

APPENDIX L: RELATED PEER-REVIEWED PUBLICATIONS AND FACT SHEETS

- Burke, J., Sharpley, A.N., L. Berry, K. Brye, M.B. Daniels, E. Gbur, K.W. VanDevender, S. King, P. Hays, and B.E. Haggard. 2018. Nutrient concentrations in Big Creek correlate to regional watershed land use. Cooperative Extension Service, Division of Agriculture, University of Arkansas. Fact Sheet FSA9537. 5 pages. Available at <https://www.uaex.edu/publications/pdf/FSA-9537.pdf>.
- Dodd, R.J., A.N. Sharpley, and L.G. Berry. 2018. Organic phosphorus can make an important contribution to phosphorus loss from riparian buffers. *Agricultural & Environmental Letters* 3:180002. Available at <https://dl.sciencesocieties.org/publications/ael/articles/3/1/180002>.
- Jarvie, H.P., A.N. Sharpley, J.V. Brahana, T. Simmons, A. Price, C. Neal, A. Lawlor, D. Sleep, S. Thacker, and B. Haggard. 2014. Phosphorus retention and remobilization along hydrological pathways in karst terrain. *Environ. Sci. Technol.* 48:4860–4868. Available at <http://pubs.acs.org/doi/pdf/10.1021/es405585b>
- Jarvie, H.P., A.N. Sharpley, T. Kresse, P.D. Hays, R.J. Williams, S.M. King, and L.G. Berry. 2018. Coupling high-frequency stream metabolism and nutrient monitoring to explore biogeochemical controls on downstream nitrate delivery. *Environ. Sci. Technol.* 52:13708-13717. Available at <https://pubs.acs.org/doi/abs/10.1021/acs.est.8b03074>.
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Nutrient Concentrations in Big Creek Correlate to Regional Watershed Land Use

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In the Ozark Mountain karst region, nutrient concentrations in streams of the Buffalo, Upper Illinois and Upper White River watersheds increase as the percent of land in pasture and urban use increases. Averaged over the last three years, nutrient concentrations in Big Creek above and below the C&H Farm are similar to concentrations found in other watersheds where there is a similar amount of pasture and urban land use.

Background

Land use within watersheds influences the quantity and quality of water draining from a watershed. As land disturbance increases and use intensifies, there is a general increase in stormwater runoff and nutrient inputs that leads to a greater potential for nutrient discharge to receiving waters. For instance, with urban growth, more impervious surfaces increase the flashiness of runoff, stream flows and wastewater treatment discharge. Also, as areas of agricultural production grow, more fertilizer is applied to achieve optimum production. Thus, as the percent of a watershed drainage area in pasture, row crop or urban use increases, there is a general increase in nutrient concentrations in storm and base flows.

In this fact sheet, we show the effect of land use on nitrogen (N) and phosphorus (P) concentrations in streams of the Ozark Highlands and Boston Mountains, northwest Arkansas, by combining previously published data for the Upper Illinois River Watershed (Haggard et al., 2010), Upper White River Watershed (Giovannetti et al., 2013) and ongoing

monitoring in the Buffalo River Watershed. The location of these watersheds is shown in Figure 1. The relationships between stream nutrient concentrations and land use for the region are used to determine if a permitted concentrated animal feeding operation (CAFO) in Big Creek Watershed, a sub-watershed of the Buffalo River Watershed, has affected stream water quality. Land use in these watersheds is given in Table 1.

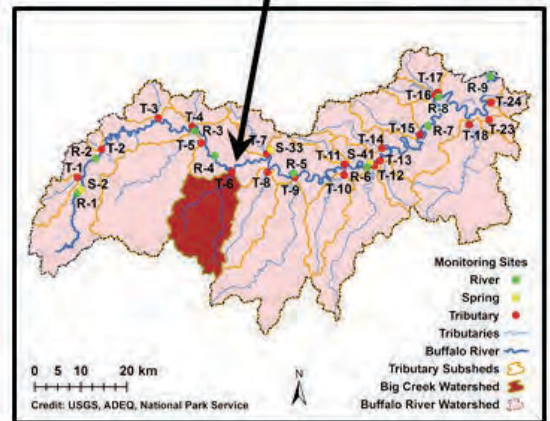
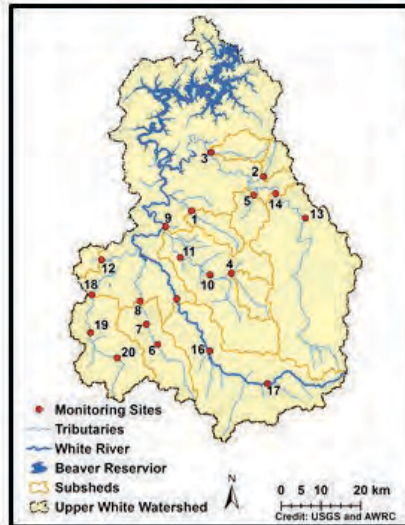
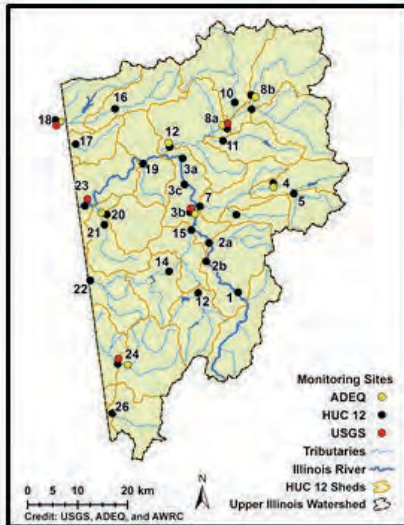
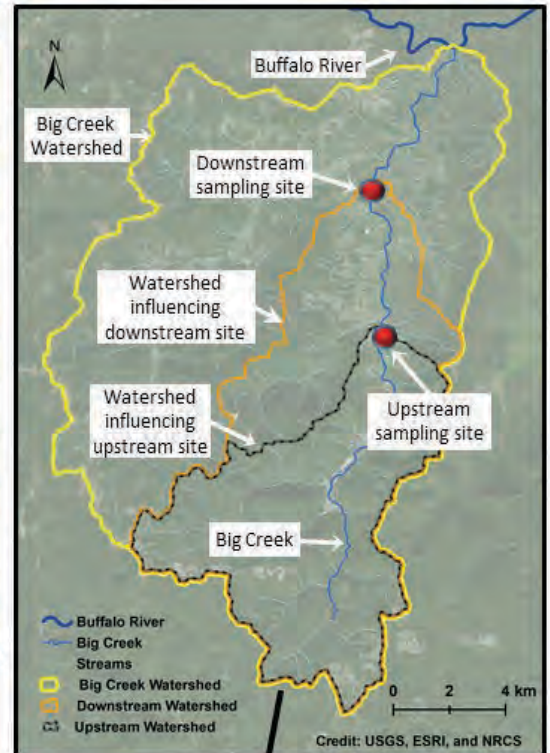
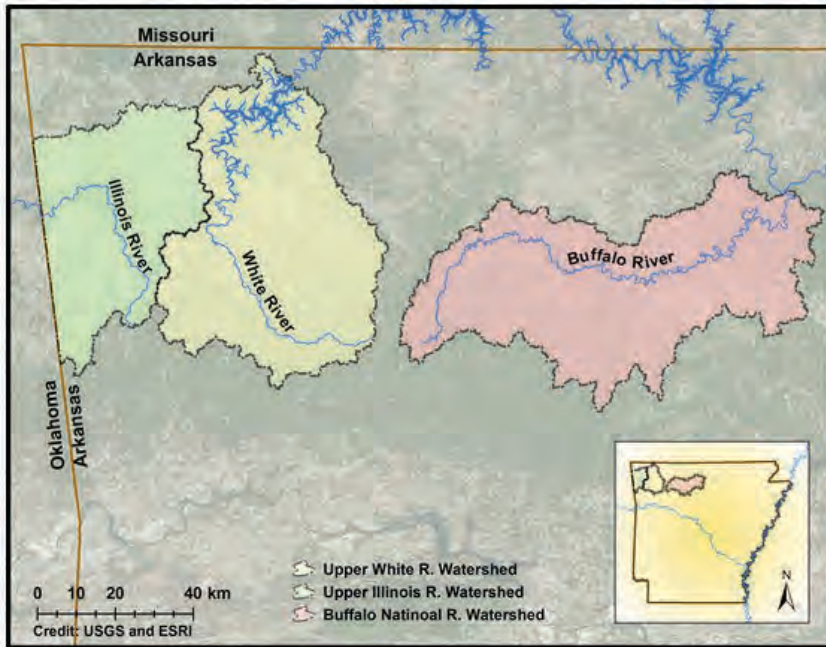
Nitrate-N, total N, dissolved P and total P concentrations have been measured over varying periods during base flow at the outlet of sub-watersheds in the Big Creek (two sites, 2014 to 2017), Buffalo (20 sites, 1985 to 2017), Upper Illinois (29 sites, 2009) and Upper White River Watersheds (20 sites, 2005 to 2006) (Figure 1).

Data from Big Creek were paired with discharge available from a gaging station just downstream from the swine CAFO, where the USGS developed the rating curve; discharge information was only available from May 2014 through December 2017. The data were then used to look at changes in flow-adjusted nutrient concentrations^[A] in Big Creek (White et al., 2004).

^[A]Concentration is defined as the mass of a substance (M), such as a nutrient, over the volume of water (V) in which it is contained, or $C = M/V$. “**Flow-adjusted nutrient concentrations**” – when looking at how concentrations change over time in streams, we have to consider how concentrations might also change with stream flow (volume of water) and not just change in mass; nutrient concentrations often have some type of relation to flow, maybe increasing or even decreasing as stream flow increases. We have to flow-adjust concentrations so we can remove the variability in concentrations that flow might cause to see how things are changing over time.

Study Watersheds in the Ozark Highlands Ecoregion

Big Creek Watershed



Upper Illinois River Watershed

Upper White River Watershed

Buffalo River Watershed

Figure 1. Location of the Big Creek, Buffalo River, Upper Illinois River and Upper White River watersheds in the Boston Mountains and Ozark Highlands ecoregion. Information from U.S. Geological Survey (USGS), Environmental Systems Research Institute (ESRI) and National Aeronautics and Space Administration (NASA).

Table 1. Percent of forest, pasture and urban land use in the Big Creek, Buffalo River, Upper Illinois and Upper White River watersheds.

Watershed	Forest	Pasture	Urban
	----- % -----		
Big Creek*			
Upstream	89.5	8.0	2.6
Downstream	79.5	17.0	3.5
Buffalo River	52 - 99	0 - 25	0 - 1
Upper White River	34 - 90	7 - 55	0 - 44
Upper Illinois River	2 - 70	27 - 69	3 - 61

*Up and downstream of CAFO operation and fields permitted to receive manure.

Putting Stream Nutrient Concentrations Into Context at Big Creek

Geometric mean concentrations^[B] of stream P and N are related to the percent of watershed drainage area in pasture and urban land use for the Buffalo, Upper Illinois and Upper White River watersheds (R^2 of 0.56 to 0.81 where the number of observations is 71; Figure 2)^[C]. The dashed lines on Figure 2 represent the upper and lower thresholds concentrations, where there is a 95 percent confidence that a stream draining a watershed with a specific percent pasture and urban land use will have a P and N concentration within those thresholds.

The relationship between land use and stream nutrient concentrations is not a model that can be used to predict concentration. Given the large variability observed in these relationships, they simply show trends between two variables, land use and stream nutrient concentrations. Continued monitoring of stream concentrations in Big Creek will continue to more reliably define trends.

As the percent pasture and urban land (i.e., land use intensity) increases, so does stream P and N concentrations (see Figure 2). The general increase in nutrient concentrations is consistent with the fact that fertilizer (as mineral and manure sources) is routinely applied to pastures to maintain forage production, as well as deposition of nutrients by grazing cattle.

Watersheds

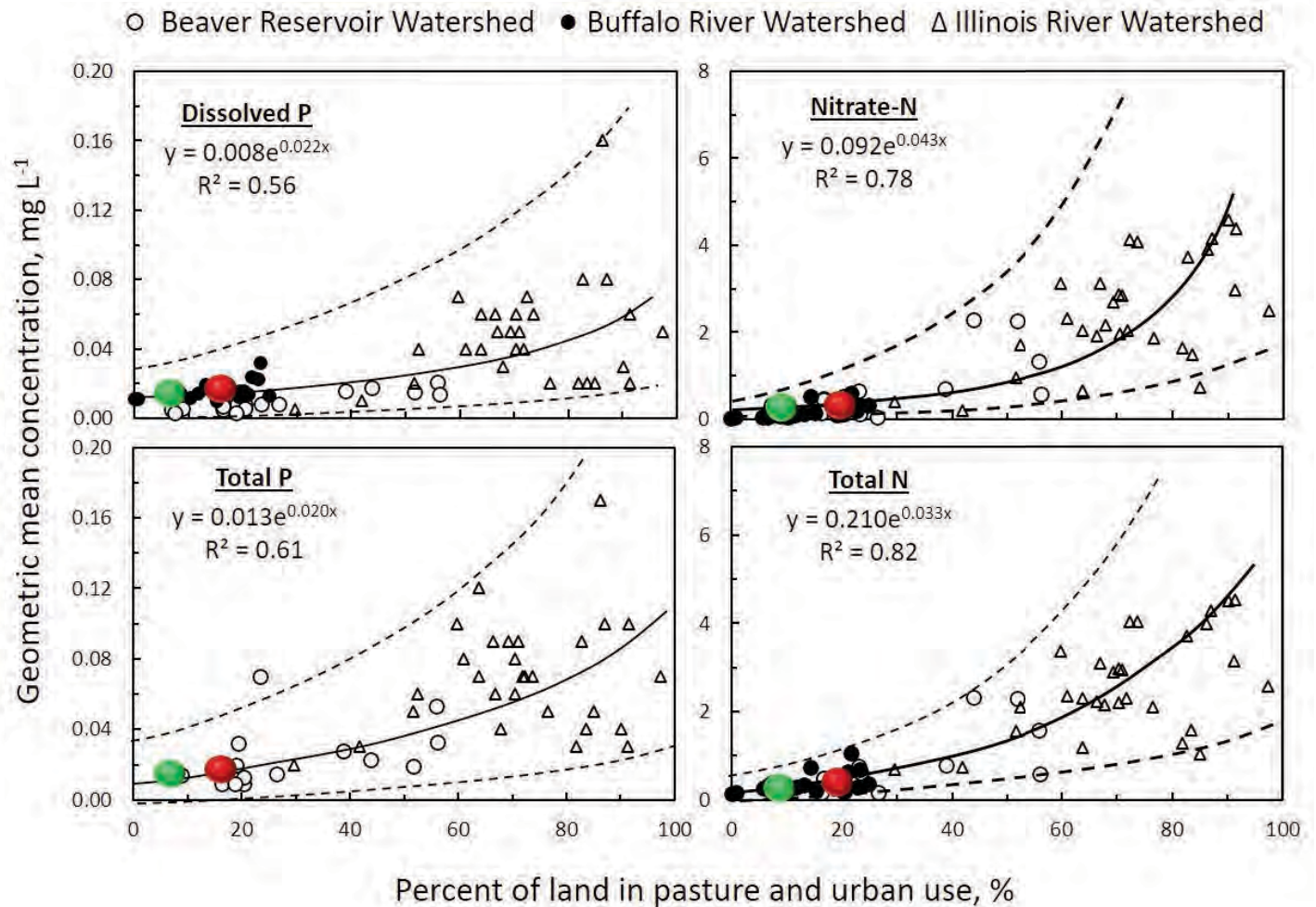


Figure 2. Relationship between land use and the geometric mean N and P concentrations (mg L⁻¹) in the Buffalo, Upper Illinois and Upper White River watersheds. Dashed lines represent the 95 percent confidence intervals for the estimated mean (solid line). Green points are geometric mean concentrations measured upstream of the CAFO on Big Creek and red points are geometric mean concentrations measured downstream of the CAFO on Big Creek.

[B]“Geometric means” – There are many ways to calculate the central or typical value of a data set, like the average or median. With water quality data, the geometric mean is often used because it minimizes the influence of really low or high values on the average.

[C]“R²” is the **coefficient of determination** – the proportion of variance in the dependent variable (i.e., vertical axis) that is predictable from the independent variable (i.e., horizontal axis). The closer to 1 the value is, means less variability and the better the relationship between the two variables is.

In the Big Creek watershed, the percent of land influenced by human activities (i.e., pasture plus urban) doubles from ~10 percent to ~20 percent in the drainage area upstream and downstream of the CAFO. In Big Creek itself, upstream of the swine production CAFO, the geometric mean concentrations of dissolved P, total P, nitrate-N and total N during base flow were 0.009, 0.030, 0.10 and 0.20 mg L⁻¹, respectively, between September 2013 and December 2017. Directly downstream of the CAFO, the geometric mean concentrations in Big Creek during base flow over the same period were 0.011, 0.030, 0.25 and 0.37 mg L⁻¹, respectively.

Geometric mean nutrient concentrations in Big Creek above and below the swine production CAFO and its current potential sphere of influence from slurry applications are similar to or lower than concentrations measured in rivers draining other sub-watersheds in the Upper Illinois and Upper White River watersheds with similar proportions of agricultural land use. (See Figure 2.)

Have Nutrient Concentrations Changed in the Short Term at Big Creek?

