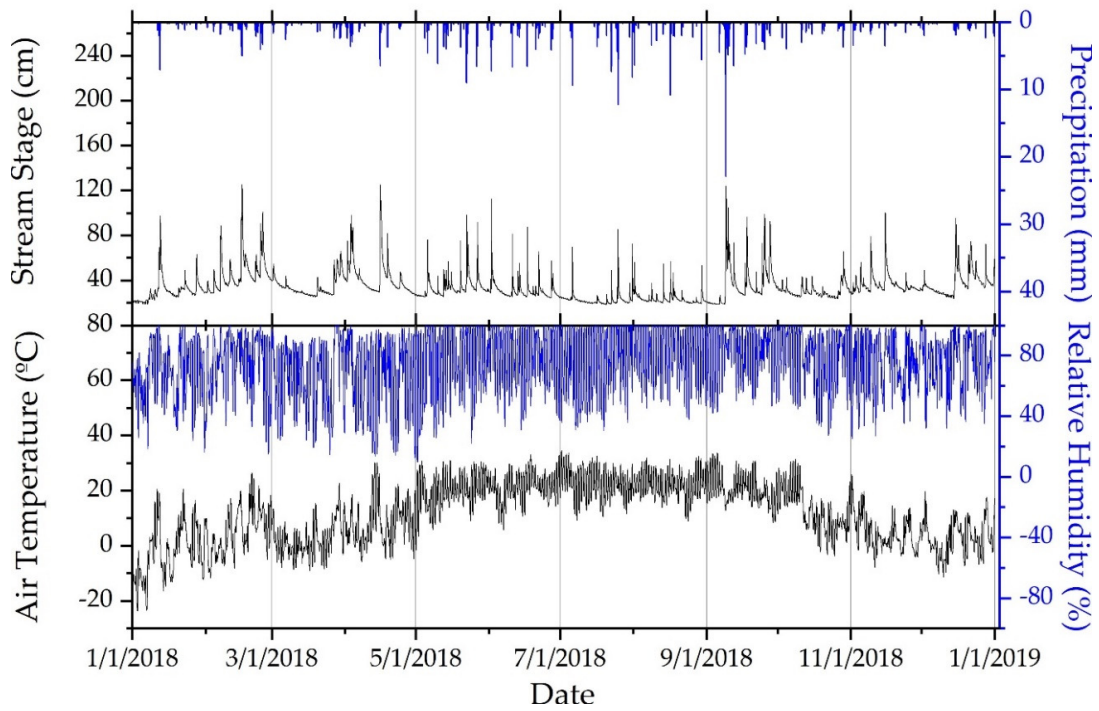
### 2.3. Data Analysis

Descriptive statistics were generated for *E. coli* and SPM concentrations and aggregated for the study period. Average percentage differences between sites were determined by comparing the average SPMs and average *E. coli* concentrations between sites. Statistical analyses were conducted using Origin Academic 2018 (OriginLab Corporation). Normality testing was completed using the Anderson Darling Test [65]. Land use practices were reclassified (lumped) into three major categories prior to analysis, namely mixed development, agriculture, and forested [20]. Mixed development constituted roads, impervious surfaces, mixed developments and barren areas. Agriculture included low vegetation, hay pasture and cultivated crops. Forested land use included mine grass, forest, mixed mesophytic forest, dry mesic oak forest, dry oak (pine) forest and small stream riparian habitats. Annual data were also analyzed in four quarter data subsets, comprising all weekly samples collected in three month blocks starting on January 1st, 2018, to analyze seasonal variation. Thus, Quarter 1 included 2 January 2018–27 March 2018 (winter), Quarter 2 included 3 April 2018–26 June 2018 (Spring), Quarter 3 included 3 July 2018–25 September 2018 (summer), and Quarter 4 included 2 October 2018–1 January 2019 (fall). Spearman correlation tests, with a significance threshold of  $\alpha = 0.05$  [66], were used to analyze the relationship between *E. coli* concentration, suspended sediment, and land use practices at all twenty-two sites, as per Petersen et al. [20] for the complete annual data set and the four quarterly data subsets. Finally, principal component analysis (PCA) was used to investigate the relationships between *E. coli* concentrations, SPM and land use practices (presented in biplots) across all 22 sampling locations for the annual data set and the four quarterly data subsets.

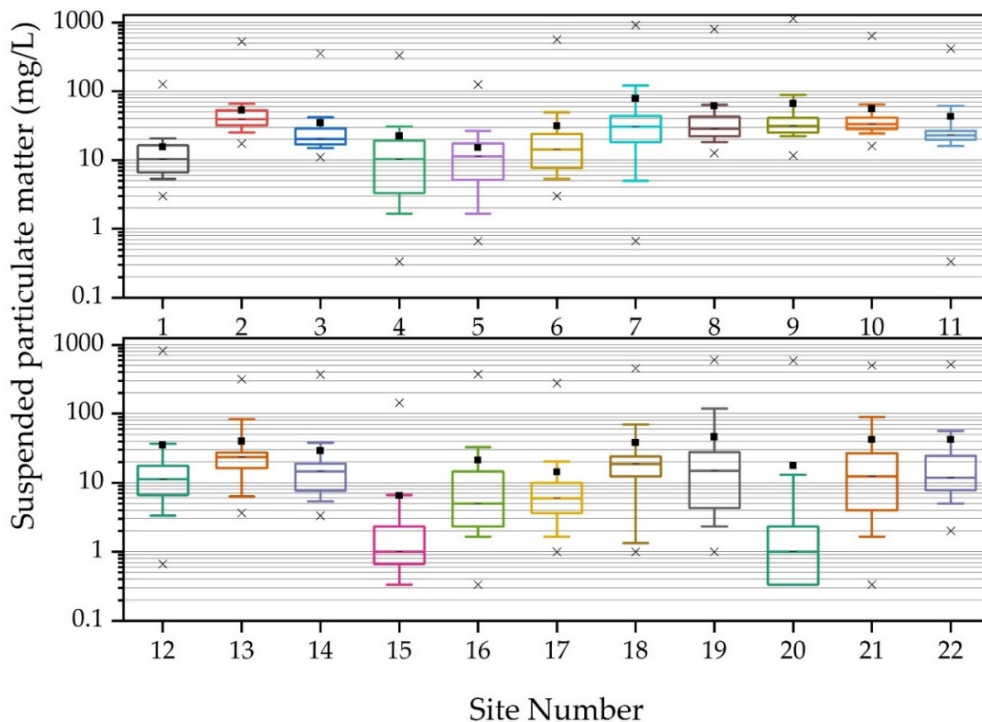
## 3. Results and Discussion

### 3.1. Climate during Study

Total precipitation was 1378 mm in 2018 in WRW. This was approximately 20% more precipitation than the historic annual average (1096 mm) dating back to 2007 [67]. September (186 mm) and October (47 mm) were the wettest and driest months, respectively, during 2018 (Figure 2). Approximately 14% of the annual precipitation was received in September. This was more than double the historic average precipitation (80 mm) for that month [67]. The average air temperature, during the study period was approximately 12 °C, which is close to the historic average of 11 °C [67]. July (22 °C) and January (−4 °C) comprised the warmest and coldest average monthly temperatures, respectively, in WRW during 2018. Relative humidity was characteristically high during 2018 (Figure 2), comprising a yearly average of 76%. Generally, climate during the period of study (2 January 2018–1 January 2019) was predictably variable and consistent with historic trends (Figure 3), including humid and warm weather during the summer months, with temperatures decreasing over the transition to winter (Figure 3). As is typical of the region, there was no dry season; however, large precipitation events during Quarters 2 (spring; e.g., May 6th: 24 mm) and 3 (summer; e.g., September 9th; 60 mm) resulted in greater quarterly (seasonal) variation in precipitation (Figure 2) [67]. Quarters 2 and 3 (spring and summer; 850 mm) therefore received 67% more precipitation than Quarters 1 and 4 (winter and fall; 510 mm).



**Figure 2.** Thirty-minute time series of climate variables during the study period (2 January 2018–1 January 2019) in West Run Watershed, West Virginia, USA. Note: stream stage was measured in the primary stream of WRW, West Run Creek, within approximately 30 m of Site #13 and West Run Creek.



**Figure 3.** Box and whisker plots of suspended particulate matter ( $\text{mg L}^{-1}$ ;  $\log^{10}$  scale) at each sampling location ( $n = 22$ ) during the study period (2 January 2018–1 January 2019) in West Run Watershed, Morgantown, West Virginia, USA. Boxes delineate 25th and 75th percentiles; lines denotes medians; squares shows means; whiskers describe 10th and 90th percentiles; x shows maxima and minima when above and below, respectively. Note: different box colors represent data from different sites.



### 3.2. Annual Suspended Particulate Matter, *E. coli* Concentrations and Land Use Practices

The results showed that forested sub-catchments had the highest average (Site #7; 55% forested; 78.4 mg/L), maximum (Site #9; 53% forested; 1140 mg/L) and minimum (Site #8; 52% forested; 12.7 mg/L) SPM concentrations (Table 2; Figure 3). Notably, these sub-catchments constituted one of the paired watersheds of the paired study design and were in close proximity to each other (Figure 1). Consequently, these sub-catchments were subject to similar land use activities and processes leading to elevated SPM in this region of the watershed. For example, the agricultural land use practices in the headwaters of sub-catchments #7 and #8 (Figure 1) could have elevated the SPM in the entire paired catchment (Sites #7, #8 and #9) as previous work has reported increased SPM in agricultural areas [19–22]. SPM concentrations were also elevated in West Run Creek (combined average of sites in West Run Creek, 39 mg/L) relative to sites located in the first and second order confluence tributaries (combined average, 35 mg/L) (Table 2; Figure 3). The increased SPM in West Run Creek was attributable to (1) the greater volumetric streamflow in West Run Creek relative to its tributaries, as increased streamflow can increase the SPM concentration [68]; and (2) increased SPM sources due to an increased drainage area relative to its tributaries (23 km<sup>2</sup> and 15 km<sup>2</sup> respectively) (Table 1; Figure 1). Conversely, SPM concentrations were decreased in mixed development sub-catchments (Sites #15: 70% mixed development and #20: 89% mixed development) comprising the lowest average (6.5 mg/L), lowest median (1 mg/L), and lowest minimum (0 mg/L) (Table 2; Figure 3). Site #15 also had low SPM concentrations during previous work conducted in the WRW, thereby supporting the results from the current investigation [20]. Mixed development areas can comprise decreased exposed soil surfaces and subsequent reductions in the SPM sources relative to other land use types, which can account for the decreased SPM concentrations [69].

**Table 2.** Descriptive statistics of suspended particulate matter (mg L<sup>-1</sup>) at each sampling location (n = 22) during the study period (2 January 2018–1 January 2019) in West Run Watershed, WV, USA. Note: all average values presented in the current work constitute arithmetic means.

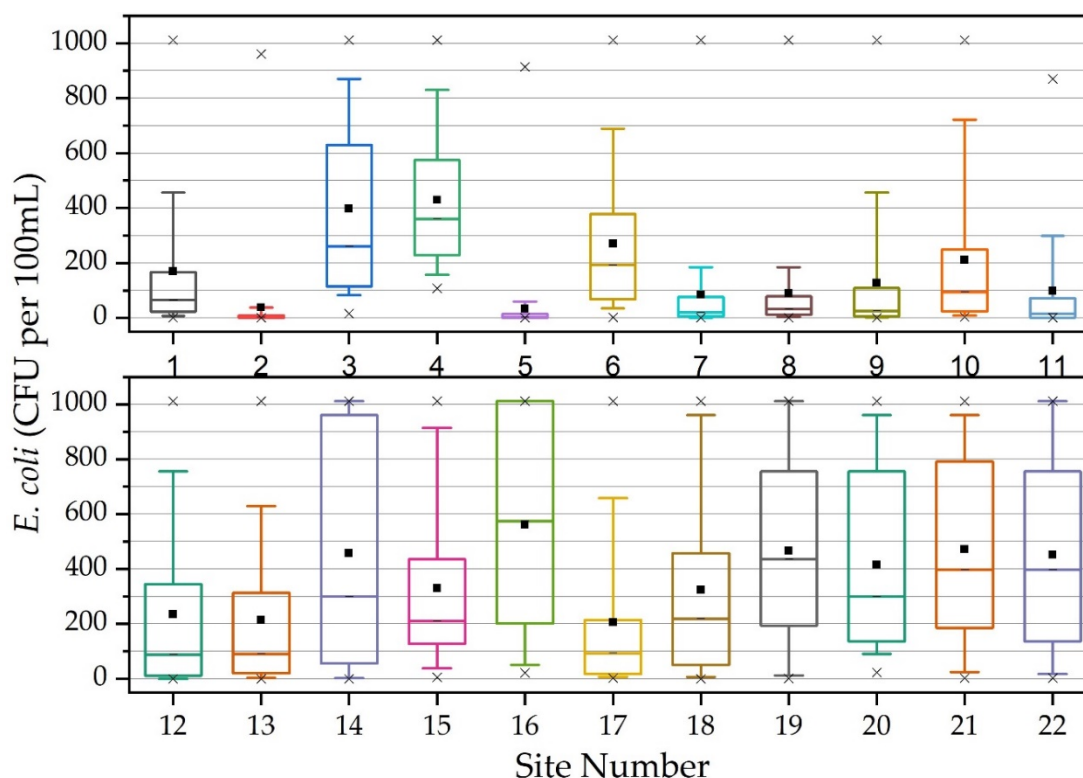
	Site Number										
	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11
Avg.	15.6	53.0	34.5	22.2	15.2	31.3	78.4	61.1	66.4	55.7	42.9
Med.	10.3	39.3	20.3	10.3	11.3	14.3	30.7	28.7	31.7	33.3	23.0
Min.	3.0	17.3	11.0	0.3	0.7	3.0	0.7	12.7	11.7	16.0	0.3
Max.	126.7	528.7	357.0	332.3	125.3	569.7	928.3	803.3	1140.0	642.0	417.3
Std. Dev.	20.8	69.8	57.5	49.5	19.0	79.9	176.5	123.5	159.7	93.3	77.2
	Site Number										
	#12	#13	#14	#15	#16	#17	#18	#19	#20	#21	#22
Avg.	35.0	40.1	29.1	6.5	21.1	14.4	38.2	46.1	17.7	41.9	42.0
Med.	11.3	23.3	14.7	1.0	5.0	6.0	18.7	15.0	1.0	12.3	11.8
Min.	0.7	3.7	3.3	0.0	0.3	1.0	1.0	1.0	0.0	0.3	2.0
Max.	819.3	316.0	370.7	144.7	376.0	277.3	456.7	603.3	590.0	502.0	518.0
Std. Dev.	116.4	62.0	59.7	21.5	59.3	38.6	75.3	103.7	83.3	101.1	99.1