Long-term (e.g., decadal scale) water quality data are needed to reliably assess how stream nutrient concentrations have changed in response to watershed management and climate variations (Hirsch et al., 2015). The literature shows that stream nutrient concentrations can change relatively quickly in response to effluent management (e.g., Haggard, 2010; Scott et al., 2011), but seeing a response (i.e., decrease or increase in concentrations) from landscape management can take decades or more (Green et al., 2015; Sharpley et al., 2013). A myriad of factors may influence observed nutrient concentrations in streams, including discharge, biological processes and climactic conditions (i.e., drought and floods), and dominant transport pathways. Thus, we need to use caution when interpreting trends in water quality over databases that only cover a limited time-frame. Flow-adjusted concentrations showed no

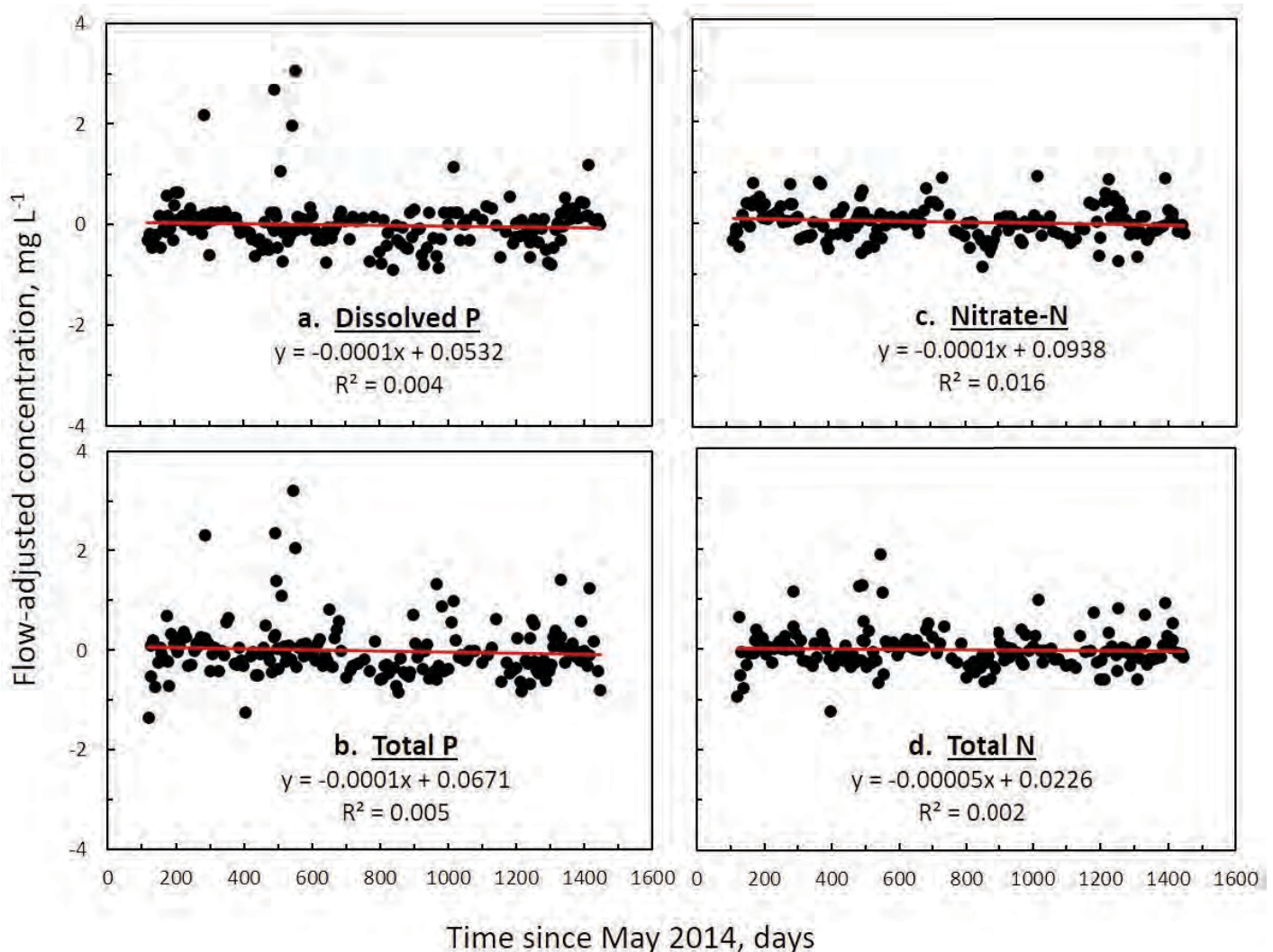


Figure 3. Change in flow-adjusted concentration of (a) dissolved P, (b) total P, (c) nitrate-N and (d) total N over time since May 2014, when monitoring in Big Creek started.

statistically significant increasing or decreasing trends in dissolved P, total P, nitrate-N and total N ($R^2 < 0.016$); where number of observations is 182) over the current monitoring period (Figure 3).

Summary

Nutrient concentrations at Big Creek upstream and downstream of the swine CAFO, and indeed most tributaries of the Buffalo River, are low relative to other watersheds in this ecoregion (Figure 2). This provides a starting point to build a framework to evaluate changes in nutrient concentrations of streams as a function of land use and management.

The evaluation of flow-adjusted concentrations over time showed that nutrients in Big Creek were not increasing over the short duration of monitoring for which concentration and discharge data were

available (May 2014 through April 2017). At this point in time, it is evident that nutrient concentrations in Big Creek have not increased at the monitored site. However, flow and nutrient concentration data over a longer period are needed to reliably quantify water quality trends and characterize sources, and monitoring needs to continue for at least a decade to evaluate how discharge, season and time influence nutrient fluxes.

Stream nutrient concentration-land use relationships are not a predictive tool. However, use of these relationships provides a method to determine if nutrient concentrations in a given watershed are similar to observed nutrient concentration-land use gradients in other watersheds of the Ozark Highlands and Boston Mountains. Over time, tracking these relationships provides a mechanism to note and evaluate changes in nutrient concentrations.

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Organic Phosphorus Can Make an
Important Contribution to Phosphorus
Loss from Riparian Buffers

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Core Ideas

- Forested and vegetative buffers can retain P runoff from adjacent fields.
- High concentrations of molybdate unreactive P were detected in soil water extracts.
- With time, these buffer soils can be a source of soluble inorganic and organic P.
- High microbial activity in buffer soils suggests biologically mediated P release.

Abstract: Vegetative buffer strips (VBS) and managed or unmanaged riparian zones between the edge of field and receiving watercourse are widely adopted conservation practices aimed at reducing nonpoint nutrient pollution. However, their effectiveness at decreasing phosphorus (P) loss has been mixed. This study investigated the effectiveness of a VBS and a forested riparian zone (FRZ) in decreasing P loss from pasture soils receiving swine manure and aimed to determine the potential factors controlling P release, using water extractable P (WEP) as a proxy for P loss. The inorganic WEP concentrations were significantly greater in the fertilized pasture zone soils than the VBS or FRZ soils. However, there was no significant difference between the field and riparian soils for total WEP due to increased contribution from organic WEP in these soils. Degree of P saturation, which is a function of soil test P, was a good predictor of inorganic WEP, but not organic WEP, where the variation in concentrations was better explained by variables involved in biotic P release.

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FORESTED riparian zones (FRZs) or vegetated buffer strips (VBS) are composed of a zone of managed or unmanaged vegetation between the edge of the field and the receiving watercourse. They are widely used to decrease nutrient and sediment runoff leaving agricultural fields and entering adjacent flowing waters. The main functions of these areas are to slow the flow of surface runoff, promoting sedimentation and infiltration, and to act as a filter to trap sediment and reduce dissolved nutrient concentrations through soil sorption and plant uptake (Hoffmann et al., 2009). Numerous studies have demonstrated the effectiveness of VBS at decreasing particulate P loss; however, their effect on dissolved P is less clear (Dodd and Sharpley, 2016). Detailed reviews of the literature have highlighted studies where such VBS have become sources rather than sinks of P where soil P concentrations are elevated (e.g., Hoffmann et al., 2009; Roberts et al., 2012; Sheppard et al., 2006).

Three possible mechanisms for the release of P from VBS have been suggested (Roberts et al., 2012): (i) decreased P sorption capacity due to saturation of P sorption sites, (ii) desorption of P from soil surfaces or dissolution of precipitated P, and (iii) biological cycling through the plant and microbial pools. Compared to much-studied geochemical processes, relatively little is known about processes involved in the microbial P cycle or the impact of differing land management strategies on these. Furthermore, the contribution of dissolved organic P forms to P loss from VBS is often overlooked (Dodd and Sharpley, 2015), and we suggest that organic forms could make up a substantial proportion of dissolved P in soils with active microbial P cycling. This study aims to address this research gap.

Abbreviations: DPS, degree of soil phosphorus saturation; FPZ, fertilized pasture zone; FRZ, forested riparian zone; M3-P, Mehlich extractable P; MBC, microbial biomass C; MBN, microbial biomass N; MBP, microbial biomass P; TC, total C; TN, total N; TP, total P; TWEP, total water extractable phosphorus; VBS, vegetated buffer strips; WEP, water extractable phosphorus; WEPI, inorganic water extractable phosphorus; WEPo, organic water extractable phosphorus.

The Buffalo River is an important recreation area in northwest Arkansas. In 2013, a concentrated animal feeding operation (CAFO) was permitted to operate in this watershed (Arkansas Department of Environmental Quality, 2017), raising concerns of potential impairment of area waters for recreational use. In this operation, swine manure is land applied to pasture land, either grazed by cattle or hayed. Fields adjacent to a stream have a 30-m buffer, to which no manure or fertilizer can be applied, providing an ideal opportunity to investigate the fate and cycling of soil P along a gradient of a fertilized pasture zone (FPZ), grass VBS, and a forested riparian zone (FRZ). Three fields with different management histories, soil properties, and slope were selected to investigate the potential for dissolved P release, as measured by water extractable P, across these three zones. We addressed two main objectives:

1. To determine the effect of landscape position on the potential for P release as both dissolved inorganic and organic P.
2. To investigate the soil chemical and biological properties that control the release of dissolved P from these soils.

While it is acknowledged that vegetation can be an additional source of P loss, especially from forested riparian areas, where there may be accumulation of litter material, quantification of its contribution was beyond the scope of this study, which focuses on the release of soil P to water.

Materials and Methods

The study site is located in Mount Judea, AR (Fig. 1). Three fields were sampled (Fields 1, 5a, and 12; Fig. 2). Dominant soil types, along with management, for these are listed in Table 1. All three fields received poultry litter once every 2 yr in March from 2004 to 2012 ($4.5 \text{ Mg ha}^{-1} \text{ yr}^{-1}$; approximately 50 kg P and $120 \text{ kg N ha}^{-1} \text{ yr}^{-1}$). Fields 1 and 12 currently receive only swine manure. In 2014, Field 1 received a total of 47 kg P ha^{-1} and 94 kg N ha^{-1} and Field 12 received 65 kg P

ha^{-1} and 128 kg N ha^{-1} . In 2015, Field 1 received a total of 7.3 kg P ha^{-1} and 32 kg N ha^{-1} and Field 12 received 35 kg P ha^{-1} and 146 kg N ha^{-1} . While no swine manure has been applied to Field 5a, diammonium phosphate fertilizer was applied annually since 2012 at 11 kg P and 25 kg N ha^{-1} .

On Fields 1 and 12, receiving swine manure, a required application buffer of 30 m from the field edge is in place. Field 1 has a steep topography and drains into an ephemeral stream located within the riparian zone and connected to Big Creek. Fields 5a and 12 have slopes of $<2\%$. These fields border Big Creek and are prone to flooding during large storm events. Field 1 is continuously grazed by cattle, whereas grass is cut for silage in Fields 5a and 12.

At each field, three transects were laid across the site running through the FPZ, VBS, and into the FRZ. For each transect, soil samples were taken at the 0- to 10-cm depth from three locations within the FPZ, one location within the manure application VBS, and one location in the FRZ, which borders the stream. Soil sampling at all fields was performed over the course of 1 d on four occasions, October 2014, January 2015, April 2015, and July 2015, corresponding to autumn, winter, spring, and summer to account for seasonal variability.



Fig. 1. Buffalo River watershed location in Arkansas, USA.

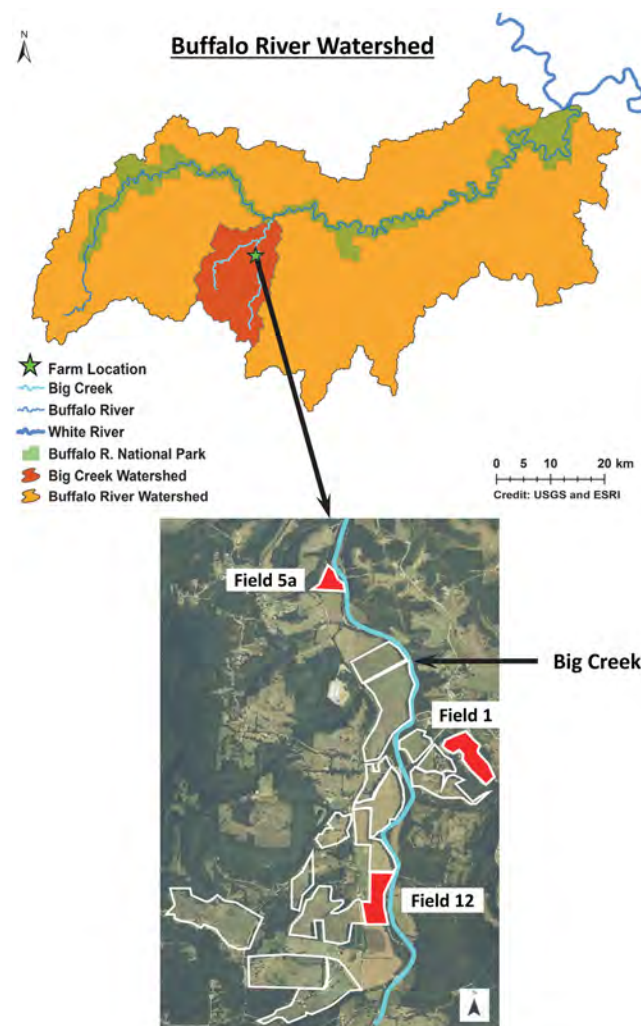


Fig. 2. Location of Big Creek watershed, farm, and fields studied.

Table 1. Field properties and management.

Site	Soil series	Area	Range in slope	Management
		ha	%	
Field 1	Noark very cherty silt loam	6.3	2.0–20.0	Grazed at 0.5 animal units ha ⁻¹
Field 5a	Razort loam	10.8	0.2–1.0	Hayed and grazed at 0.3 animal units ha ⁻¹
Field 12	Spadra loam	9.6	0.5–2.0	Hayed and grazed at 0.3 animal units ha ⁻¹

As much of the vegetation mat as possible was removed in the field. Soil samples were separated into two subsamples for biotic and abiotic analysis. Samples for biotic analysis (microbial biomass and enzyme activity) were sieved <2 mm and stored at 4°C until analysis. Samples for abiotic analysis were air-dried, ground, and sieved <2 mm. Additional plant material, shoots, and roots were removed by hand before sieving.

Soil samples were analyzed for the following properties using the methods outlined in Table 2: total WEP (TWEP), inorganic WEP (WEPI), organic WEP (WEPo), Mehlich extractable P (M3-P), degree of soil P saturation (DPS), total P (TP), total C (TC), total N (TN), pH, microbial biomass P, C, and N (MBP, MBC, MBN), and phosphatase enzyme activities. Phosphorus concentration for all analyses except M3-P was determined using the molybdate blue method of Watanabe and Olsen (1965); M3-P concentrations were determined via inductively coupled plasma.

The P content determined colorimetrically directly following extraction with water is more accurately described as molybdate reactive P and consists mainly of orthophosphate ions and a small proportion of easily hydrolyzable inorganic and organic P. The difference between this value and that determined following digestion is more accurately described as molybdate unreactive P and consists mainly of organic P forms but also a smaller fraction of condensed inorganic P,

such as polyphosphates (Haygarth and Sharpley 2000). Due to the dominance of inorganic P in molybdate reactive P and organic P in molybdate unreactive P, WEPI and WEPo have been used to distinguish between these two forms of P to avoid confusion and allow a clear message to be presented.

Before all statistical analysis, the data were examined for normality and the following parameters were log-transformed: TWEP, WEPI, and WEPo. To examine the differences in soil properties across the three landscape positions, the data from the three samples taken along each transect within the FPZ were averaged to provide one value for each zone (FPZ, VBS, and FRZ) for each transect. Data from each of the three fields and from each of the transects within the fields were treated as replicates, giving nine location replicates per zone. These data were subjected to a one-way ANOVA by zone blocked by season, providing four seasonal replicates for each location replicate and a total replication of 36 data points per zone. For all parameters, a Tukey test was used to determine significant differences between the zones at the $p < 0.05$ level of significance.

To determine which soil properties were contributing to the release of WEP and which soil parameters are important in regulating the release of P, a stepwise regression was undertaken using the following parameters: acid phosphomonoesterase, alkaline phosphomonoesterase, phosphodiesterase, total phosphatase, MBN, MBP, MBC, M3-P, DPS, pH,

Table 2. Summary of analytical methods used.