The study results showed that *E. coli* concentrations were the highest at sub-catchments comprising the greatest percentage agricultural land use area (Site #16: 59% agricultural). These results are similar to those of previous investigations in WRW reporting increased *E. coli* concentrations in agricultural land use sub-catchments [20]. This predominantly agricultural sub-catchment comprised the highest average (560 CFU per 100 mL) and median (575 CFU per 100 mL) (Table 3; Figure 4) *E. coli* concentrations over the period of investigation. Previous investigations in the USA (California and Ohio) reported increased fecal contamination with agricultural land use practices [31,70], and a significant correlation ( $p < 0.04$ ) between agricultural land use and *E. coli* concentrations [20], thereby supporting the results recorded during the current work. The lowest *E. coli* concentrations were recorded at two forested sites (Site #2: 74% forested and Site #5: 51% forested) comprising the lowest median (3 CFU per

100 mL) and average (34 CFU per 100 mL) amongst the sites respectively (Table 3; Figure 5). These two sites, and the forested sub-catchments comprising one of the paired watersheds (Sites #7, #8 and #9) located in the headwaters of WRW (Figure 1), were heavily impacted by acid mine drainage (AMD) from historic mining activities [20,22,53], which likely, at least in part, explains the low *E. coli* concentrations observed at these sites. This is an important finding given that previous studies showed that AMD lowers the pH of receiving waters [71] and that the current results indicate that AMD may also lower *E. coli* concentrations. Forested sites generally had lower *E. coli* concentrations (e.g., Site #17: 86% forested; average *E. coli* concentration: 206 CFU per 100 mL) during the study period than sites comprising other land use practices (e.g., Site # 20: 89% mixed development; average *E. coli* concentration: 415 CFU per 100 mL) (Figure 4). These results align well with previous studies reporting decreased fecal contamination in forested areas [70] and are attributable to the increased quality of receiving waters in forested areas [72]. Consequently, both forested land use practices and AMD lowered *E. coli* concentrations in WRW during the investigation. Notably, the low average *E. coli* concentrations recorded during the study period (2 January 2018–1 January 2019), specifically in the headwaters, affirms the study objective of analyzing samples collected between storm events that comprise lower *E. coli* concentrations. Additionally, in no other study has there been such high spatial and temporal resolution sampling over a full annual year. This allowed for a more comprehensive analysis of *E. coli* concentration regimes and relationships with SPM and land use, including accounting for seasonality, than is available in the literature surrounding contemporary mixed land use watersheds. The current study therefore lends greatly needed confirmation through high spatial and temporal resolution of previous studies.

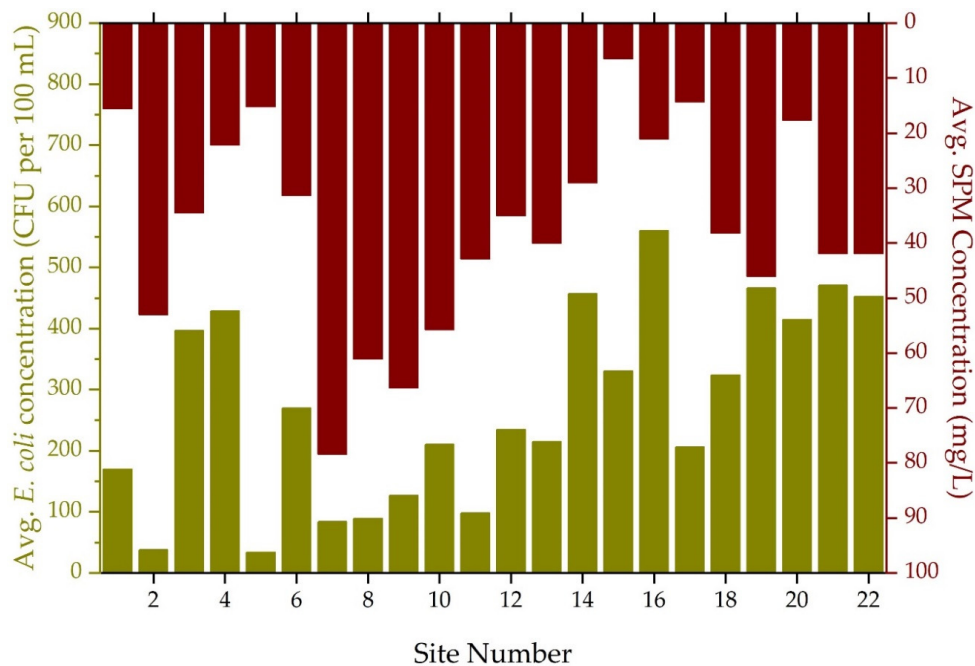
**Table 3.** Descriptive statistics of *E. coli* concentration (CFU per 100 mL) at each sampling location (n = 22) during the study period (2 January 2018–1 January 2019) in West Run Watershed, WV, USA.

	Site Number										
	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11
Avg.	170	38	397	429	34	269	84	89	127	210	98
Med.	66	3	260	361	4	194	20	32	25	93	16
Min.	0	0	15	107	0	2	0	0	0	3	0
Max.	1011	961	1011	1011	914	1011	1011	1011	1011	1011	870
Std. Dev.	251	139	315	249	129	276	179	180	241	273	202
	Site Number										
	#12	#13	#14	#15	#16	#17	#18	#19	#20	#21	#22
Avg.	234	215	457	330	560	206	324	466	415	471	452
Med.	88	91	299	211	575	93	218	436	299	397	397
Min.	0	0	0	5	22	3	0	1	23	2	3
Max.	1011	1011	1011	1011	1011	1011	1011	1011	1011	1011	1011
Std. Dev.	305	266	406	293	373	288	342	339	340	342	345

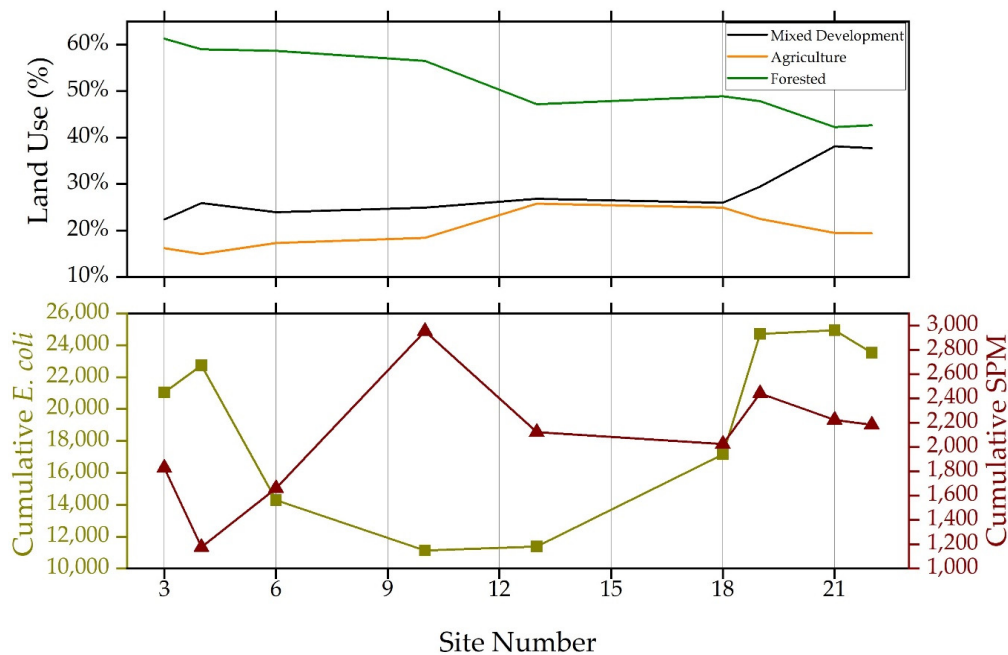


**Figure 4.** Box and whisker plot of *E. coli* concentration (CFU per 100 mL) at each sampling location ( $n = 22$ ) during the study period (2 January 2018–1 January 2019) in West Run Watershed, Morgantown, West Virginia, USA. Boxes delineate 25th and 75th percentiles; lines denote medians; squares show means; whiskers describe 10th and 90th percentiles; x shows maxima and minima when above and below, respectively. Note: different box colors represent data from different sites.

*E. coli* concentrations showed a general increase from the headwaters of WRW to the confluence of the Monongahela River, with larger average concentrations typically being observed in the lower portions of the watershed (Figure 5). In the current work, AMD may account for the lower *E. coli* concentrations in the upper watershed (as discussed above). However, in the lower elevations of WRW, land use practices may be the predominant factor influencing *E. coli* concentrations. For example, in West Run Creek (Sites #13–#21), there was a notable increase in cumulative *E. coli* concentrations and a simultaneous increase in agricultural and mixed development land use practices (Figure 6). Previous work reported on the increased fecal contamination associated with increased agricultural and urban areas [31,70,73,74], commonly attributed to increased sources (livestock and manure) [23] and increased (concentrated flow) run-off during precipitation events, respectively [73], and urban stream syndrome [75], thereby supporting the results from the current investigation. The inter-site relationship between *E. coli* concentrations and SPM was not clearly discernable based on average values (Figure 5) or cumulative values in West Run Creek (Figure 6), as increases in SPM were not always accompanied by similar increases or decreases in *E. coli* between the different sampling locations. A potential explanation for these results may be that SPM and *E. coli* concentrations are affected by different factors at different sites (e.g., geochemistry, land use and antecedent soil water conditions). Thus, in WRW, there may exist a spatial disconnect regarding the factors influencing *E. coli* concentrations and subsequently impacting the relationship between *E. coli* and SPM in the watershed. To account for the spatial disconnect, site specific analysis of *E. coli* and SPM correlations, including separate analysis for different time periods in the year (quarters), was required to improve the current understanding regarding this relationship and to assess the use of SPM as a proxy for fecal contamination.



**Figure 5.** Average suspended particulate matter ( $\text{mg L}^{-1}$ ) and *E. coli* concentration (CFU per 100 mL) at each sampling location ( $n = 22$ ) during the study period (2 January 2018–1 January 2019) in West Run Watershed, Morgantown, West Virginia, USA.