Parameter†	Analytical method	Reference
TWEP	1:20 soil-to-water extraction followed by centrifugation, filtration <0.45 µm, and acid persulfate digestion	Self-Davis et al. (2009) and Rowland and Haygarth (1997)
WEPI	1:20 soil-to-water extraction followed by centrifugation and filtration <0.45 µm	Self-Davis et al. (2009)
WEPo	Assumed to be the difference between TWEP and WEPI	—
M3-P	1:10 soil-to-Mehlich-3 extractant and centrifugation	Mehlich (1984)
DPS	Calculated from M3-P, Fe, and Al according to the equation $DPS = M3-P/0.5 \times (M3-Fe + M3-Al) \times 100$	Adapted from Schoumans (2009)
TP	Alkaline oxidation	Dick and Tabatabai (1977)
TC	Combustion on an Elementar VarioMax CN	Provin (2014)
TN	Combustion on an Elementar VarioMax CN	Provin (2014)
pH	1:2 soil-to-water extraction	—
MBP	Chloroform-fumigation extraction	Adapted from Brookes et al. (1985) and McLaughlin et al. (1986)
MBC	Chloroform-fumigation extraction	Vance et al. (1987)
MBN	Chloroform-fumigation extraction	Vance et al. (1987)
Acid P _{mono}	Enzyme assays using 5mM <i>para</i> -nitrophenyl phosphate as the substrate buffered at pH 6.5	Tabatabai (1994)
Alk P _{mono}	Enzyme assays using 5mM <i>para</i> -nitrophenyl phosphate as the substrate buffered at pH 11	Tabatabai (1994)
Pdi	Enzyme assays using 1 mM bis- <i>para</i> -nitrophenyl phosphate as the substrate buffered at pH 8	Tabatabai (1994)
Total phosphatase	Sum of acid P _{mono} , alk P _{mono} , and Pdi	—

† acid P_{mono}, acid phosphomonoesterase; alk P_{mono}, alkaline phosphomonoesterase; DPS, degree of soil phosphorus saturation; M3-P, Mehlich extractable P; MBC, microbial biomass C; MBN, microbial biomass N; MBP, microbial biomass P; Pdi, phosphodiesterase; TC, total C; TN, total N; TP, total P; TWEP, total water extractable phosphorus; WEPI, inorganic water extractable phosphorus; WEPo, organic water extractable phosphorus.

TP, TC, and TN. All analyses were performed using the SPSS statistical package version 22 (IBM, 2013).

Results and Discussion

Water extractable soil P concentration has been shown to be directly related to the potential for dissolved P release from soils to surface runoff (Pote et al., 1996; Sharpley, 1995). The total, inorganic, and organic WEP (TWEP, WEPi, WEPo) concentrations across the three fields was significantly lower in the VBS than the FPZ, reflecting the larger inputs of P to the FPZ (Fig. 3). However, there was no significant difference in TWEP between the pasture and FRZs despite a decrease in WEPi. This is a result of the significantly higher concentrations of WEPo present in the FRZ, where WEPo made up 57% of TWEP in these soils compared with just 24% in the pasture soils. This suggests that dissolved organic P can contribute to total P release in riparian soils.

The release of soil P to water can occur through abiotic and biotic processes. Desorption and dissolution reactions dominate the abiotic release mechanisms and are governed by soil chemical properties and number of available sorption sites (Arai and Sparks, 2007). Microbial soil biomass can contain a significant pool of P in temperate pastures (Oberson and Joner, 2005). This pool is in constant flux, immobilizing P from or replenishing P in the soil solution during microbial growth or cell death. Phosphorus release from this pool

occurs through three main mechanisms: (i) mineralization (Oehl et al., 2001), (ii) cell lysis in response to environmental stress (e.g., desiccation) (Turner and Haygarth, 2001), or (iii) predation by soil fauna (Bonkowski, 2004). Biotic processes can also control the form of dissolved P in solution through the exudation of phosphatase enzymes, which can catalyze the conversion of soluble organic P compounds to ortho-phosphate ions for plant uptake (Richardson et al., 2011).

Table 3 shows the abiotic and biotic soil properties of the soils across the three landscape zones. The FPZ soils had significantly higher concentrations of TP and M3-P, but lower concentrations of TC and TN, compared with the FRZ soils. Additionally, the DPS was significantly higher in the FPZ soils compared with the VBS and FRZ soils, indicating more of the P sorption sites had become saturated. Surprisingly, we saw no significant difference in microbial biomass P, C, or N concentrations among zones, despite expected increased leaf litter inputs in the FRZ. However, the total phosphatase activity was 17% higher in the FRZ soils than the field soils, suggesting increased microbial activity.

To determine which pools of P and which soil properties were key in controlling the release of P to water, we performed a stepwise regressions for TWEP, WEPi, and WEPo using data from all three sites and four sampling dates. The results from this analysis are shown in Table 4. Both TWEP and WEPi were well predicted by the model (adjusted $r^2 =$

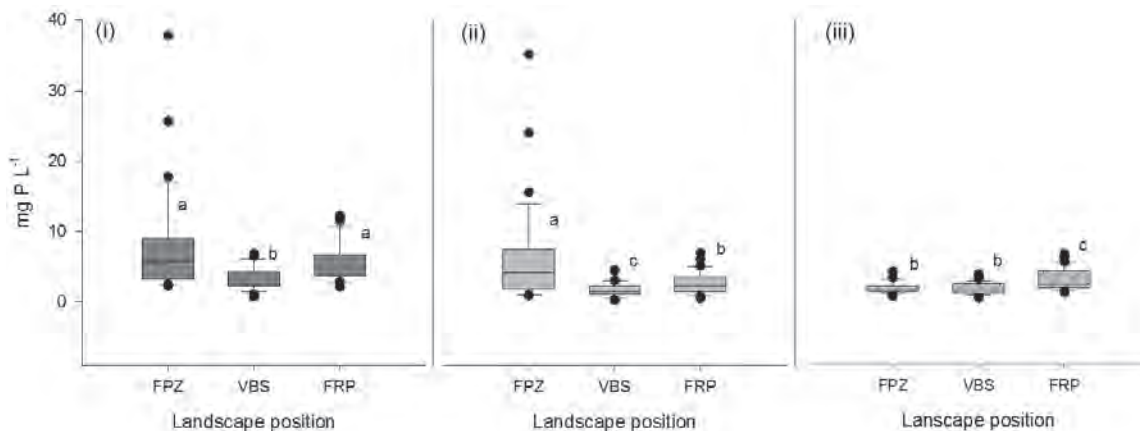


Fig. 3. Box plots showing the concentration of (i) total water extractable P, (ii) inorganic water extractable P, and (iii) organic water extractable P across the three zones (fertilized pasture zone [FPZ], vegetative buffer strip [VBS], and forested riparian zone [FRZ]). Letters denote significant differences between zones at $p < 0.05$ as determined by Tukey's test of multiple comparisons.

Table 3. Difference in mean soil properties across the different landscape zones, fertilized pasture zone (FPZ), vegetative buffer strip (VBS), forested riparian zone (FRZ): pH, total C (TC), total N (TN), total P (TP), Mehlich-3 P (M3-P), degree of P saturation (DPS), microbial biomass P (MBP), microbial biomass C (MBC), microbial biomass N (MBN), acid phosphomonoesterase (acid P_{mono}), alkaline phosphomonoesterase (alk P_{mono}), phosphodiesterase (Pdi), and total phosphatase activities.

Zone	Soil chemical properties						Soil biological properties						
	pH	TC	TN	TP	M3-P	DPS	MBP	MBC	MBN	Acid P_{mono}	Alk P_{mono}	Pdi	Total phosphatase
		%		$mg\ kg^{-1}$		%	$mg\ kg^{-1}$			$\mu mol\ pNP\ g^{-1}\ h^{-1}$			
FPZ	5.87b‡	2.06b	0.23b	640a	67a	7.48a	26	371	85	3.00a	1.33b	1.20b	5.53b
VBS	5.71b	1.88b	0.21b	584ab	48b	4.89b	23	386	79	2.59ab	0.93b	0.96b	4.56b
FRZ	6.40a	3.21a	0.26a	521b	28c	4.57b	28	356	101	2.33b	2.05a	2.05a	6.66a
<i>p</i> value	<0.001	<0.001	<0.05	<0.01	<0.005	<0.001	NS	NS	NS	<0.005	<0.001	<0.001	<0.001

† pNP, *para*-nitrophenyl phosphate.

‡ Means followed by the same letter are not significantly different at the $p < 0.05$ level of significance according to Tukey's test for multiple comparisons.

Table 4. Model predictions of total water extractable P (TWEP), inorganic water extractable P (WEPI), and organic water extractable P (WEPO) from stepwise regression across all data. The *F* statistic for all three model predictions is <0.001.

TWEP			WEPI			WEPO		
<i>r</i> ² _{adjusted}	Predictor	Relative importance	<i>r</i> ² _{adjusted}	Predictor	Relative importance	<i>r</i> ² _{adjusted}	Predictor	Relative importance
0.66	Degree of soil P saturation	0.80	0.65	Degree of soil P saturation	0.93	0.37	Total phosphatase activity	0.78
	Total phosphatase activity	0.18		Total phosphatase activity	0.07		Microbial biomass N	0.17
	M3-P	0.02					pH	0.05

0.66 and 0.65, respectively), and variation in these concentrations was mostly explained by DPS, with a small contribution from total phosphatase activity, an indicator of biologically mediated P release.

In contrast to WEPI, variation in WEPO was less closely related to any of the measured parameters (adjusted *r*² = 0.37). Furthermore, DPS was not included in the selected model, and total phosphatase activity explained most of the variation in WEPO. While total phosphatase activity was greatest in FRZ soils, the activity of the different types of enzyme varied across the landscape positions (Table 3). Acid phosphomonoesterase activity was highest in the FPZ soils and of a similar magnitude to that found agricultural soils with a history of poultry manure application and high soil test P **Spelled out STP; correct???** concentrations (Tomlinson et al., 2008). Acid phosphomonoesterase is thought to be mainly released by plant roots and some microbes, and there is evidence that high concentrations phosphatase enzymes can be present in manures (Nannipieri et al., 2011). Furthermore, these enzymes have been shown to sorb strongly onto soil particles (Burns, 1986; Nannipieri et al., 2011); hence, the large acid phosphomonoesterase activities found in the FPZ may be directly due to manure application. In contrast, alkaline phosphomonoesterase and phosphodiesterase activities were greatest in the FRZ, in keeping with the small increase in pH (Table 3). These enzymes are thought to be released by soil microorganisms rather than plant roots. The differences in phosphatase activities across the zones suggest that release of P from riparian soils is likely to be controlled in part by the biologically mediated release of organic P.

This study demonstrates that the significant decrease in soil test P concentrations in FRZ soils compared with regularly fertilized FPZ does not necessarily translate to a reduction in the total amount of P, which can be released to runoff due to the increase in WEPO. Furthermore, while DPS, of which soil test P is a component, was a good predictor of WEPI release, additional factors relating to biological cycling need to be considered when trying to account for the potential release of organic P.

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Phosphorus Retention and Remobilization along Hydrological Pathways in Karst Terrain

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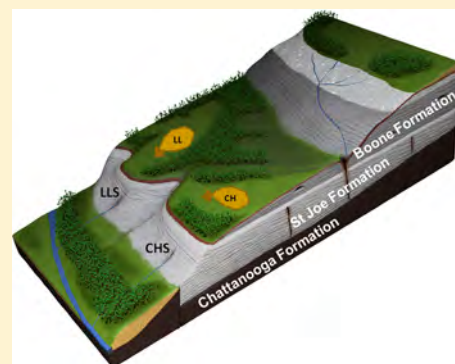
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Supporting Information

ABSTRACT: Karst landscapes are often perceived as highly vulnerable to agricultural phosphorus (P) loss, via solution-enlarged conduits that bypass P retention processes. Although attenuation of P concentrations has been widely reported within karst drainage, the extent to which this results from hydrological dilution, rather than P retention, is poorly understood. This is of strategic importance for understanding the resilience of karst landscapes to P inputs, given increasing pressures for intensified agricultural production. Here hydrochemical tracers were used to account for dilution of P, and to quantify net P retention, along transport pathways between agricultural fields and emergent springs, for the karst of the Ozark Plateau, midcontinent USA. Up to ~70% of the annual total P flux and ~90% of the annual soluble reactive P flux was retained, with preferential retention of the most bioavailable (soluble reactive) P fractions. Our results suggest that, in some cases, karst drainage may provide a greater P sink than previously considered. However, the subsequent remobilization and release of the retained P may become a long-term source of slowly released “legacy” P to surface waters.



INTRODUCTION

More than 25% of the world's population either lives on or obtains its drinking water from karst aquifers. Karst underlies 30% of the land area of China, 30% of Europe, and 20% of the United States.^{1,2} Karst aquifers exert an important control on the quality and ecology of surface waters in these areas.³ The complexity of subsurface drainage^{4,5} and the difficulties in deconvoluting flow pathways and groundwater contributing areas⁶ have been a significant barrier to detailed studies of nutrient transport and fate in karst systems.^{7,8} Nevertheless, it is widely assumed that karst drainage systems (formed by dissolution of carbonate rocks, mainly limestone) are highly vulnerable to phosphorus (P) impairment from agricultural sources.

This vulnerability is assumed to arise from the low nutrient buffering capacity of the thin cherty soils which overlie karst and the rapid transmission of surface runoff through conduits enlarged by dissolution,^{9,10} which is thought to bypass the zones where key processes of P retention occur.^{11–13} Nonetheless, highly intensive monitoring of Irish karst springs, in areas of livestock, demonstrated major P attenuation (reduction in P concentrations) relative to agricultural runoff,^{14,15} with low P concentrations in spring discharge, even during storm events

when agricultural P losses are expected to be highest. This attenuation was attributed to a combination of both hydrological dilution and P retention during infiltration and transmission of runoff along groundwater conduit pathways.

Crucially, we lack information on the extent to which P attenuation is controlled by P retention processes during transit along karst flow paths,¹⁴ or by hydrological dilution of agricultural runoff by cleaner groundwater sources.¹⁶ This is of strategic importance for understanding the P buffering capacity and wider resilience of karst landscapes to nutrient inputs.^{10,17,18} Many karst lands have traditionally been used for low-intensity livestock farming, owing to poor soils and their unsuitability for arable production.⁹ However, there is increasing pressure for intensive livestock production, as global demands for greater efficiency in food production intensify.^{19,20} Given the move toward more intensive livestock production systems, which accumulate P,^{21,22} and the perceived vulnerability of karst drainage systems to P loss, there is now a pressing and strategic

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Table 1. Summary of Concentrations of Soluble Reactive Phosphorus (SRP), Total Phosphorus (TP), Potassium (K), Rubidium (Rb), and Calcium (Ca) in Field Runoff and Spring-Water Samples

		field runoff ($\text{m}^3 \text{ha}^{-1}$) spring flow (L s^{-1})	SRP (mg L^{-1})	TP (mg L^{-1})	Rb ($\mu\text{g L}^{-1}$)	K (mg L^{-1})	Ca (mg L^{-1})
Langle Field (LL)	mean	38.0	2.21	2.57	6.97	10.4	5.12
	median	35.5	1.87	2.12	5.96	10.2	4.94
	range	3.4–91.5	0.59–5.02	0.8–5.53	0.93–20.6	2.04–26.3	2.11–9.87
Copperhead Field (CH)	mean	23.1	0.68	1.09	2.94	6.11	3.45
	median	14.6	0.57	1.03	2.52	5.11	3.43
	range	1.8–79.9	0.47–1.22	0.63–1.91	0.58–8.76	1.4–14.7	1.95–7.34
Langle Spring (LLS)	mean	13.1	0.029	0.057	1.06	1.54	37.5
	median	9.38	0.012	0.034	0.878	1.14	36.7
	range	1.24–59	0–0.403	0.002–0.608	0.195–3.57	0.534–4.92	12.2–65.9
Copperhead Spring (CHS)	mean	22.5	0.019	0.041	1.08	1.37	40.5
	median	2.62	0.017	0.032	1.1	1.4	42.9
	range	0.19–253	0.001–0.12	0–0.58	0.328–1.9	0.84–2.17	14.5–61.5

need for better understanding of the fate and transport of P in karst landscapes. Here this shortfall is addressed for karst terrain in south-central USA. Hydrochemical tracers and endmember mixing analysis^{23–26} were used to assess the vulnerability to P loss, by accounting for the hydrological dilution of agricultural runoff and directly quantifying net P retention, during infiltration through the soil, and along karst transport pathways, through to the emergent springs.