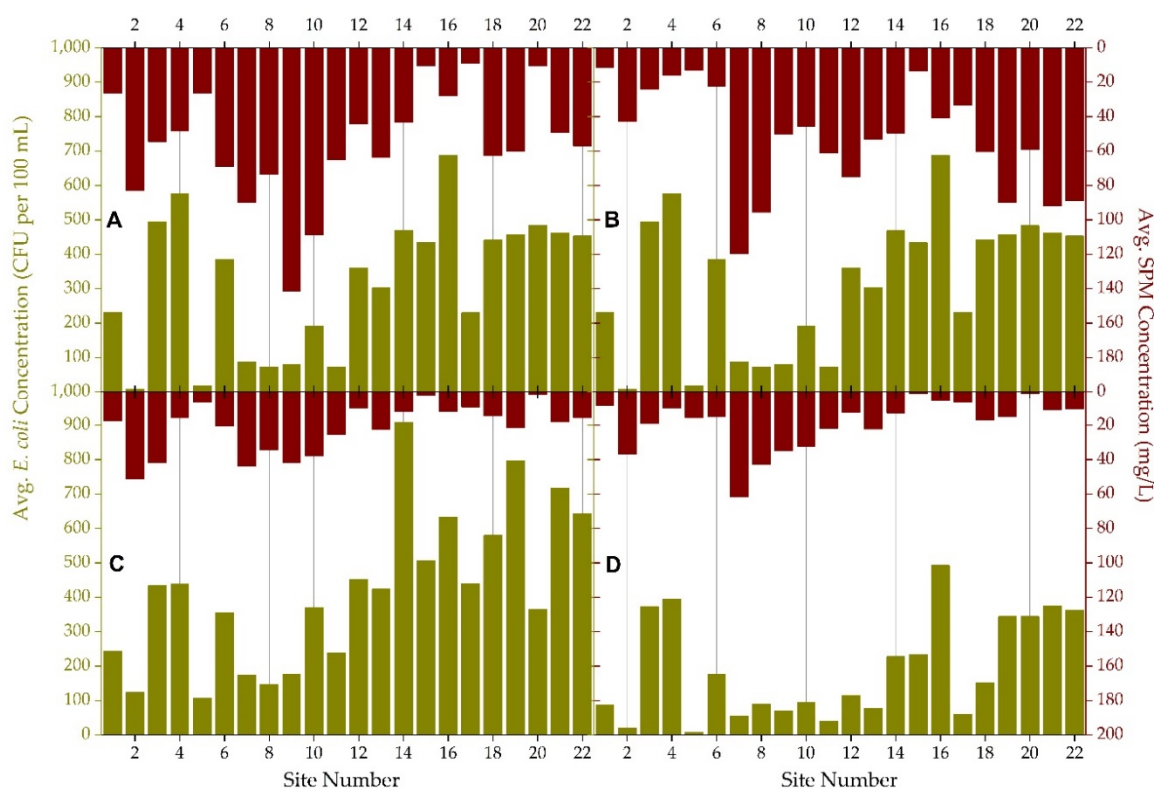


**Figure 6.** Land use percentage relative to cumulative annual *E. coli* concentration (CFU per 100 mL) and SPM concentration ( $\text{mg/L}$ ) at West Run Creek monitoring sites ( $n = 9$ ) during the study period (2 January 2018–1 January 2019) in West Run Watershed, Morgantown, West Virginia, USA. Note: West Run Creek included the following site numbers: #3, #4, #6, #10, #13, #18, #19, #21 and #22.

### 3.3. Quarterly Suspended Particulate Matter, *E. coli* Concentrations and Land Use Practices

Average *E. coli* and SPM concentration data showed notable temporal variation during 2018, based on quarterly analysis (Figure 7). *E. coli* concentrations were elevated during Quarter 2 (spring; 3 April 2018–26 June 2018) and Quarter 3 (summer; 3 July 2018–25 September 2018) of 2018, coinciding with the

warmer spring and summer months (average maximum daily temperatures: Quarters 2 and 3 = 26 °C; Quarters 1 and 4 = 9 °C) of the year and higher stream flows induced by larger and more frequent precipitation events (Figure 3). Previous work noted correlations between elevated air temperatures and *E. coli* concentrations in freshwater [74], thereby supporting the results from the current work reporting greater *E. coli* concentrations during warmer months. Furthermore, the second and third quarters included some of the largest precipitation events of 2018 (Figure 3). These precipitation events could, at least in part, account for the elevated *E. coli* concentrations recorded during this time period, as previous work linked precipitation events to elevated *E. coli* concentrations in receiving waters [32,74]. Conversely, the highest average SPM concentrations were recorded during Quarters 1 (winter) and 2 (spring), with average concentrations decreasing during the second half of the year (Figure 7). The high SPM recorded during this time period may be attributable to decreased vegetation cover throughout WRW, leading to increased exposed soil surfaces, owing to the seasonal changes in vegetation (i.e., many plant species senesce during the cold winter months) [76,77]. Once vegetation throughout WRW increases during Quarter 3 (summer), exposed soil surfaces and sources of SPM become more limited. Quarter 4 (fall) comprised low *E. coli* and SPM concentrations. During this time period, WRW received less precipitation than in the preceding quarters (Figure 2), which could have limited the transport of pollutants (i.e., *E. coli* and SPM) to receiving waters [78]. Additionally, *E. coli* concentrations in the receiving water could have been suppressed by the colder temperatures [79] and decreased nutrient availability owing to the drier antecedent conditions and greater infiltration [77,80].



**Figure 7.** Quarterly average *E. coli* concentration (CFU per 100 mL) and SPM concentration (mg/L) at each sampling location ( $n = 22$ ) during the study period (1/2/18–1/1/19) in West Run Watershed, Morgantown, West Virginia, USA. Note: (A) represents Quarter 1 (winter: 2 January 2018–27 March 2018); (B) represents Quarter 2 (spring: 3 April 2018–26 June 2018); (C) represents Quarter 3 (summer: 3 July 2018–25 September 2018); (D) represents Quarter 4 (fall: 2 October 2018–1 January 2019).

### 3.4. Non-Parametric Statistical Results

Normality test results indicated that the *E. coli* concentration data were non-normally distributed, thus Spearman correlation coefficients (nonparametric version of the Pearson product moment

correlation) were used to quantify the relationships between *E. coli* concentration, SPM concentration, and land use at each site. *E. coli* concentrations and SPM concentrations were not significantly correlated at all sites; however, nine of the 22 sites (Sites #4, #7, #8, #9, #11, #15, #16, #17 and #20) did have significant correlations (Table 4). Notably, Sites #7, #8 and #9, which had the highest SPM during the investigation (Table 2; Figure 3), displayed statistically significant positive correlations ( $p < 0.01$  for all three sites) between *E. coli* concentrations and SPM concentrations. Based on these relationships, SPM may serve as a relatively accurate proxy for *E. coli* concentrations in similarly physiographic catchments. Mixed development and forested sub-catchments did not display consistent significant correlations ( $p < 0.05$ ) between *E. coli* and SPM concentrations. For example, Sites #15 (70.3% mixed development) and #17 (85.8% forested) both comprised statistically significant relationships between SPM and *E. coli* concentrations ( $p < 0.01$  and  $p < 0.05$ , respectively) despite comprising different predominant land use practices. However, Site #1 (53.2% mixed development) and Site #5 (51.1% forested) displayed statistically insignificant correlations, despite including approximately similar dominant land use practices to Sites #15 and #17. As discussed in the preceding sections, AMD in the headwaters of WRW could be affecting *E. coli* concentrations, thereby creating inconsistency in the correlations between the *E. coli* and SPM concentrations. Therefore, the *E. coli* and SPM correlation results imply a spatial disconnect in terms of the influence of land use practices—in particular, mixed development and forested areas—on the use of SPM as a proxy for fecal contamination. Notably, it seems likely, based on these results, that legacy effects (AMD) of historic land use practices (coal mining) may be impacting *E. coli* concentrations and affecting the observed relationships between *E. coli* and SPM concentrations. Conversely, both of the predominantly agricultural sites (Site #11 and #16) displayed significant correlations between SPM concentrations and *E. coli* concentrations, supporting previous work that reported elevated *E. coli* and SPM concentrations in the receiving waters of agricultural areas [19,20]. Therefore, in agricultural areas, SPM could be implemented as a proxy for *E. coli* with greater accuracy and less difficulty than in other land use areas.