EXPERIMENTAL METHODS

Study Area. The study was undertaken at the University of Arkansas long-term Savoy Experimental Watershed (SEW), NW Arkansas, USA.²⁷ The SEW is located in the Illinois River Watershed, a mixed land-use watershed ($\sim 4330 \text{ km}^2$), which spans the states of Arkansas and Oklahoma.^{28,29} The SEW covers 1250 ha and is typical of the karst terrain of the Ozark Plateau of midcontinental USA (Figure SI-1a, Supporting Information). The soils of the SEW are predominantly silt loams (see Supporting Information). Around 70% of the land is native forest, with the remaining 30% rolling pasture grazed by beef cattle (~ 2 cows ha^{-1}). The SEW also supports poultry production, with the resulting poultry litter used to fertilize pastures. There are no septic tanks or settlements in the SEW, and agricultural runoff from pastures grazed by cattle provides the overwhelmingly dominant P source in the watershed.³⁰

The stratigraphy of the SEW^{30–32} (see Figure SI-1c, Supporting Information) includes (a) the limestone aquifer of the St. Joe Formation, (b) the Boone Formation, an impure limestone which mantles the St. Joe Formation and forms “epikarst”, and (c) a layer of regolith (vadose zone) which overlies the Boone Formation. Karst drainage has a major control on water quality in the Illinois River;^{29,33} 67% of annual river flow comes from karst springs, rising to 80% of flow in the summer and fall.³⁴

Sample Collection and Analysis. Surface runoff and spring-water chemistry and flow monitoring (Figure SI-1a and c, Supporting Information) were undertaken at the following: (1) two adjacent karst springs (Langle Spring, LLS, and Copperhead Spring, CHS), which flow continually from the St. Joe Formation (focused conduit flow) springs; (2) two surface runoff field plots (Langle, LL, 1.07 ha, and Copperhead, CH, 1.05 ha), which are located above and within the watershed (recharge zone) of the LLS and CHS springs. These runoff plots are located on Razort silt loams which make up most of the grazed pastures of the SEW. All pastures are treated similarly in terms of grazing

intensity and maintenance fertilizer applications (30 kg P ha^{-1} every two years as either poultry litter or diammonium phosphate).

Flows at the karst springs (LLS and CHS) were monitored on 15-min intervals (see Supporting Information). Karst spring water was sampled weekly, with stage-triggered, subdaily automated sampling using an ISCO sampler during storm events. Figure SI-2 (Supporting Information) shows the distribution of samples collected on the rising and falling stage of the storm hydrographs. The volume of surface runoff from both fields was automatically measured, and samples were collected on a flow-weighted basis by an ISCO autosampler. All water samples were filtered within 24 h of the water being sampled and were analyzed following EPA standard protocols, as described below (and in the Supporting Information). Filtered ($<0.45 \mu\text{m}$) samples were analyzed for soluble reactive phosphorus (SRP), by colorimetric analysis,³⁵ and for a full suite of major cations (including potassium, K, and calcium, Ca) and trace elements (including lanthanum, La, and rubidium, Rb) (see Supporting Information). Unfiltered samples were analyzed for total phosphorus (TP), after acid-persulfate digestion, by colorimetric analysis.^{35,36} These measurements are consistent with standard protocols for TP and SRP analysis.³⁷

Use of Conservative Tracers and Endmember Mixing Analysis. Conservative chemical tracers and endmember mixing models were used to apportion water sources, and to differentiate the effects of hydrological dilution from the biogeochemical processes, which retain and cycle P during transit through the karst drainage system. Chemical tracers have been widely used in watershed hydrology for tracing water sources and flow pathways,³⁸ owing to their conservative behavior (chemical inertness). Here we made use of chemical tracers already in the watershed to apportion water sources. Using the hydrochemical monitoring data, tracers were chosen which had elevated concentrations in either base flow groundwater or in agricultural runoff. First, two-component endmember mixing models^{23,39} were used to link the spring-water chemistry to sources within the watershed, by (a) quantifying the relative proportions of surface runoff and groundwater and (b) estimating the contribution of surface runoff from the agricultural grazed land. Second, comparing the mixing patterns of P in spring water with a conservative tracer of agricultural runoff allowed us to directly evaluate whether P was behaving nonconservatively (i.e., being taken up or released) along the hydrological pathways in the karst drainage system.

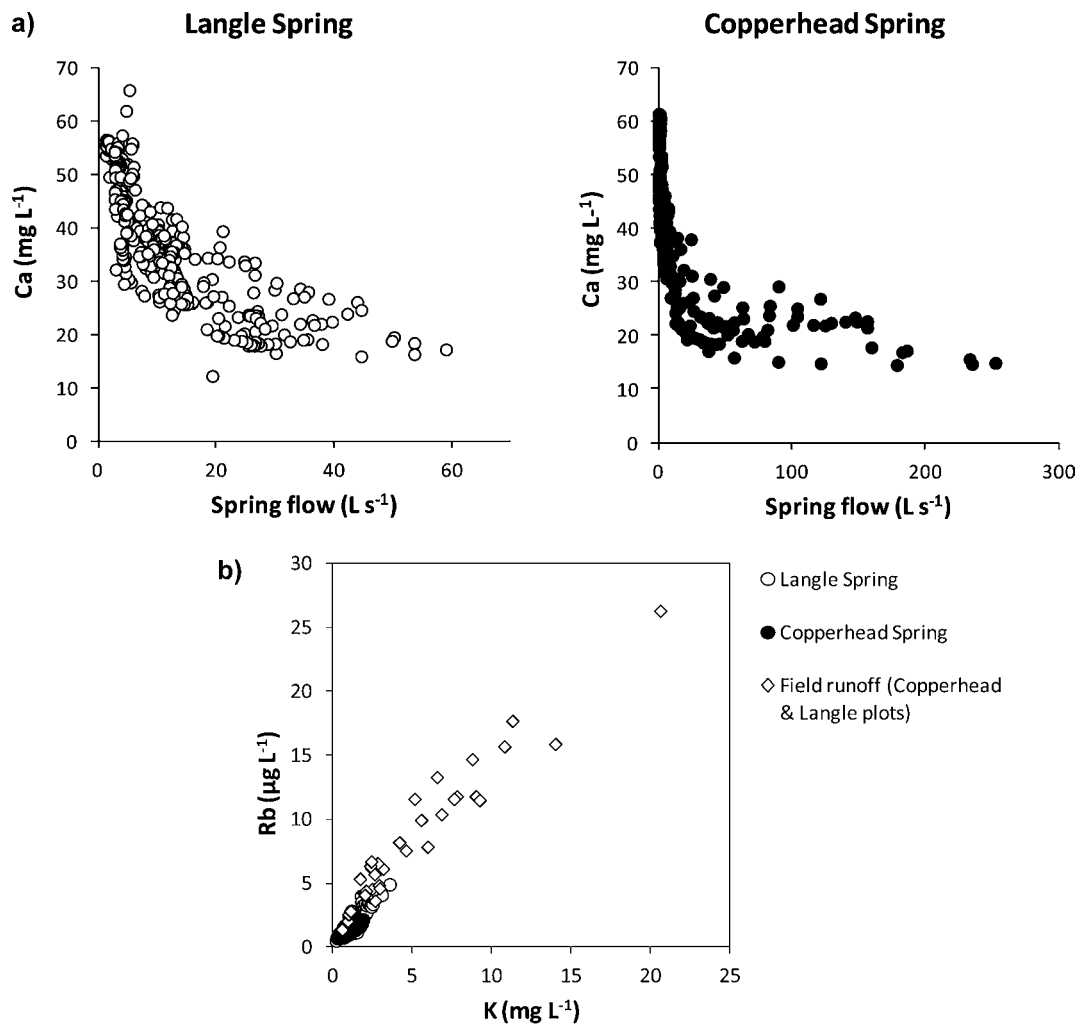


Figure 1. (a) Relationships between calcium (Ca) concentrations and flow at Langle and Copperhead springs. (b) Relationship between rubidium (Rb) and potassium (K) concentrations in field runoff and spring-water samples.

RESULTS AND DISCUSSION

Comparison of Agricultural Runoff and Spring-Water Chemistry. Concentrations of TP, SRP, K, and Rb were consistently highest in field runoff, relative to the springs (Table 1), and runoff from the grazed fields provides the greatest concentrations of P, K, and Rb within the SEW. In contrast, Ca concentrations were consistently highest in the springs, compared with runoff. This indicates a dominant base flow groundwater source of Ca, from dissolution of limestone, which is diluted by surface runoff (Figure 1a).

Concentrations of SRP, TP, K, and Rb were all higher in field runoff at LL compared with CH. This likely reflects higher cattle grazing density at LL (2.5 cows ha⁻¹) than at CH (1.0 cows ha⁻¹), as well as higher runoff per unit area that likely led to greater solute and particulate entrainment and transport capacity compared with CH. This may also reflect a larger hydrologically active area contributing runoff at LL, linked to greater soil compaction from more intensive cattle grazing.

For the springs, there was a greater variability in SRP, TP, K, and Rb concentrations at LLS than at CHS, despite a much lower variability in spring flow at LLS (Table 1). However, concentrations of TP, SRP, K, and Rb did not correlate with flow at either of the springs. For most storm events at LLS, concentrations of TP, SRP, K, and Rb increased dramatically

above base flow concentrations, especially on the rising stage of the storm hydrograph (Figure SI-2, Supporting Information). These high concentrations on the rising stage are likely due to upstream point recharge of surface runoff from pasture land into the underlying St. Joe aquifer in locations where the confining chert layer is breached. At CHS, the response of TP, SRP, K, and Rb to storm events was more mixed. Small initial increases in concentration occurred with the onset of higher flows, followed by marked reductions in concentration, reflecting substantial dilution by a water source with relatively low SRP, TP, K, and Rb concentrations, most likely from the nonagricultural (ungrazed and forested) parts of the watershed. Indeed, karst inventories have verified that this part of the flow regime reflects runoff from areas which are not grazed by livestock.^{30,31}

To evaluate the attenuation (i.e., the reductions in concentrations) of TP, SRP, K, and Rb during transit through the karst, the median concentrations in agricultural runoff were compared with the corresponding median concentrations in CHS and LLS springs (Table 1). The average attenuation of TP and SRP concentrations ranged from 96% to 99%. In contrast, the average attenuation of K and Rb concentrations was lower, at 56% to 89%. Correspondingly, under storm flow conditions, comparisons of average field runoff concentrations and the 90th percentile concentrations in spring water (which typically correspond with the rising stage of the storm hydrographs of

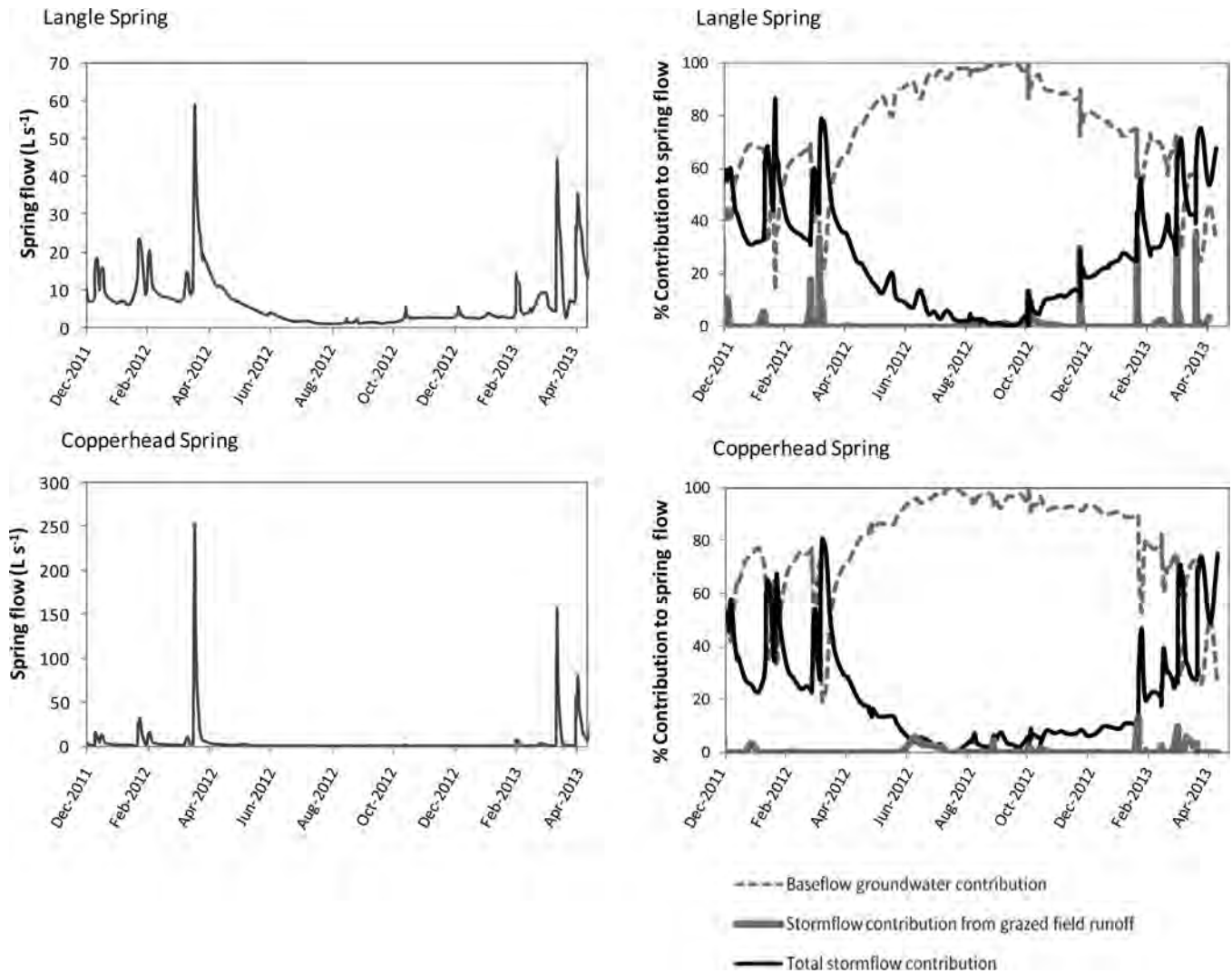


Figure 2. Hydrographs and water source apportionment for Langle and Copperhead springs.

the springs) revealed that storm flow attenuation of TP and SRP ranged from 93% to 96%, compared with 46% to 74% for K and Rb. Across all flow conditions, the higher rates of attenuation of P concentrations, relative to K and Rb, reflect the nonconservative behavior of P during transit through the karst.

K and Rb show high correlation (Figure 1b) due to their similar hydrogeochemistry (group 1a monovalent base cations of relatively small hydration size). Figure 1b shows a dominant two-component mixing series between a high concentration “endmember” (i.e., surface runoff from fertilizer and grazed pastures in runoff) and a low concentration spring-water “endmember” (i.e., runoff from nonagricultural and forested areas, which have no grazing or fertilizer inputs). Both K and Rb are highly soluble monovalent ions, and once transmitted into the karst drainage system, chemical interactions will be relatively small. Therefore, the attenuation of K and Rb during transport through the karst will be largely controlled by hydrological dilution, without retention mechanisms (with only possibly a small attenuation or release within the epikarst where there is a high proportion of clays^{31,40}). In contrast, P behaves non-conservatively, reflected by the higher rates of attenuation of P relative to K and Rb.

Spring Hydrology and Water-Source Apportionment. Comparing the hydrology of the two springs (Figure 2), base

flows at CHS were consistently lower than at LLS; the median flow at CHS was 2.62 L s^{-1} , compared with 13.1 L s^{-1} at LLS (Table 1). Further, CHS exhibited a more flashy flow regime than LLS, and storm flows were dramatically higher at CHS. For instance, the average of the highest 10% of flows was 139 L s^{-1} at CHS, compared with 40 L s^{-1} at LLS. This discrepancy reflects the following: (i) LLS being the “underflow” spring (3 cm lower than CHS), with a much larger groundwater drainage area under low-flow conditions than CHS, which accounts for the higher base flows at LLS; (ii) water capture (spring “piracy”) by CHS during storm events, which has been shown to result in a dramatic expansion in the watershed drainage area for CHS relative to LLS.^{32,33}

Contributions to spring water at LLS and CHS were apportioned by two-component endmember mixing analysis.^{2,3,41} Here Ca was used as a tracer of groundwater and K as a tracer of agricultural runoff, based on the observed dominant groundwater source of Ca and the dominant agricultural runoff source of K. For the mixing model, endmembers were defined as the following:

- (i) A base flow groundwater endmember with elevated Ca, and a storm flow endmember with low Ca concentrations.
- (ii) Runoff endmember from agricultural land with high K concentration, and a spring base flow low K endmember.

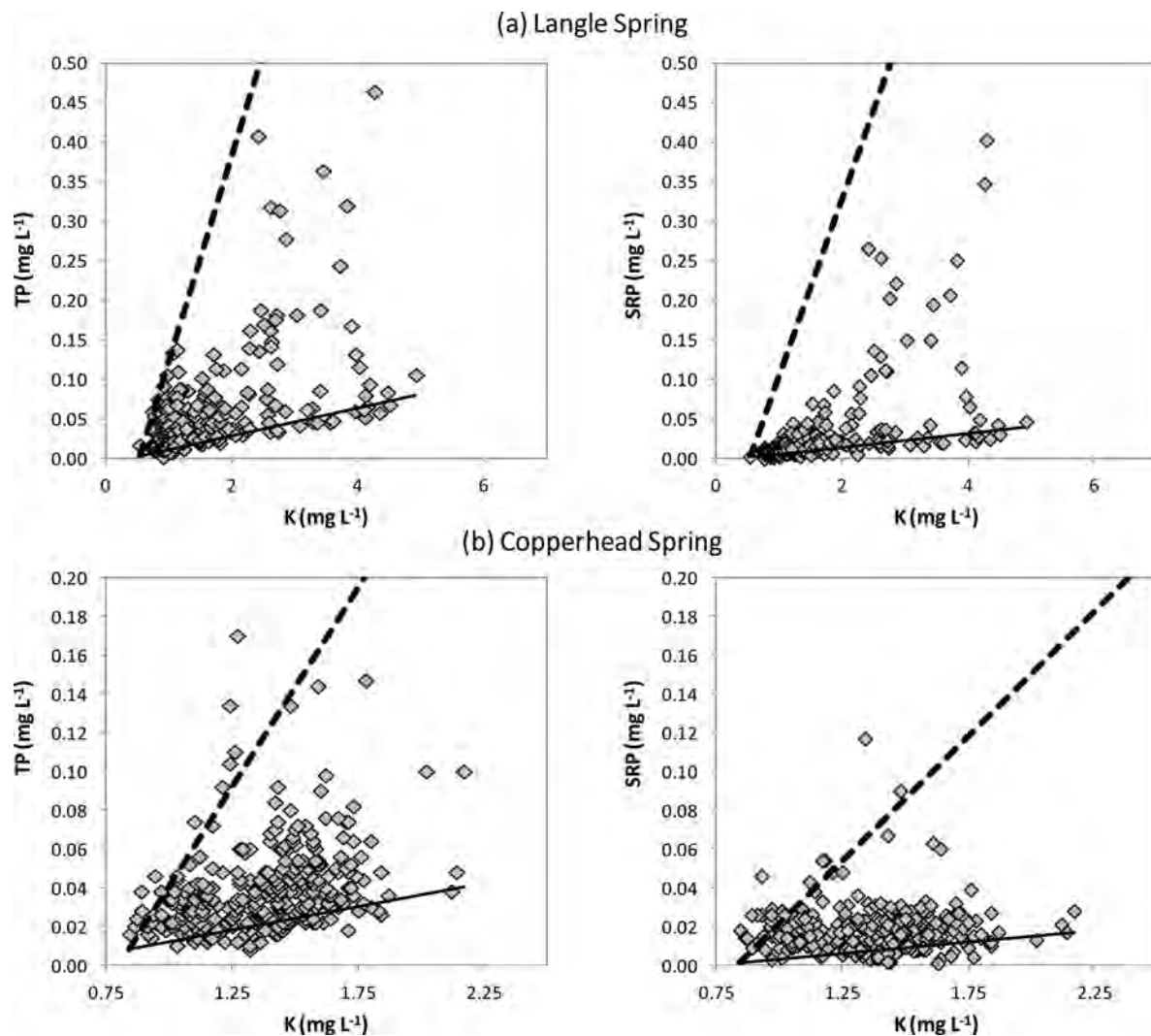


Figure 3. Relationships between total phosphorus (TP), soluble reactive phosphorus (SRP), and potassium (K) for (a) Langle Spring and (b) Copperhead Spring. The dashed line denotes the conservative mixing line, and the solid line denotes a line of maximum P retention (see text for explanation).

Applying a simple two-component mixing model^{23,41} (eq 1) and the endmembers identified above, Ca concentrations were used to partition the contributions to spring flow at LLS and CHS from base flow groundwater (the high concentration endmember) and from stormwater runoff (the low concentration endmember). Then a second two-component mixing model was used for K, to quantify the contributions from grazed pasture runoff (eq 2).

$$\begin{aligned} & \% \text{ total storm runoff} \\ & = 100 \times (Ca_{\text{gw}} - Ca_{\text{m}}) / (Ca_{\text{gw}} - Ca_{\text{ro}}) \end{aligned} \quad (1)$$

$$\begin{aligned} & \% \text{ agricultural runoff} \\ & = 100 \times (K_{\text{bf}} - K_{\text{m}}) / (K_{\text{bf}} - K_{\text{ag}}) \end{aligned} \quad (2)$$

where Ca_{gw} was the groundwater Ca concentration (high concentration base flow endmember), defined here as the average Ca concentration for the lowest 10% of flows sampled, Ca_{m} was the measured spring-water Ca concentration, Ca_{ro} was the stormwater (agricultural runoff) endmember, defined here as the average field runoff Ca concentration, K_{bf} was the base flow endmember (average K concentration for the lowest 10% of

spring flows sampled), K_{m} was the measured spring-water K concentration, and K_{ag} was the agricultural runoff endmember, defined here as the average field runoff K concentration. The values used to define the endmember concentrations at LLS and CHS are shown in Table SI-1, Supporting Information.

The water source apportionment for LLS and CHS (Figure 2) showed similar percentage contributions from base flow groundwater and total storm flow at LLS and CHS for most of the year and particularly during storm events. During winter and spring storm events, a much greater proportion of flow at LLS was derived from agricultural (grazed field) runoff (up to approximately a third of flow). This greater contribution of water from pastures than from nonagricultural land at LLS accounted for the higher storm-event concentrations of K and Rb at LLS. Agricultural runoff contributed a much lower proportion of winter and spring storm event flow at CHS (typically less than 10%). These results and the much higher storm flow discharges at CHS suggest that the water “piracy” at CHS, during storm events, captured water sources, which had a lower K and Rb concentration, from the nonagricultural (ungrazed and forested) areas.

Table 2. Measured and “Conservative” Annual Loads, and Mean Daily Base Flow and Storm Flow Loads, of Total Phosphorus (TP) and Soluble Reactive Phosphorus (SRP) in Langle and Copperhead Springs, with Net and Percentage TP and SRP Retention

		measured P load (kg y ⁻¹ or g d ⁻¹)	“conservative” P load (kg y ⁻¹ or g d ⁻¹)	net P retention (kg y ⁻¹ or g d ⁻¹)	% net P retention
Langle Spring (LLS)	annual TP load (kg y ⁻¹)	7.01	22.3	15.3	69
	annual SRP load (kg y ⁻¹)	1.85	19.0	17.2	90
Copperhead Spring (CHS)	annual TP load (kg y ⁻¹)	2.65	5.7	3.1	54
	annual SRP load (kg y ⁻¹)	0.98	3.3	2.3	70
Langle Spring (LLS)	avg base flow TP load (g d ⁻¹)	10.3	23.3	13.0	56
	avg base flow SRP load (g d ⁻¹)	2.21	19.8	17.6	89
Copperhead Spring (CHS)	avg base flow TP load (g d ⁻¹)	1.27	3.55	2.28	64
	avg base flow SRP load (g d ⁻¹)	0.45	2.14	1.69	79
Langle Spring (LLS)	avg storm flow TP load (g d ⁻¹)	112	1448	1336	92
	avg storm flow SRP load (g d ⁻¹)	51.4	1240	1189	96
Copperhead Spring (CHS)	avg storm flow TP load (g d ⁻¹)	445	971	527	54
	avg storm flow SRP load (g d ⁻¹)	175	567	392	69

Quantifying Net P Retention in Karst Drainage.

Endmember mixing analysis^{23–26} was applied using the “conservative” tracer, K, to explore the net P retention and release along karst hydrological pathways from infiltration through the soil, to spring discharge. First, concentrations of TP and SRP were plotted against K as the “conservative” tracer (Figure 3). Two dominant and distinct sources of spring water (both with different TP, SRP, and K concentrations) are hypothesized (Table SI-1, Supporting Information): (i) a high concentration agricultural endmember source (K_{ag} , TP_{ag} , SRP_{ag}), defined here as the average concentrations (of K, TP, and SRP) in agricultural field runoff at the LL and CH field plots, and (ii) a low concentration (nonagricultural) endmember (K_{na} , TP_{na} , SRP_{na}). As the source of this low concentration runoff could come from a wide range of nonagricultural sources (ungrazed and forest land) across the watershed, the most reliable means of capturing the integrated low-concentration endmember signal was to use the minimum measured spring-water K, TP, and SRP concentrations at LLS and CHS.

A theoretical linear two-component mixing series, i.e., a “conservative mixing line” between the high concentration and low concentration endmembers (Figure 3), would be observed if P behaved conservatively during mixing of the two endmember water sources during transport through the karst. In contrast, the observed relationships between TP and K, and SRP and K, in spring water were highly scattered at LLS and CHS (Figure 3). Most of the samples plot well below the conservative mixing line, showing predominantly net retention of TP and SRP relative to K. A few isolated samples plotted above the conservative mixing line, which are indicative of some sporadic net P release relative to the K tracer. The mixing patterns between TP, SRP, and K concentrations in Figure 3 had a well-defined lower boundary of samples with the lowest P concentrations relative to K (shown in Figure 3 as a “line of maximum P retention”). This line of maximum P retention probably represents a secondary endmember mixing line, between the same low concentration nonagricultural runoff endmember and a secondary agricultural

field runoff endmember, with high K but lower P concentrations as a result of P retention processes filtering out P. We posit that the majority of this P was “filtered” out during diffuse recharge of water as through the soil and the epikarst, into the karst aquifer. The spring-water samples which lie between the line of maximum retention and the conservative mixing series therefore likely reflect the *net* effects of P retention and remobilization processes for runoff water entering the karst drainage system via a mixture of diffuse and point recharge.

By comparing the observed spring-water TP and SRP versus K relationships with the theoretical linear conservative mixing series, the *net* effects of P retention and release can be directly quantified (Figure 3). By applying the theoretical conservative mixing series (TP versus K and SRP versus K) to the measured spring-water K concentrations at LLS and CHS, “conservative” TP and SRP concentration time series were derived (Figure SI-3a,b, Supporting Information) and converted to loads, using the corresponding spring flow data. By taking the difference between measured and “conservative” TP and SRP loads, we calculated net TP and net SRP retention on an annual basis, as well as for base flows (lowest 10% of flows) and storm flows (highest 10% of flows) (Table 2).

Annual net TP retention ranged from 69% at LLS to 54% at CHS. Net percentage P retention was consistently higher for SRP compared with TP, not only on an annual basis but also under storm and base flow conditions. This indicated preferential retention of more labile SRP fractions by sorption/uptake and greater mobility of TP organic and particulate P fractions. Similar patterns of soluble and particulate P retention have also been observed in other karst soils and drainage systems.^{7,11,13} Highest percentage net P retention occurred during storm events at LLS (92% TP retention and 96% SRP retention). However, the two springs showed very different patterns in P retention under storm and base flow conditions. At LLS, net P retention was greatest during storm flows than under base flow conditions, reflecting a high efficiency of P retention from agricultural runoff at LLS. In contrast, at CHS, a greater percentage of the P load was retained

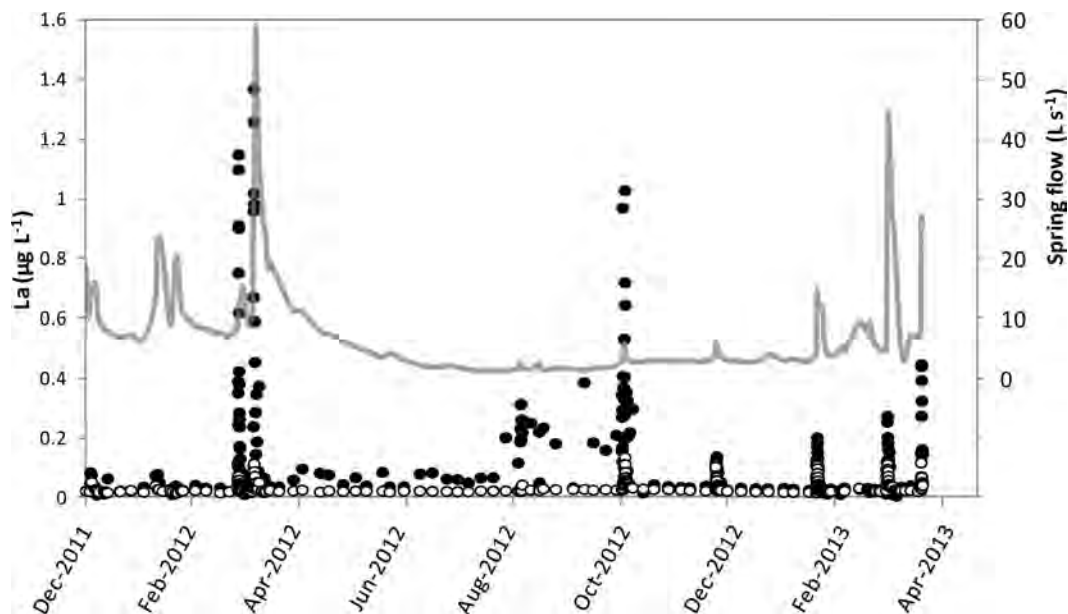


Figure 4. Time series of measured and “conservative” lanthanum (La) concentrations and flow at Langle spring. Measured La concentrations are denoted by solid circles; “conservative” La concentrations are denoted by open circles. See text for explanation of how “conservative” La concentrations were calculated.

under base flow than during storm flow. This reflects much lower base flows at CHS, which increase water residence time and promote particulate sedimentation and P retention, and higher storm flows linked to stream piracy, which provide greater flushing from nonagricultural areas, where flows have a low P concentration.

Contaminant Residence Times in Karst Drainage. While monitoring P relative to a conservative tracer provides us with valuable information on rates of annual and storm flow/base flow net retention, it provides no information about the residence times of P within the karst, or the time scales over which retention and remobilization may occur. This is of strategic concern in relation to the “legacy” of P within watersheds,^{42,43} whereby time-lags in release of retained P may mask the effects of conservation measures on receiving water quality. By measuring a full suite of trace elements using ICP-MS, a “serendipitous” observation was made, which may help provide clues about the wider contaminant residence times within the karst drainage. Concentrations of “dissolved” (<0.45 μm) lanthanum (La) in storm flow spring discharge at LLS were more than an order of magnitude higher than could be accounted for by the runoff sources measured within the SEW. Figure 4 shows the concentrations of La in the spring discharge at LLS and a “conservative” (maximum) concentration from runoff, which accounts for the dilution of agricultural runoff during transit through the karst drainage, using K as a tracer. The high storm flow La concentrations observed at LLS are likely a “legacy” signal from a past tracer experiment. In 2001, lanthanum-labeled montmorillonite clays were injected into a losing stream at SEW as part of a study to examine clay and bacterial transport.⁴⁴

While the La tracer was detected at LLS around 16 h after it was injected,⁴⁴ our monitoring suggests the La tracer was also retained within the karst drainage system and continues to be remobilized and released during storm events more than 10 years later. Unfortunately, it is impossible to perform a mass balance to quantify how much of the La applied in the tracer study remains within the karst drainage system and how long a La “legacy” might persist, as no La measurements were made in the

intervening 10 years between the tracer injection in 2001 and our monitoring which started in November 2011. Within the scope of this study, it was also not possible to determine whether the La concentrations measured were truly dissolved or a <0.45 μm colloidal/clay fraction or whether La geochemistry is sufficiently similar to be used as an indicator of P transport. However, these results indicate that La, a tracer expected to be flushed rapidly through the karst, was retained and continues to be remobilized and released during storm events, more than 10 years later. This indicates the potential for contaminant retention in the subsurface karst drainage system, where contaminant storage and gradual rerelease may occur over time scales of at least a decade.

Wider Implications. Hydrochemical tracers of agricultural runoff allowed us to directly evaluate the nonconservative behavior of P, within karst drainage, and quantify net P retention. Our results challenge the widely held assumption that karst landscapes are always highly vulnerable to P loss and suggest that, in some cases, karst drainage may provide a greater sink for P than previously considered. P from agricultural runoff was attenuated by hydrological dilution from cleaner (nonagricultural) sources during transport through karst drainage. However, there was also a high capacity for net P retention, especially for Langle Spring, which was subject to the highest agricultural P loadings. Here ~70% of the annual TP flux and ~90% of the annual SRP flux was retained. Moreover, the buffering within the soils and karst drainage not only retained a high proportion of incoming fluxes of P from agricultural runoff but preferentially retained the most bioavailable P fractions. For instance, much research has documented the capacity of soil to retain applied P in various inorganic (Al, Fe, Ca complexes) and organic forms of varying stability.^{45,46} The long-term accumulation of P in soil, however, can be released slowly to soil water.^{28,47}

The mechanisms of P retention were not investigated here but likely include varying combinations of processes including adsorption onto clays, coprecipitation of P with CaCO_3 , and binding with particulate humic substances^{11–13} in the soil, in epikarst, and within the fractures and conduits. These adsorption

products and precipitates will be physically retained as the water velocity slows and will be deposited as sediment along the base of the conduit flow paths. With the recurrence of high flow, these sediments are resuspended by turbulent flow and moved along the flow path, until redeposited, or eventually resurged at the base-level spring. Given the potential importance of CaCO_3 -P coprecipitation for P retention in karst terrain, and the possibility of reductions in the efficiency of this coprecipitation mechanism under higher P and dissolved organic carbon (DOC) concentrations,^{12,48,49} further work is needed to examine any unforeseen impacts of increasing agricultural intensification on this “self-cleansing” P retention mechanism. However, in this study, the site with the higher livestock intensity and with higher manure-enriched runoff actually demonstrated greater efficiency of P retention. This may indicate that critical P and DOC thresholds for inhibition of CaCO_3 precipitation were not reached or that other P retention process mechanisms were occurring.

The patterns in spring-water La concentrations suggest continued release of La from springs more than 10 years after a tracer injection and indicate the potential for long-term contaminant retention, storage, and subsequent release. Indeed, the complex nature of karst hydrological pathways can result in large distributions in water and contaminant residence times, and lag times for discharge to surface waters may be much longer than expected.^{50–52} Our findings indicate that retention of P within karst drainage may reduce the risk of acute episodic storm-driven losses of agricultural P. However, the potential buffering of P in the epikarst, and within the fracture and conduit drainage system, can provide a slow, but long-term, source of P released via springs to surface waters. Further work is needed to determine the ecological impacts of such patterns of P release to receiving streams and the ability of those streams to assimilate those inputs, compared with higher pulse inputs during storm flows.