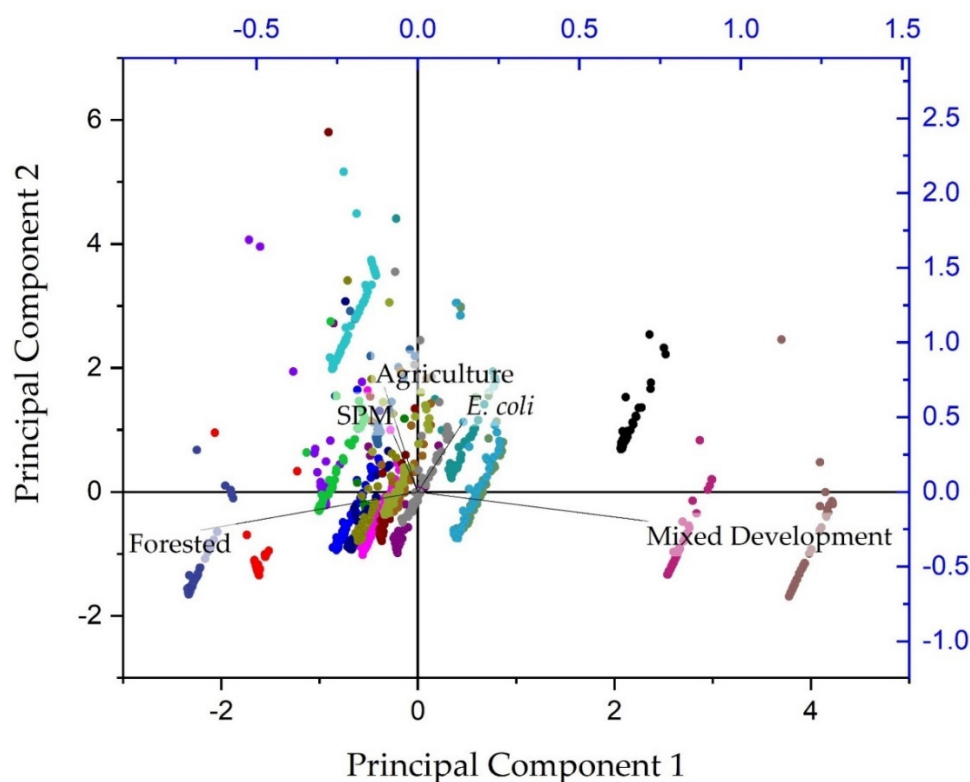
**Table 4.** Results of Spearman’s correlation test, including annual *E. coli* concentration (CFU per 100 mL) and annual SPM concentration (mg/L) at each sampling location ( $n = 22$ ) during the study period (2 January 2018–1 January 2019) in West Run Watershed, WV, USA. Note: bold values indicate significant correlations ( $p < 0.05$ ).

	Site Number										
	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11
SCC	0.19	0.18	0.04	<b>0.30</b>	−0.11	0.04	<b>0.46</b>	<b>0.52</b>	<b>0.73</b>	0.24	<b>0.56</b>
<i>p</i> -value	0.17	0.19	0.77	<b>0.03</b>	0.42	0.78	<b>&lt;0.01</b>	<b>&lt;0.01</b>	<b>&lt;0.01</b>	0.09	<b>&lt;0.01</b>
	Site Number										
	#12	#13	#14	#15	#16	#17	#18	#19	#20	#21	#22
SCC	0.00	0.13	−0.25	<b>0.42</b>	<b>0.64</b>	<b>0.27</b>	0.02	−0.14	<b>0.55</b>	0.01	0.17
<i>p</i> -value	0.99	0.34	0.07	<b>&lt;0.01</b>	<b>&lt;0.01</b>	<b>0.05</b>	0.88	0.33	<b>&lt;0.01</b>	0.94	0.24

SCC = Spearman correlation coefficient.

Explanatory variables that account for the maximal variance in a data set can be identified via principle component analysis (PCA), through the computation of multiple principal components and their respective Eigenvalues [81]. Components comprising the highest Eigenvalues are assumed principal components, given that Eigenvalues represent the variance of the data in that direction [81]. A principle component is a linear function of the variables in an original data set that successively maximize variance and that are uncorrelated with each other [82]. Multiple principal components are typically calculated and ranked based on their Eigenvalues as most data cannot be well-described by a single principal component [81]. For the current work, the results showed three principal components with Eigenvalues exceeding 1 (an accepted threshold of importance [22,83]), including Principal Component 1 (Eigenvalue = 1.83), Principal Component 2 (Eigenvalue = 1.22) and Principal

Component 3 (Eigenvalue = 1.17). These three principle components explained approximately 85% of the cumulative variance of the data set. Conversely, Principal Components 4 and 5 accounted for approximately 16% and 0% of the variance of the data set. For the current work, the principle component biplots showed distinct spatial distributions for study sites along Principal Components 1 and 2 (Figure 8). The idealized biplot vector space defined by Principal Components 1 and 2 is characterized by the grouping of the sites. Given these results, it can be concluded that land use practices are the primary factors influencing the grouping of the data in the biplot, given the similarity of the sites in terms of geology, topography and climate, and their close proximity to each other [52]. The strongest correlation illustrated by the biplot is between SPM concentration and agricultural land use practices (Figure 8). However, *E. coli* concentration is also closely related to both, attributable to agricultural land use practices (i.e., the rearing of livestock, manure application, soil tillage and increased exposed soil surfaces), as discussed in the preceding sections and reported by previous investigations [19–23]. Ultimately, the PCA results were analogous to the Spearman correlation coefficient results, indicating that SPM could potentially serve as proxy for *E. coli* in agricultural areas, especially during periods with lower levels of fecal contamination between storm events.



**Figure 8.** Results of principal component analysis, including biplots, for extracted principal components of annual *E. coli* concentration (CFU per 100 mL) and annual SPM concentration (mg/L) at 22 monitoring sites (indicated by the different colors) during the study period (2 January 2018–1 January 2019) in West Run Watershed, West Virginia, USA.

The Spearman correlation test results based on the quarterly analysis of data indicated predictable temporal variation in the correlation between *E. coli* and SPM concentrations, with only Site #9 showing significant correlations ( $p < 0.05$ ) throughout all four quarters ( $p < 0.01$  Quarters 1 to 3;  $p = 0.02$  Quarter 4) (Table 5). Quarter 1 displayed the most significant correlations (10; Site #2, #7, #8, #9, #10, #11, #12, #13, #15, and #18), whereas Quarter 4 had the fewest significant correlations (two; Site #9 and #16) (Table 5). The temporal variation can be explained by the different impacts seasonal variation have on *E. coli* and SPM concentrations. For example, as discussed above, SPM concentrations will be influenced by changes in vegetation, with elevated concentrations typically occurring when

vegetation cover decreases and decreasing as vegetation cover increases [76,77]. Therefore, elevated SPM concentrations can be expected during and immediately after the cold winter months, with decreased concentrations during the warmer summer months. Conversely, previous work linked elevated *E. coli* concentrations with warmer water (and air) temperatures [84–86]. Consequently *E. coli* concentrations can be expected to be elevated during the warmer summer months and decrease during the colder winter months. Ultimately, there is a temporal (seasonal) difference between periods of elevated SPM and *E. coli* concentrations. For example, Quarter 3 included the highest average (424 CFU per 100 mL) *E. coli* concentrations across all 22 sampling locations, while Quarter 1 comprised the lowest average (187 CFU per 100 mL) *E. coli* concentration. Thus, between Quarter 1 and 3 there was more than a 100% increase in average *E. coli* concentrations across the 22 sampling sites. Conversely, Quarter 1 had the highest average (55.7 mg/L) SPM across all 22 sampling locations, while Quarter 4 comprised the lowest average (18.2 mg/L) SPM. Consequently, there was more than a 65% decrease in average SPM, across the 22 sampling locations, between Quarter 1 and Quarter 4. These temporal differences, driven by changes in precipitation [78], antecedent conditions [87], seasonal land cover [76] or land use practices [29] may account for the variable *E. coli* and SPM concentrations correlations identified in Table 5.