■ ASSOCIATED CONTENT

● Supporting Information

Map of the SEW and the karst water flow system; time series of spring-water TP, SRP, K, and Rb concentrations; table of Ca, TP, and SRP endmember concentrations; soils and geology of the Savoy Experimental Watershed; experimental methods. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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Coupling High-Frequency Stream Metabolism and Nutrient Monitoring to Explore Biogeochemical Controls on Downstream Nitrate Delivery

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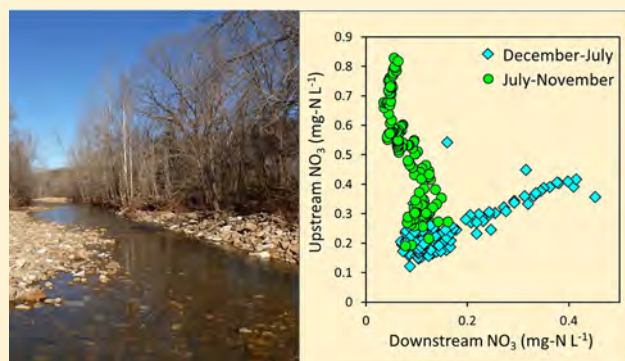
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Supporting Information

ABSTRACT: Instream biogeochemical process measurements are often short-term and localized. Here we use in situ sensors to quantify the net effects of biogeochemical processes on seasonal patterns in baseflow nitrate retention at the river-reach scale. Dual-station high-frequency in situ nitrate measurements, were coupled with high-frequency measurements of stream metabolism and dissolved inorganic carbon, in a tributary of the Buffalo National River, Arkansas. Nitrate assimilation was calculated from net primary production, and combined with mass-balance measurements, to estimate net nitrification and denitrification. The combined net effects of these instream processes (assimilation, denitrification, and nitrification) removed >30–90% of the baseflow nitrate load along a 6.5 km reach. Assimilation of nitrate by photoautotrophs during spring and early summer was buffered by net nitrification. Net nitrification peaked during the spring. After midsummer, there was a pronounced switch from assimilatory nitrate uptake to denitrification. There was clear synchronicity between the switch from nitrate assimilation to denitrification, a reduction in river baseflows, and a shift in stream metabolism from autotrophy to heterotrophy. The results show how instream nitrate retention and downstream delivery is driven by seasonal shifts in metabolic pathways; and how continuous in situ stream sensor networks offer new opportunities for quantifying the role of stream biota in the dynamics, fate, and transport of nitrogen in fluvial systems.



1. INTRODUCTION

Nutrients, including nitrogen (N), phosphorus (P), and carbon (C) from agriculture and domestic wastewater, are a major source of water-quality impairment.¹ Excessive nutrient inputs to rivers, streams, and lakes can accelerate growth of nuisance and harmful algae. Resulting increases in microbial activity and depletion of dissolved oxygen (DO) have profound negative consequences for invertebrates and fish, potable water supply, and recreation.^{2,3} However, biogeochemical processes in streams also play an important role in regulating downstream nutrient transport, with stream biota retaining and removing nutrients from the water column, reducing downstream ecological impacts.^{4–6}

Streams can provide a major sink for nitrate (NO₃⁻) through uptake (assimilation) by primary production and

through denitrification.^{7,8} The effectiveness of these processes varies throughout the year and between streams, but conventional methods for estimating NO₃⁻ uptake are based on relatively few, short-term experimental nutrient additions and isotope measurements,^{9–11} making results difficult to extrapolate in space and time.¹² Continuous high-frequency in situ measurements offer new opportunities to explore NO₃⁻ source dynamics,^{13–17} and instream processes have been inferred from single-station diurnal concentration cycles,^{12,18,19} longitudinal profiling,^{20–23} and nested sensor networks.²⁴

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In this study, we used in situ sensors to quantify the net effects of biogeochemical processes on seasonal patterns in baseflow NO_3^- retention at the river-reach scale. The approach employed here is novel because it combines dual-station high-frequency NO_3^- measurements, with high-frequency measurements of stream metabolism (analysis of diurnal DO curves to calculate primary production and respiration), dissolved inorganic carbon (DIC), and excess partial pressure of carbon dioxide (EpCO_2), to explore the capacity of instream biogeochemical processes to retain and remove NO_3^- . High-frequency in situ monitoring of water chemistry and streamflow was undertaken along a 6.5 km experimental reach of Big Creek, a tributary of the Buffalo National Scenic River, Arkansas, U.S.A, and were used to calculate a NO_3^- mass balance along the reach. Net primary production was used to calculate NO_3^- assimilation by photoautotrophs. Daily NO_3^- removal rates and rates of NO_3^- assimilation by photoautotrophs were used to calculate net nitrification and denitrification. The biogeochemical controls on NO_3^- removal were then evaluated in relation to wider ecosystem drivers including streamflow, DO, and stream ecological function, to explore how seasonal shifts in metabolic pathways influence instream NO_3^- retention and downstream NO_3^- delivery.

2. MATERIALS AND METHODS

2.1. Site Description and Water-Quality Monitoring.

Big Creek, a tributary of the Buffalo National Scenic River, Arkansas (Figure 1), is the subject of detailed water-quality

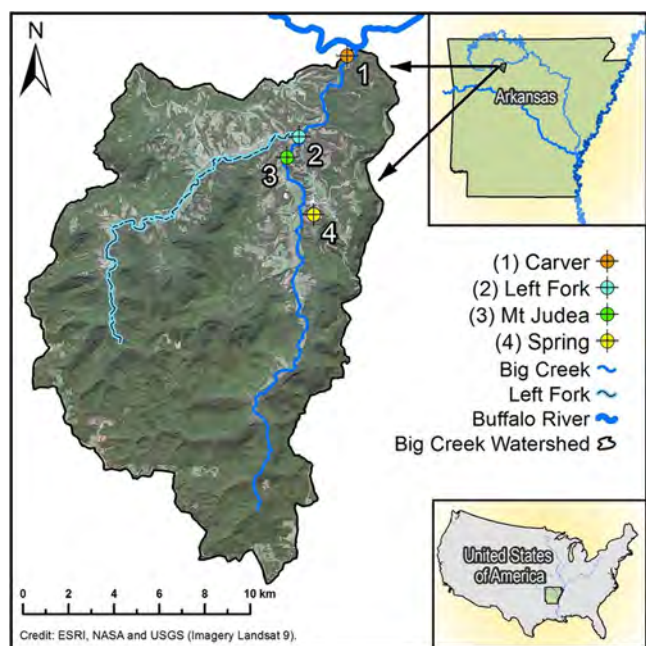


Figure 1. Map of the Big Creek watershed and its location.

monitoring because of a permitted swine concentrated animal feeding operation (CAFO) within the watershed, in operation since September 2013. The Big Creek watershed lies in the karst terrain of the Ozark Plateau of the midcontinental U.S.A. (Figure 1). The watershed area is 236 km^2 , with 79% of the land area deciduous forest, 3% evergreen forest, 14% grassland/pasture, and 3% developed land (see Supporting Information, SI, S1.1). Swine-manure slurry from the CAFO

has been land applied to permitted fields since January 1, 2014, in accordance with State regulations.

The focus of this study is an experimental reach of Big Creek, downstream of the CAFO, from an upstream monitoring station at Mt Judea (USGS site 07055790; watershed area 106 km^2) to a downstream monitoring station at Carver (USGS site 07055814; watershed area 233 km^2), 7.21 and 0.69 km from the confluence between Big Creek and the Buffalo River, respectively (Figure 1). One tributary (Left Fork) enters Big Creek between Mt Judea and Carver. The watershed is a mantled karst terrain characterized by intimate connection between groundwater and surface water; transport of surface-derived nutrients can be rapid²⁵ (see S1.2).

USGS conducted high-frequency (15 min) NO_3^- monitoring using subsensible ultraviolet nitrate probes at Carver (06/03/2014 to 04/29/2017) and Mt Judea (11/01/2014 to 11/01/2015); there was therefore one year of overlapping data (11/01/2014 to 11/01/2015), during which NO_3^- monitoring was undertaken at both Mt Judea and Carver. A water-quality sonde (YSI 6600) operating at Carver simultaneously collected 15 min interval DO, pH, specific conductance, and water temperature data. Further information about the high-frequency water-quality monitoring is provided in S1.3.

Water-quality samples, collected on a weekly basis since 09/12/2013, with additional opportunistic high-flow sampling, at Mt Judea, Left Fork and at a groundwater (spring) monitoring site (Figure 1), provided NO_3^- (by ion chromatography, Dionex ICS-1600); alkalinity (by fixed-end point acidimetric titration to pH 4.5²⁶); and conductivity (VWR Symphony B10C) data. All nitrate concentrations are reported as NO_3^- -N (mg-N L^{-1}). Water-quality data are available at <https://bigcreekresearch.org/>.

2.2. Stream-Flow Measurements and Hydrograph Separation. Stream flow was measured using established USGS streamflow gauging methods²⁷ (see S1.4). A two-component mixing model was used to partition the contributions to streamflow from groundwater and surface runoff,²⁸ using alkalinity as a conservative groundwater tracer (see S1.5).

2.3. Analysis of Diurnal Dissolved Oxygen Curves to Calculate Primary Production and Respiration. The daily average gross primary production, daily average ecosystem respiration and reaeration coefficient were calculated from the series of diurnal DO curves at Carver (see S1.6), using a piecewise solution of the mass balance, DO model²⁹ simplified for the situation where the deficit does not vary spatially (eq 1): the Delta method.^{30,31}

$$dD/dt + k_a D = ER_{av} - GPP_{av}(t) \quad (1)$$

where D is the DO deficit ($\text{mg-O}_2 \text{ L}^{-1}$), t is the time (days), k_a is the reaeration coefficient, ER_{av} is the ecosystem respiration ($\text{mg-O}_2 \text{ L}^{-1} \text{ d}^{-1}$), and GPP_{av} is the gross primary production ($\text{mg-O}_2 \text{ L}^{-1} \text{ d}^{-1}$); these are standard measures of ecosystem respiration and gross primary production in river systems.³²

Odum³³ suggested a classification system of flowing-water communities based on oxygen metabolism by using the ratio of GPP_{av} to ER_{av} (GPP/ER). Respiration is associated with both plant and microbial activity. Photosynthesis is only associated with plants. Autotroph-dominated communities are represented by GPP/ER values >1 , whereas heterotroph-dominated communities are represented by GPP/ER values <1 .

2.4. Use of the THINCARB Model for Calculating Dissolved Inorganic Carbon Concentrations and Excess

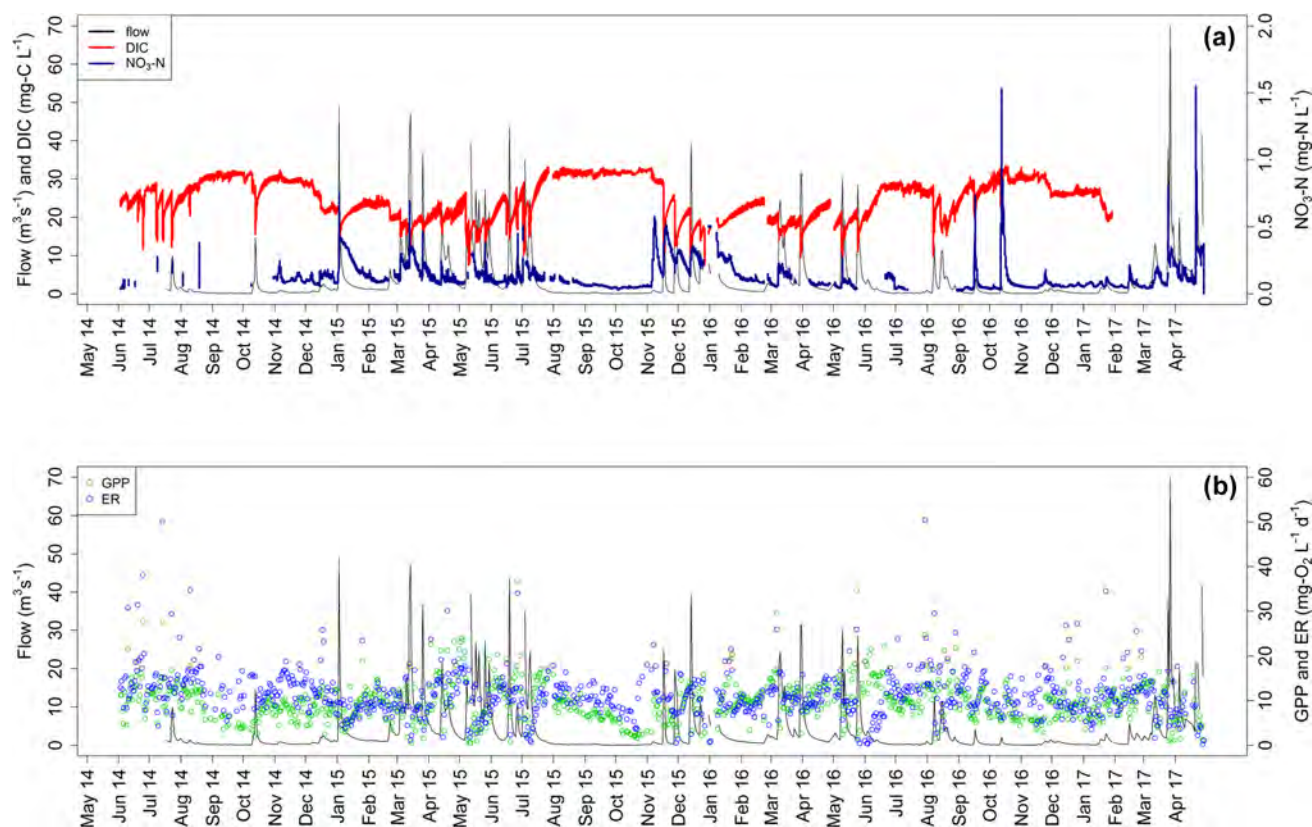


Figure 2. Time series at the downstream monitoring site (Carver), from May 2014 to May 2017, showing: (a) nitrate (NO_3^-), dissolved inorganic carbon (DIC) and streamflow; and (b) daily average gross primary production (GPP), ecosystem respiration (ER), and streamflow.

Partial Pressure of Carbon Dioxide. The THINCARB model (THERmodynamic modeling of INorganic CARBon)³⁴ uses pH, Gran Alkalinity (Alk_{Gran}) and temperature measurements to calculate dissolved inorganic carbon (DIC) concentrations and DIC speciation from the excess partial pressures of carbon dioxide (EpCO_2) in freshwaters. THINCARB is open access and is described in detail in Jarvie et al. (2017);³⁴ an outline is provided in S1.7. Prior to use, alkalinity measurements in units of $\text{mg-CaCO}_3 \text{ L}^{-1}$ were first converted to Alk_{Gran} (in $\mu\text{eq L}^{-1}$), where $1 \text{ mg L}^{-1} \text{ CaCO}_3 = 19.98 \mu\text{eq L}^{-1}$.³⁴

THINCARB was applied to the high-frequency sonde data from Carver. Specific conductance was used as a surrogate for alkalinity: using the regression relationship between Alk_{Gran} and specific conductance (κ), measured across the Big Creek watershed, including the spring, and Mt Judea, Left Fork, and Carver stream sites: $\text{Alk}_{\text{Gran}} = 8.65 (\pm 0.28) \times \kappa - 6.44 (\pm 66)$, $R^2 = 0.95$, $n = 270$, $P < 0.001$ (numbers in parentheses represent twice the standard error). By applying this regression equation to the hourly κ series, an hourly alkalinity record was derived, which was then used alongside the hourly pH and water-temperature data, to calculate a high-frequency DIC and EpCO_2 series.

2.5. Mass-Balance Calculation of Baseflow Nitrate Fluxes, Instream Losses, and Net Nitrification and Denitrification. Daily mass-balance calculations were undertaken for eight quiescent, low-flow periods (each typically of 1–2 weeks). USGS stream-velocity readings from Carver ranged from 0.457 and 1.22 m s^{-1} , and with a stream distance along the experimental reach of 6.52 km, the travel times ranged from 3.96 to 1.48 h. Therefore, daily mass balances

over a 24-h period were assumed sufficient to account for transit of NO_3^- , given: (a) the relatively short travel times; (b) the high degree of stationarity in flux transfers during quiescent baseflow conditions; and (c) that calculated daily mass balances were averaged over a 1–2 week period.

The 15 min NO_3^- measurements at Mt Judea and Carver were converted to daily means, and daily nitrate loads at each site were calculated using the corresponding gauged daily streamflow data. To account for flow accretion along the reach, the difference between the daily flow downstream at Carver, and the upstream site at Mt Judea was calculated. The increase in flows was assumed to be input from Left Fork (Figure 1).

Daily NO_3^- input loading to the reach (L_T) was calculated as the sum of the daily NO_3^- loads from Mt Judea (L_{MJ}) and Left Fork (L_{LF}):

$$L_T = L_{\text{MJ}} + L_{\text{LF}} \quad (2)$$

There was no high-resolution NO_3^- monitoring on Left Fork, so weekly NO_3^- measurements from grab samples taken at Left Fork were combined with the measured daily flow accretion to derive daily loads from Left Fork (S1.8.1). A sensitivity analysis evaluated the potential effects of under- or overestimating Left Fork NO_3^- concentrations by $\pm 50\%$ (Tables S11 and S12).