**Table 5.** Results of Spearman’s correlation test, including quarterly *E. coli* concentration (CFU per 100 mL) and quarterly SPM concentration (mg/L) at each sampling location (n = 22) during the study period (2 January 2018–1 January 2019) in West Run Watershed, WV, USA. Note: Quarter 1 represents 2 January 2018–27 March 2018; Quarter 2 represents 3 April 2018–26 June 2018; Quarter 3 represents 3 July 2018–25 September 2018; Quarter 4 represents 2 October 2018–1 January 2019. Bold values indicate significant correlations ( $p < 0.05$ ).

		Site Number										
		#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11
Quarter 1	SCC	0.40	<b>0.60</b>	0.26	0.48	0.52	0.35	<b>0.76</b>	<b>0.70</b>	<b>0.73</b>	<b>0.63</b>	<b>0.69</b>
	p-value	0.18	<b>0.03</b>	0.39	0.09	0.09	0.23	<b>&lt;0.01</b>	<b>0.01</b>	<b>&lt;0.01</b>	<b>0.02</b>	<b>0.01</b>
Quarter 2	SCC	0.19	0.18	0.03	0.26	−0.04	0.07	<b>0.78</b>	<b>0.88</b>	<b>0.78</b>	0.51	<b>0.93</b>
	p-value	0.53	0.56	0.91	0.38	0.89	0.81	<b>&lt;0.01</b>	<b>&lt;0.01</b>	<b>&lt;0.01</b>	0.08	<b>&lt;0.01</b>
Quarter 3	SCC	−0.05	−0.40	0.32	0.50	<b>0.76</b>	<b>0.69</b>	−0.27	<b>0.58</b>	<b>0.79</b>	0.25	0.54
	p-value	0.88	0.17	0.29	0.08	<b>&lt;0.01</b>	<b>0.01</b>	0.37	<b>0.04</b>	<b>&lt;0.01</b>	0.42	0.06
Quarter 4	SCC	−0.30	−0.32	−0.39	−0.04	−0.02	−0.01	−0.08	−0.09	<b>0.62</b>	−0.22	0.51
	p-value	0.31	0.27	0.17	0.89	0.95	0.98	0.78	0.75	<b>0.02</b>	0.45	0.06

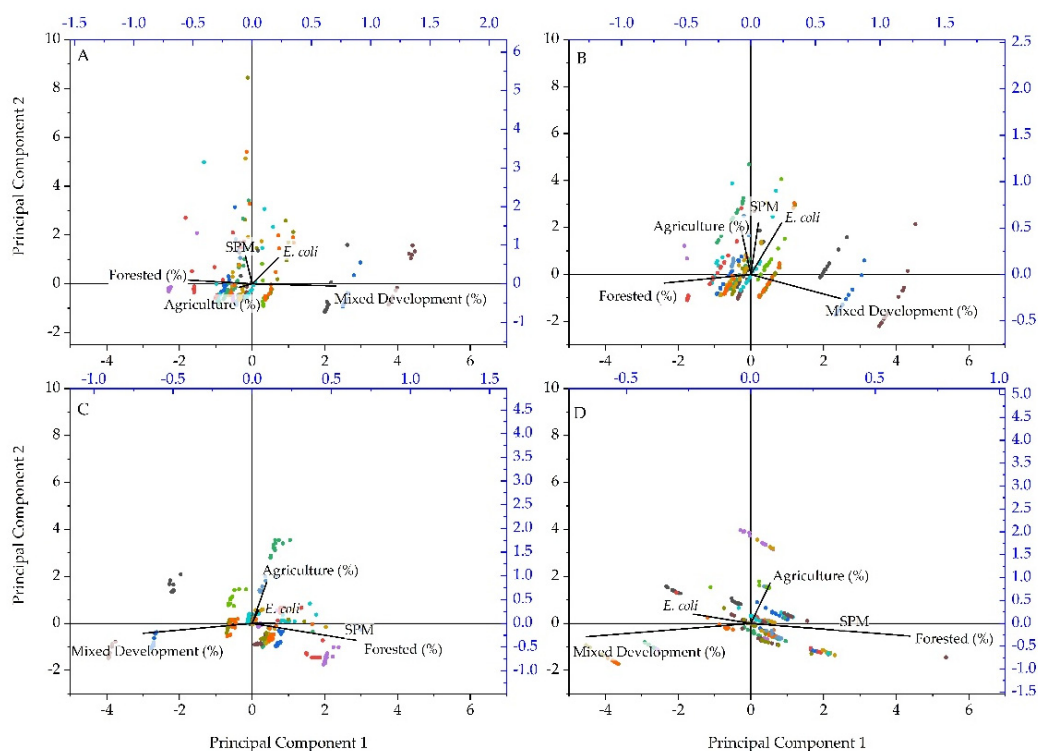
  

		Site Number										
		#12	#13	#14	#15	#16	#17	#18	#19	#20	#21	#22
Quarter 1	SCC	<b>0.63</b>	<b>0.62</b>	−0.16	<b>0.60</b>	0.36	0.10	<b>0.68</b>	0.16	0.35	0.12	0.40
	p-value	<b>0.02</b>	<b>0.02</b>	0.59	<b>0.03</b>	0.22	0.74	<b>0.01</b>	0.61	0.24	0.69	0.19
Quarter 2	SCC	0.17	0.44	<b>0.58</b>	<b>0.71</b>	<b>0.91</b>	0.42	0.54	0.24	<b>0.75</b>	0.39	0.37
	p-value	0.57	0.13	<b>0.04</b>	<b>0.01</b>	<b>&lt;0.01</b>	0.15	0.06	0.43	<b>&lt;0.01</b>	0.19	0.22
Quarter 3	SCC	<b>0.60</b>	0.45	0.22	0.29	<b>0.72</b>	<b>0.68</b>	0.51	0.34	<b>0.58</b>	0.38	0.40
	p-value	<b>0.03</b>	0.13	0.47	0.33	<b>0.01</b>	<b>0.01</b>	0.07	0.26	<b>0.04</b>	0.19	0.18
Quarter 4	SCC	0.09	−0.16	−0.39	−0.28	<b>0.56</b>	0.07	−0.31	−0.18	0.38	0.19	0.28
	p-value	0.75	0.58	0.17	0.33	<b>0.04</b>	0.82	0.27	0.53	0.19	0.53	0.34

The varying correlations displayed in Table 5 constitute important results regarding the use of SPM as a proxy for *E. coli*. Temporal changes in correlation significance indicate that SPM cannot be a consistently accurate proxy for fecal contamination throughout all the quarters (seasons) of a year. Even agricultural sub-catchments displayed insignificant correlations during certain quarters (Quarter 3 and 4 for Site #11 and Quarter 1 for Site #16), despite being significantly correlated for the annual time period. These results differ from previous investigations that showed strong correlations between *E. coli* and SPM concentrations [15,16], particularly small SPM particles [22]. However, these studies did not comprise high frequency or sufficiently long sampling regimes that would allow for quarterly (seasonal) analysis. Therefore, the current work is among the first to include seasonal analysis of the relationship (correlation) between *E. coli* and SPM concentrations in receiving waters.