Within this karst watershed, some of the flow accretion will arise from direct groundwater input into Big Creek. Discharge data were not available from the Left Fork tributary, and direct apportionment of contributions from Left Fork and groundwater was not possible. We therefore evaluated a second, alternative “endmember” case scenario whereby all of flow

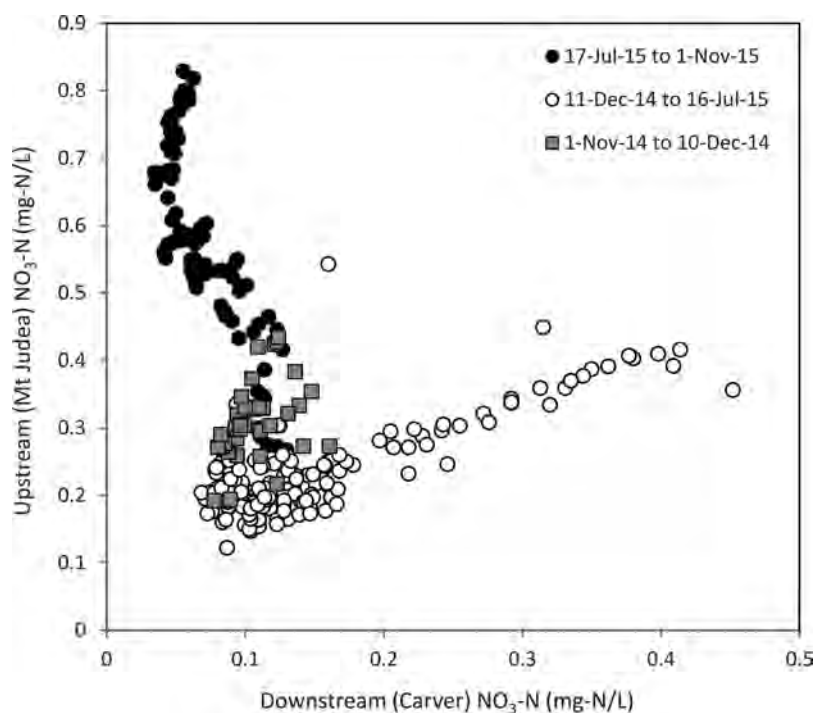


Figure 3. Scatter plot showing the relations between mean daily nitrate concentrations upstream at Mt Judea and downstream at Carver.

accretion was attributed to direct groundwater contribution (S1.8.2).

The daily instream NO_3^- load removal (L_R) along the reach was calculated as the difference between the daily input NO_3^- loading (L_T), and the daily NO_3^- load at Carver (L_C):

$$L_R = L_T - L_C \quad (3)$$

To allow direct comparison with rates of assimilatory NO_3^- uptake by photosynthesis, L_R (kg-N d^{-1}) was then converted to a daily NO_3^- removal rate, U_T ($\text{mg-N L}^{-1} \text{d}^{-1}$). U_T incorporates both assimilatory NO_3^- uptake by photoautotrophs (U_A), heterotrophic NO_3^- removal through direct uptake and denitrification (U_D), and NO_3^- enrichment due to remineralization via nitrification (R):²⁰

$$U_T = U_A + U_D - R \quad (4)$$

U_A was estimated from the GPP_{av} measurements.^{12,35} GPP_{av} data were converted into net primary production (NPP), assuming that autotrophic respiration consumed 50% of the GPP_{av} .^{36,37} NPP data were then converted from units of O_2 uptake ($\text{mg-O}_2 \text{L}^{-1} \text{d}^{-1}$) to C uptake ($\text{mg-C L}^{-1} \text{d}^{-1}$), with a photosynthetic quotient of 1.00, then converted to NO_3^- uptake ($\text{mg-N L}^{-1} \text{d}^{-1}$), using a molar ratio of C:N of 12.³⁸ Subtracting U_T from U_A provides a measure of either net nitrification (positive values) or net heterotrophic NO_3^- removal through direct uptake and denitrification, hereafter referred to as “net denitrification” (negative values). When the river was influent, loss of NO_3^- to groundwater was accounted for, as described in S1.8.3.

3. RESULTS AND DISCUSSION

3.1. Three-Year Time Series of Nitrate, Dissolved Inorganic Carbon and Stream Metabolism. The hourly NO_3^- and DIC concentrations variations at Carver were driven by streamflow, but in opposing directions (Figure 2a). The mean and median NO_3^- concentrations were 0.128 and 0.093

mg-N L^{-1} , respectively. Nitrate concentrations at Carver were lowest during baseflow (mean $0.043 \text{ mg-N L}^{-1}$; lowest 10% of flows) and highest during storm runoff (mean $0.278 \text{ mg-N L}^{-1}$; highest 10% of flows), arising from nonpoint-source mobilization and delivery of NO_3^- during rainfall events.

The mean and median DIC concentrations were 24.8 and 25.2 mg-C L^{-1} , respectively. DIC concentrations were highest during baseflow (mean 31.7 mg-C L^{-1}), with DIC concentrations diluted by storm runoff (mean 13.2 mg-C L^{-1}). Highest DIC and lowest NO_3^- concentrations occurred during the extended low-flows between August and November 2015.

The mean and median molar C:N ratios were 356 and 305, respectively. The mean C:N ratio during baseflow was 882, and 82 during stormflow. C:N ratios greater than ~ 6.6 are indicative of stoichiometric depletion of N relative to C.³⁹ Absolute NO_3^- concentrations below $\sim 0.1 \text{ mg-N L}^{-1}$ are deemed likely to be limiting to algae, with algal growth response to NO_3^- enrichment occurring between 0.38 to 1.79 mg-N L^{-1} .⁴⁰ Therefore, under average and baseflow conditions at Carver, a clear potential exists for algal growth to be limited by low NO_3^- availability.

No longer-term trends in either NO_3^- or DIC were observed over the three years. These high-frequency monitoring results are consistent with results from near-weekly water-quality monitoring of Big Creek at Mt Judea, which showed no statistically significant increasing or decreasing trends in dissolved or particulate forms of P and N concentrations since 2013.⁴¹

Earlier studies⁶ have shown that Ozark streams can be very effective at retaining available nutrients, and buffering additional nutrient inputs. Therefore, the absence of any increasing trend in nutrients in the water column may result from the rapid and efficient uptake of nutrient inputs by stream biota. Consequently, high-resolution stream metabolism and nutrient measurements were used here to detect whether increased photosynthesis or respiration rates resulted from increased

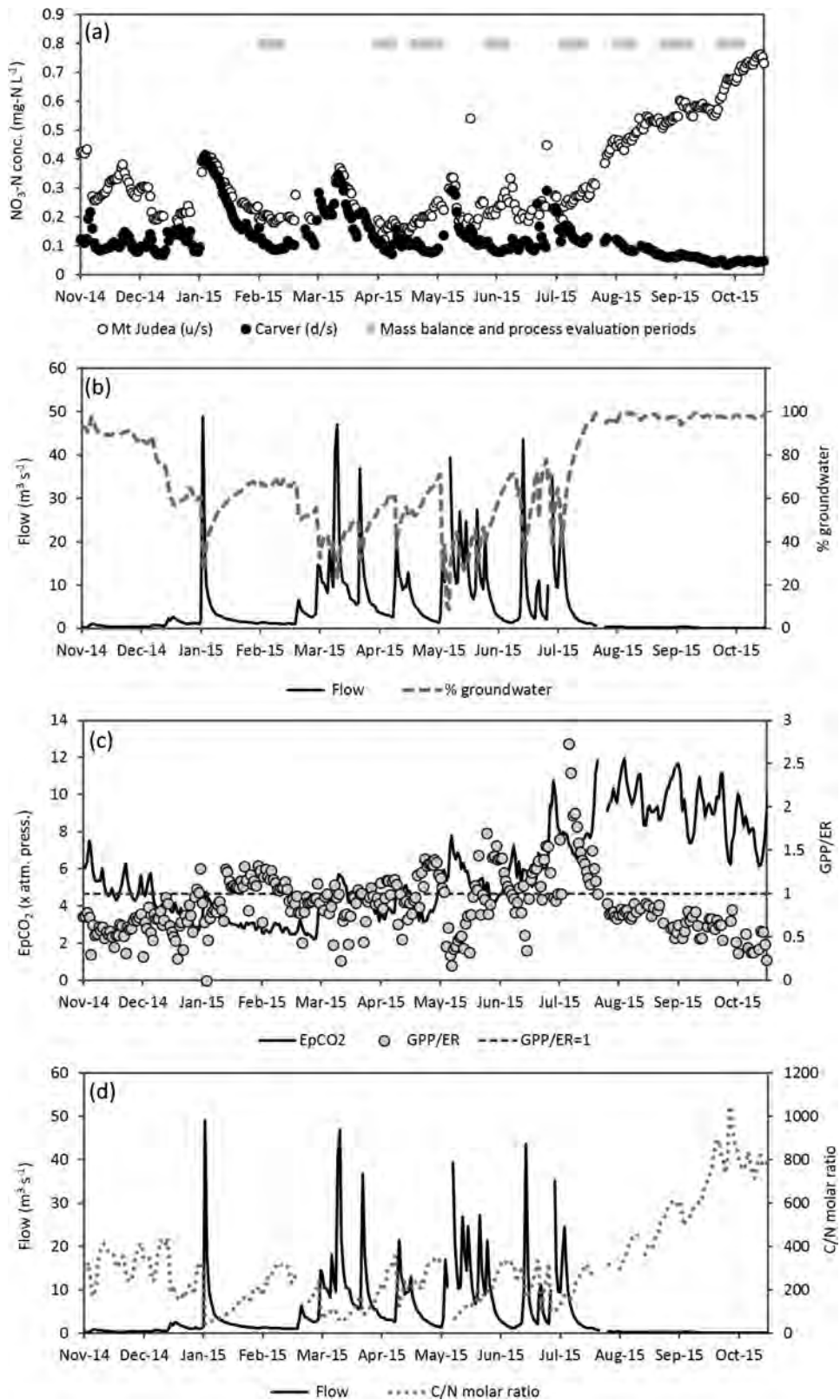


Figure 4. Time series from 1 November 2014 to 1 November 2015, showing: (a) Nitrate concentrations upstream at Mt Judea and downstream at Carver, and the lower-flow time periods used for mass balance calculation and evaluation of biogeochemical processes; (b) streamflow at Carver and the percentage groundwater contribution to streamflow; (c) daily ratio of gross primary production: ecosystem respiration (GPP/ER) downstream at Carver (horizontal dashed line shows GPP/ER of 1, i.e., balance between heterotrophy and autotrophy), and excess partial pressure of carbon dioxide (EpCO₂); and (d) streamflow and the molar C:N ratio (DIC, dissolved inorganic carbon/NO₃-N) downstream at Carver.

nutrient assimilation, even where no increases in water-column nutrient concentrations could be observed.

The time series in daily rates of GPP_{av} and ER_{av} at Carver (Figure 2b), showed no definitive long-term trends between

Table 1. Seasonal Patterns in Mean Daily NO_3^- Input Loadings (L_T) to Big Creek, Mean Daily Instream NO_3^- Load Removal (L_R) along the 6.5 km Experimental Reach, Under Low-Flow Conditions, and Mean Daily NO_3^- Load Removal As a Percentage of NO_3^- Inputs (U_E)^a

season	date range	NO_3^- input loading to reach (L_T) (kg-N d ⁻¹)	instream NO_3^- removal along reach (L_R) (kg-N d ⁻¹)	instream NO_3^- removal (L_R) as % of NO_3^- input loading (L_T) (U_E)
winter	4–13 Feb 2015	17.3 (1.12)	7.68 (0.46)	44.7 (4.09)
spring 1	5–12 Apr 2015	44.1 (6.35)	19.0 (2.82)	43.9 (9.53)
spring 2	24 Apr–5 May 2015	37.9 (15.3)	16.9 (3.85)	47.6 (8.93)
early summer	2–10 Jun 2015	49.2 (23.6)	24.1 (8.54)	51.2 (5.34)
mid summer	11–21 Jul 2015	61.7 (44.2)	14.6 (2.82)	32.1(14.1)
late summer	7–16 Aug 2015	7.56 (1.22)	5.57 (0.59)	74.2 (4.66)
autumn 1	1–14 Sept 2015	5.81 (1.23)	4.49 (0.81)	77.8 (2.39)
autumn 2	1–11 Oct 2015	2.98 (0.29)	2.82 (0.25)	94.8 (1.20)

^aStandard deviations are shown in parentheses.

2014 and 2017. GPP_{av} declined rapidly in response to major storm runoff events, but typically recovered within a couple of weeks. Highest GPP_{av} tended to occur during quiescent baseflow or recessionary streamflow conditions during the summer (May through August). Both GPP_{av} and ER_{av} declined during the autumn (September through December), reflecting reductions in stream biological activity, and GPP_{av} tended to decline at a faster rate than ER . This was particularly apparent during the extended low-flows between August and December 2015, suggesting a decline in primary production relative to microbial activity and a transition from net autotrophic to net heterotrophic stream communities. During winter baseflows (November through January), ER_{av} tended to exceed GPP_{av} . During the 3-yr monitoring, no CAFO-related impacts on either stream nutrient concentrations or metabolism are discernible at Carver.

3.2. Temporal and Spatial Variability in NO_3^- Concentrations, Relative to Other Key Environmental Variables. Mean daily NO_3^- concentrations varied between baseflow and storm events at Mt Judea and Carver, during the one year of overlapping data (Figure 3). There was a clear differentiation between a higher-flow period characterized by regular storm events from mid-December 2014 to mid-July 2015, and lower-flow conditions from August to November/December 2015 (Figures 3 and 4).

During the higher-flow period, a positive correlation existed between upstream (Mt Judea) and downstream (Carver) NO_3^- , with a ratio approaching 1 (Figure 3). During this high-flow period, NO_3^- concentrations at both upstream and downstream sites ranged between ~ 0.1 and ~ 0.4 mg-N L⁻¹. Time series data show close convergence between upstream and downstream NO_3^- concentrations during storm-event peak concentrations (Figure 4a,b).

Under lower-flow conditions, NO_3^- concentrations were consistently higher upstream than downstream (Figure 3). The increase in NO_3^- concentrations at the upstream site during the summer and autumn 2015 corresponds with reductions in flow. This is typical of the longer-term hydrologically driven cycles in NO_3^- concentrations observed at the upstream site, reflecting a strong flow dependency, with highest concentrations under the lowest flows, and dilution with increasing flow (Figure S11a,b,c). The strong increase in NO_3^- concentrations during July to November 2015 therefore reflects hydrological controls, and is consistent with falling flows. The high NO_3^- concentrations in autumn 2015 subsequently declined with the onset of higher flows (Figure S11a,b).

The gap in NO_3^- concentrations between upstream and downstream sites widened with decreasing flow, particularly during the protracted low-flows between mid-July and November 2015. During this time, minimal soil water contributed to streamflow, and almost all (>95%) of streamflow was derived from groundwater (Figure 4a,b). By the end of October 2015, upstream NO_3^- concentrations reached ~ 0.75 mg-N L⁻¹, whereas downstream NO_3^- concentrations were ~ 0.05 mg-N L⁻¹. Between July and November 2015, downstream NO_3^- concentrations exhibited a much lower range (~ 0.05 to ~ 0.15 mg-N L⁻¹) as compared with upstream (~ 0.1 to ~ 0.8 mg-N L⁻¹) (Figure 3). This reduction in both magnitude and range of downstream NO_3^- concentrations under baseflow conditions could arise either from dilution of NO_3^- , as a result of downstream accretion of water sources with much lower NO_3^- concentrations, or by removal of NO_3^- through biogeochemical processes, necessitating a mass-balance evaluation (see section 3.3).

The widening gap in NO_3^- concentrations between upstream and downstream sites after mid-July 2015 corresponded with a decline in GPP/ER , which fell below 1, indicating a change to net heterotrophy (Figure 4c). During the low-flow period from mid-July to November 2015, Big Creek was heterotrophic for $\sim 90\%$ of days. Daily streamwater EpCO_2 doubled between mid-July and November 2015, from 4.5 to 9.1 times atmospheric pressure, independently confirming an increase in rates of respiration (CO_2 release), relative to photosynthesis (CO_2 uptake).

During the higher-flow period from mid-January to mid-July, Big Creek was predominantly net autotrophic ($\text{GPP}/\text{ER} > 1$ for 52% of days). Net heterotrophic conditions prevailed predominantly during lower-flow intervals between storm events, with $\text{GPP}/\text{ER} < 1$ typically during and immediately after storm events.

Molar C:N ratios at Carver also increased markedly after mid-July, from ~ 300 to >800 (Figure 4d). This stoichiometric depletion of N, along with persistence of low NO_3^- concentrations below 0.1 mg-N L⁻¹ (falling to <0.04 mg-N L⁻¹), suggests that algal growth may have been limited by low N availability at Carver over the late summer and autumn of 2015.

3.3. Nitrate Reach Mass Balance to Quantify Seasonal Nitrate Removal during Baseflow Conditions. Mean daily NO_3^- mass balances for the eight seasonal quiescent baseflow periods between February and October 2015 are presented in Table 1. Mean daily NO_3^- input loadings to the reach (L_T) increased from 17.3 kg-N d⁻¹ in February to 61.7 kg-N d⁻¹ in

Table 2. Seasonal Patterns in Mean Daily NO_3^- Removal Rate (U_T) along the 6.5 km Experimental Reach of Big Creek, Under Low-Flow Conditions, And Mean Daily Assimilatory Uptake of NO_3^- by Photoautotrophs (U_A)^a

season	date range	instream NO_3^- removal rate (U_T) (mg-N L ⁻¹ d ⁻¹)	assimilatory NO_3^- uptake (U_A) (mg-N L ⁻¹ d ⁻¹)
winter	4–13 Feb 2015	0.077 (0.006)	0.212 (0.035)
spring 1	5–12 Apr 2015	0.072 (0.017)	0.256 (0.050)
spring 2	24 Apr–5 May 2015	0.082 (0.018)	0.355 (0.067)
early summer	2–10 Jun 2015	0.090 (0.014)	0.269 (0.045)
mid summer	11–21 Jul 2015	0.066 (0.030)	0.259 (0.040)
late summer	7–16 Aug 2015	0.284 (0.026)	0.180 (0.016)
autumn 1	1–14 Sept 2015	0.229 (0.019)	0.115 (0.038)
autumn 2	1–11 Oct 2015	0.656 (0.029)	0.076 (0.028)

^aStandard deviations are shown in parentheses.