Similarly to in the annual results, Quarter 1 (winter) had three principal components with Eigenvalues exceeding 1 (Eigenvalues = 1.86, 1.35 and 1.20, respectively), which accounted for 88% of the cumulative variance in the data. Quarters 2 and 3 also included three principal components with Eigenvalues exceeding 1 (1.85, 1.29 and 1.15; and 1.88, 1.22 and 1.16, respectively). The principal components explained 86% of the variance in Quarter 2 and 85% of that in Quarter 3. Two principal components, accounting for 63% of the data variance, were identified for Quarter 4, comprising Eigenvalues of 1.95 and 1.21. The quarterly PCA results illustrated the correlation between agricultural land use and *E. coli* and SPM during Quarters 2 (spring) and 3 (summer) (Figure 9). The agricultural land use impacts on *E. coli* and SPM concentrations during these quarters were subjected to the largest precipitation events during the study period (Figure 3). Precipitation has been reported to exacerbate the impact of land use practices on receiving waters [19,74,88]; therefore, land use impacts (particularly agriculture) were elevated during Quarters 2 (spring) and 3 (summer). The elevated impacts of land use practices during precipitation events are attributable to the increased transport of both *E. coli* and SPM during runoff events and subsequent increased *E. coli* and SPM concentrations in the receiving waters [88]. Conversely, during Quarters 1 (winter) and 4 (fall), none of the land use classes were closely correlated with either SPM or *E. coli* concentrations (Figure 9). Small and fewer precipitation events during Quarters 1 (winter) and 4 (fall) (Figure 3) may account for these results as fewer runoff events would lead to decreased concentrations of *E. coli* and SPM in the associated receiving waters [88]. The reduced precipitation during Quarters 1 and 4 would also have led to drier antecedent soil water conditions [89], leading to greater infiltration during subsequent precipitation events [90], further reducing the transport of both *E. coli* and SPM to the receiving waters. These results highlight the varied seasonality of land use impacts on *E. coli* and SPM concentrations and thus advance the science-based understanding of temporal fluctuations in *E. coli* concentration regimes.



**Figure 9.** Results of principal component analysis, including biplots, for extracted principal components of quarterly *E. coli* concentration (CFU per 100 mL) and quarterly SPM concentration (mg/L) at each sampling location ( $n = 22$ ) during the study period (2 January 2018–1 January 2019) in West Run Watershed, WV, USA. Note: (A) represents Quarter 1 (winter: 2 January 2018–27 March 2018); (B) represents Quarter 2 (spring: 3 April 2018–26 June 2018); (C) represents Quarter 3 (summer: 3 July 2018–25 September 2018); (D) represents Quarter 4 (fall: 2 October 2018–1 January 2019).

### 3.5. Study Implications and Future Work

The scale-nested experimental watershed study design and the high spatial and temporal sampling period implemented during the current work allowed for the collection of a unique data set. PCA biplots illustrated the close correlation between agricultural land use practices and both *E. coli* and SPM concentrations, relative to mixed development and forested land use practices. Additionally, spatial and temporal variability in the significant correlations between *E. coli* and SPM concentrations indicated that SPM would not be a suitable proxy for fecal contamination. The recorded lack of consistent *E. coli* and SPM relationships constitutes an important result for the development of accurate predictive fecal pollution models. The investigation emphasized the efficacy of the nested-scale experimental watershed study design to elucidate land use influences on fecal pollution in receiving waters. Future work should expand on the results from the current investigation by attempting to determine the precise tipping points associated with different land use practices' influence on *E. coli* and SPM concentrations. Implementing a similar study design, as in the current work, in mixed land use watersheds not impacted by legacy land use impacts (e.g., AMD) could provide useful information regarding the precise tipping points for various land use practices. Furthermore, due to the results of the current investigation indicating that *E. coli* concentrations are not solely influenced by land use practices and given the previously reported influence of physicochemical parameters (e.g., pH and water temperature) on *E. coli* [84–86], future work should focus on the identification of additional variables (e.g., physicochemical and geochemical) influencing *E. coli* concentrations in receiving waters. The incorporation of a multi-year study period would also allow future work to expand on the results of this study to account for annual variations in climate.

## 4. Conclusions

A 22-site, nested-scale, experimental watershed study design was implemented to investigate *E. coli* concentrations in a mixed land use watershed in the Appalachian region of the eastern United States. Specific focus was given to the relationship between *E. coli* concentrations, SPM concentrations and land use practices, including an evaluation of the potential use of SPM concentrations as a proxy for *E. coli* concentrations. Agricultural land use sub-catchments comprised elevated *E. coli* concentrations (avg. 560 CFU per 100 mL) compared to adjacent mixed development (avg. 330 CFU per 100 mL) and forested (avg. 206 CFU per 100 mL) sub-catchments. Annual *E. coli* and SPM concentration data displayed a statically significant relationship ( $p < 0.01$ ) in agricultural areas. However, quarterly SCC analysis highlighted fluctuations between significance ( $p < 0.05$ ) and insignificance ( $p > 0.05$ ) in the correlations between *E. coli* and SPM concentrations across all land use classes. Therefore, SPM lacked the consistent significant correlations with *E. coli* concentrations required to be a suitable proxy for fecal contamination. The annual PCA results illustrated the influence of agricultural land use practices on both *E. coli* and SPM concentrations, serving as validation for previous investigations, which typically included less temporally and spatially robust sampling regimes. The quarterly PCA results highlighted the seasonal variability of land use impacts on both *E. coli* and SPM concentrations, with Quarters 2 and 3's biplots displaying greater correlations between agricultural land use practices, *E. coli* and SPM concentrations than Quarters 1 and 2. Combined, Quarters 2 and 3 received 67% more precipitation (850 mm) than Quarters 1 and 2 (510 mm), accounting for the temporal variation in land use impacts depicted by the quarterly biplots. Ultimately, the current investigation advances the understanding of the influence of land use practices on *E. coli* and SPM concentrations, thereby contributing to the current understanding of fecal contamination regimes in contemporary mixed land use watersheds. The results better inform model builders, policy makers and land use managers regarding the factors influencing freshwater fecal contamination, thereby aiding in effective decision making and effective water quality management.

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