Table 3. Seasonal Patterns in Mean Daily NO_3^- Concentration Gains by Net Nitrification (+) and Losses by Net Denitrification (–) along the Experimental Reach of Big Creek, under Low-Flow Conditions; Mean Daily Values of the Ratio between Gross Primary Production and Ecosystem Respiration (GPP/ER); Excess Partial Pressure of Carbon Dioxide (EpCO₂); Dissolved Oxygen (DO); Streamflow; and the Percentage of Groundwater Contribution to Streamflow^a

season	date range	net nitrification (+) or denitrification (–) (mg-N L ⁻¹ d ⁻¹)	GPP/ER	EpCO ₂ (× atm. press.)	DO (mg-O ₂ L ⁻¹)	flow (m ³ s ⁻¹)	% groundwater
winter	4–13 Feb 2015	+0.135 (0.032)	1.14 (0.09)	2.80 (0.20)	11.9 (0.49)	1.15 (0.07)	66.5 (1.34)
spring 1	5–12 Apr 2015	+0.184 (0.039)	1.06 (0.13)	3.64 (0.20)	10.2 (0.33)	3.10 (0.37)	58.6 (2.38)
spring 2	24 Apr–5 May 2015	+0.273 (0.058)	1.25 (0.16)	3.81 (0.59)	10.3 (0.50)	2.61 (1.16)	61.7 (5.79)
early summer	2–10 Jun 2015	+0.179 (0.044)	1.34 (0.15)	4.71 (0.49)	9.39 (0.42)	3.30 (1.72)	58.0 (6.48)
mid summer	11–21 Jul 2015	+0.193 (0.024)	1.97 (0.78)	7.15 (0.46)	8.98 (0.29)	2.54 (1.28)	82.8 (7.21)
late summer	7–16 Aug 2015	–0.104 (0.032)	0.78 (0.05)	10.6 (0.83)	6.95 (0.35)	0.23 (0.04)	98.8 (0.98)
autumn 1	1–14 Sept 2015	–0.102 (0.027)	0.62 (0.10)	9.85 (1.65)	6.50 (0.54)	0.24 (0.06)	96.6 (1.42)
autumn 2	1–11 Oct 2015	–0.592 (0.015)	0.57 (0.23)	8.17 (1.50)	7.85 (0.64)	0.04 (0.004)	97.8 (0.64)

^aStandard deviations are shown in parentheses.

July, then declined rapidly to 7.56 kg-N d⁻¹ in August, which also corresponded with an order of magnitude reduction in baseflow discharge. By October, L_T had fallen to only 2.98 kg-N d⁻¹. Instream NO_3^- removal (L_R) followed a similar pattern to L_T , with highest mean daily instream NO_3^- removal during June (24 kg-N d⁻¹), then decreasing during the late summer and autumn, and falling to 2.82 kg-N d⁻¹ in October. However, the efficiency of instream NO_3^- removal (U_E , i.e., L_R expressed as a percentage of L_T) increased markedly during the late summer and autumn, from 32% in July to 74–95% between August and October.

The fluvial mass balance therefore confirmed that the observed downstream reductions in NO_3^- concentrations under baseflow were a result of net instream removal of NO_3^- by biogeochemical processes, rather than a dilution effect.

Although L_T and L_R were greatest during the winter to early summer period, U_E and the instream NO_3^- removal rate (U_T) increased dramatically during the low flows of the late summer and autumn; U_T increased from ≤ 0.09 mg-N L⁻¹ d⁻¹ (February through July), to >0.2 mg-N L⁻¹ d⁻¹ in August and September, and 0.66 mg-N L⁻¹ d⁻¹ in October (Table 2). By autumn 2015, $>75\%$ of the NO_3^- inputs were removed by biogeochemical processes (Table 1).

We also assessed the efficiency of NO_3^- removal under the alternative scenario, where the increase in flow along the experimental reach was solely from direct groundwater input (S1.6.2). This made relatively little difference to the U_E , which also increased markedly during the late summer and autumn, from 46% in July to 72–94% between August and October (Table SI3). The sensitivity analysis (Tables SI1 and SI3) showed that a 50% increase or decrease in either Left Fork or

groundwater NO_3^- concentrations made little difference to these findings: a consistent increase in efficiency of NO_3^- removal was observed after July, with August to October U_E values consistently ~ 70 – 95% .

3.4. Biogeochemical Controls on Nitrate Delivery: Accounting for Assimilatory Nitrate Uptake to Calculate Net Nitrification and Net Denitrification. From February to July, assimilatory NO_3^- uptake by photosynthesizing plants (U_A) consistently exceeded U_T (Table 2) indicating, first, that assimilation of NO_3^- by photoautotrophs was the dominant process removing NO_3^- from the water column; and second that assimilation was partially balanced by net nitrification NO_3^- gains. In contrast, from August to October, U_T exceeded U_A , indicating that heterotrophic NO_3^- removal through direct uptake and denitrification was removing NO_3^- along the reach in late summer and autumn.

Table 3 shows that net nitrification gains to the reach ranged from 0.135 mg-N L⁻¹ d⁻¹ in February to 0.273 mg-N L⁻¹ d⁻¹ in April/May. However, after July, a pronounced switch from net nitrification gains to net denitrification losses occurred. During late summer and autumn, denitrification losses of NO_3^- increased from ~ 0.100 mg-N L⁻¹ d⁻¹ in August and September to 0.592 mg-N L⁻¹ d⁻¹ in October. These estimates were based on using an average periphyton C:N molar ratio of 12 for U.S.A. streams.^{35,38} We also evaluated the effects of using an average periphyton molar C:N ratio of 8.6, from research in northern European streams.¹⁷ This increased U_A values by $\sim 39\%$, but did not alter our findings of a switch between net nitrification between February and July, to net denitrification from August to October. By changing the C:N stoichiometry from 12 to 8.6, net nitrification ranged from

+0.218 mg-N L⁻¹ d⁻¹ in February to +0.414 mg-N L⁻¹ d⁻¹ in April/May, with net denitrification ranging from -0.033 mg-N L⁻¹ d⁻¹ in August to -0.562 mg-N L⁻¹ d⁻¹ in October.

Net nitrification and denitrification rates were compared with mean daily GPP/ER, EpCO₂, streamflow and percentage groundwater discharge (Table 3). The shift from net nitrification to net denitrification corresponded directly with (1) a change in stream metabolism from net autotrophic (GPP/ER in July was 1.97) to net heterotrophic (GPP/ER fell below 1, to 0.78 in August, 0.62 in September, and 0.57 in October); and (2) an increase in EpCO₂ and a reduction in DO arising from the increases in microbial respiration relative to photosynthesis.

The alternative scenario where flow accretion between Mt Judea and Carver was attributed to direct groundwater discharge to Big Creek also had no effect on the timing of the shift from net nitrification to denitrification (S1.6.2, Table S14). Sensitivity analysis (Tables S12 and S14) also showed that, irrespective of a 50% increase or decrease in either Left Fork or groundwater NO₃⁻ concentrations, the same consistent switch between net nitrification and net denitrification was observed after July.

The consistency in this observed switch between instream NO₃⁻ production and instream NO₃⁻ removal, and its synchronicity with measured changes in stream metabolism, provides compelling evidence that the marked change in instream NO₃⁻ processing and delivery after July was linked to changes in stream metabolism from net autotrophy to net heterotrophy.

The karst streams of the Ozarks are characterized by a large hyporheic zone,^{42,43} a hotspot of nitrogen transformation.⁴⁴ Water residence times and redox conditions provide a key control on changes between NO₃⁻ removal and NO₃⁻ production with hyporheic zone sediments.⁴⁵⁻⁴⁸ In Big Creek, the winter to midsummer period was characterized by higher baseflows (at least an order of magnitude greater than late summer/autumn baseflows), and net autotrophy resulting in higher instream DO concentrations. Rapid movement of well-oxygenated water throughout the water column, and into the hyporheic zone, promotes aerobic metabolism of organic matter and release of NO₃⁻ through nitrification.^{46,49} From winter to midsummer, net nitrification was observed in Big Creek, and nitrification in the hyporheic zone may have been responsible for buffering the effects of photosynthetic assimilatory uptake of NO₃⁻.

Under the more sluggish flow conditions during late summer and autumn, available oxygen is depleted as a result of increased heterotrophic activity. The reduced movement of water and oxygen through the hyporheic zone favors a shift to respiratory pathways with denitrification (conversion of nitrate to N₂O and/or N₂ gas).^{50,51} Unlike assimilation of NO₃⁻ into plant biomass, which retains N temporarily, denitrification results in a permanent loss of bioavailable N. The low baseflows of late summer and autumn 2015, resulted in higher water residence times and a greater proportion of flow moving through the hyporheic zone. This provides greater exposure and water contact time with microbial biofilms where denitrification occurs.⁵¹ The death and breakdown of biomass during the late summer and autumn contribute to the availability of organic matter for microbial decomposition, promoting higher rates of microbial respiration relative to photosynthesis, losses of DO, and greater availability of organic carbon as a resource for denitrifying bacteria.^{45,52,53}

Denitrification within the hyporheic zone may therefore be responsible for losses of NO₃⁻ in Big Creek during the late summer and autumn. Although denitrification can also occur on suspended sediments within the water column,^{54,55} this is likely to be a second order effect under baseflow conditions in a groundwater-fed stream, where suspended solids concentrations are low (typically <5 mg L⁻¹).

Under baseflow conditions, instream assimilatory NO₃⁻ uptake by photosynthesizing plants and hyporheic-zone denitrification along the experimental reach removed between ~30 and ~90% of the NO₃⁻ input load. During the period of monitoring (spring 2014 to spring 2017) NO₃⁻ loading to the upstream section of Big Creek (at Mt Judea) was attenuated by instream processing such that no CAFO-related impacts on either stream nutrient concentrations or metabolism were discernible at the downstream location (Carver), and thus, to the Buffalo River. Future monitoring will be needed to detect whether long-term changes in nutrients and organic carbon inputs may occur, whether this stimulates higher rates of heterotrophic and/or autotrophic activity, and any longer-term effects on the capacity of assimilation and denitrification processes to remove and buffer any increase in nutrient loadings.

The novelty of this research is the combination of continuous, high-frequency in situ stream metabolism and nitrate measurements, to apportion the net effects of assimilation, nitrification, and denitrification on changes in baseflow nitrate fluxes at the river-reach to watershed scale. In this case, we found that, during winter to midsummer periods, NO₃⁻ uptake in Big Creek was dominated by assimilation by photoautotrophs, which was partially compensated by release of NO₃⁻ from nitrification. In late summer, the predominant metabolic pathway switched to net heterotrophy and heterotrophic NO₃⁻ removal through direct uptake and denitrification became the dominant process of nitrate removal. Removal of NO₃⁻ by assimilation and denitrification provides an important “self-cleansing” ecosystem service, resulting in a pronounced shift in C:N stoichiometry and decreasing NO₃⁻ concentrations to low levels which would be expected to limit algal growth.⁵⁶

This approach provides a means of scaling up, from microscale and mesoscale process experiments and measurements, which are, by necessity, short-term and localized, to explore how river nitrate delivery responds to shifts in stream metabolism, from day-to-day and seasonal to interannual variability. This research, and the methods presented here, are applicable along the river continuum, from headwaters to large-scale fluvial systems (with large spatial and temporal variability in nutrient fluxes), and offer a valuable way forward in quantifying net process controls on the fate and transport of nitrogen in fluvial systems.

■ ASSOCIATED CONTENT

📄 Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.8b03074.

S1, Methods: Land use and cover data; site hydrogeology; high-frequency water-quality monitoring; streamflow measurement; hydrograph separation; analysis of diurnal dissolved oxygen curves to calculate primary production and respiration; calculation of DIC and EpCO₂; nitrate mass-balance calculations; and S2,

Results: Figure S11 time series of nitrate and flow and nitrate plotted against stream flow for Mt Judea; Tables S11 and S12, sensitivity analysis based on estimates of NO_3^- load inputs from Left Fork; Tables S13 and S14, alternative scenario sensitivity analysis based on estimates of NO_3^- load inputs from groundwater; and additional references (PDF)

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Notes

The authors declare no competing financial interest.

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***E. COLI* IN FLOWING WATERS**

Mary Savin and BCRET members

I. Why is *Escherichia coli* (*E. coli*) being monitored? What is the concern?

Fecal pollution (from excrement of humans or animals) in the environment is of concern for many reasons, not least of which is human health risks and disease control. Other concerns related to fecal pollution of our natural waters include potential changes in the nutrient status of water, introduction of antibiotic resistance and chemical contaminants, changes in the ecological condition of waters, and degradation of natural resources on which rural economies depend.

People are concerned that the land application of swine effluent (pig excrement) will increase *Escherichia coli* in Big Creek and consequently, the Buffalo River, the first National Scenic River in the U.S. This document aims to provide better understanding of

- 1) what is involved in *E. coli* monitoring;
- 2) what the numbers mean;
- 3) some limitations in interpreting values; and
- 4) provide a context for further research that may be needed to better interpret the *E. coli* numbers being measured in flowing waters such as Big Creek and Buffalo River.

II. What is *E. coli*? Why and how is it used as an indicator?

E. coli is a species of bacteria from the coliform group - bacteria that are rod-shaped, gram negative, non-spore forming facultative anaerobes, commonly found in the feces of humans and warm blooded animals. *E. coli* occurs in human intestines - many strains with no ill effects, although certain *E. coli* cause serious human illnesses. Thus, while many *E. coli* do not harm us, there are important variants that do. There are also other species of bacteria, viruses, and other small organisms that cause disease, which if present in fecal sources polluting our waters, can make humans sick. Depending on whether *E. coli* survive as long in the environment as these other pathogens, *E. coli* may or may not adequately warn us about a disease causing agent in our waters. To date, *E. coli* is the most reliable test we have, although other indicators and tests are being investigated.

E. coli cannot be seen in streams by the human eye. Thus, it is important to determine if there is a problem of elevated *E. coli* numbers by careful water sampling and using U.S. Environmental Protection Agency (EPA) standardized methods. *E. coli* is counted in water because of the extensive studies and relationships established between the presence of *E. coli* and the number of human illnesses occurring from contact with the water (e.g. swimming) containing the bacteria. From these relationships, there have been upper limits established for bacterial numbers that correspond to the acceptable risk of people getting sick from exposure to water containing them.

Water quality standards for *E. coli* are established by the state in Arkansas Department of Environmental Quality (ADEQ) Regulation 2 (see Table 1). The *E. coli* numbers must remain below a threshold in a specified number of total samples collected. The exact upper limit that is allowed depends on the designation of the waterbody and time of year (primary or secondary contact season). Primary

Table 1. Upper limits for *Escherichia coli* counts defined in Regulation 2 of the Arkansas Department of Environmental Quality (ADEQ) as specified by contact season and waterbody designation for both single samples and geometric mean.

Contact season	Water designation	Limit of <i>E. coli</i> (MPN/100mL)	
		Single sample ¹	Geometric mean ²
Primary (May 1-Sept. 30)	Extraordinary Resource Water Ecologically Sensitive Waterbody Natural & Scenic Waterway Lakes Reservoirs	298	126
	All other water	410	NA ³
Secondary (Oct. 1-April 30)	Extraordinary Resource Water Ecologically Sensitive Waterbody Natural & Scenic Waterway Lakes Reservoirs	1490	630
	All other water	2050	NA

¹ No more than 25% of samples from no less than 8 samples per contact season may exceed the limit

² Geometric mean is calculated from at least 5 samples collected within 30 days at evenly spaced time intervals during that 30-day period

³ Not applicable

contact recreation is a designation given to a waterbody where full body contact occurs and occurs from May 1 through September 30. The ADEQ also designates any stream with a watershed (e.g. drainage basin in the landscape) exceeding 10 square miles and those with smaller watersheds on individual cases (i.e. after site verification) for primary contact recreation. Secondary contact recreation designates waterbodies where activities such as boating, fishing, and wading take place and occurs from October 1 through April 30.

E. coli is measured in samples of water collected strictly following EPA guidelines. The measurement of *E. coli* starts within 8 hours of collection. This method provides an estimate of *E. coli* presence in the sampled water that is the most probable number (MPN) of *E. coli*. Using an EPA method provides numbers that are theoretically comparable to other labs using the same method. The MPN depends on growing bacteria in the laboratory in “culture” and is an approach used routinely in microbiology. This measurement is subject to high variability because of the nature of environmental bacteria; thus, variability in the data in *E. coli* counts from streams is not unusual. For this reason, it is important to establish background levels of *E. coli* in any water resulting from various wildlife, human (e.g., septic tanks, sewers), and agricultural (e.g. pig, chicken, cattle) sources.

E. coli thresholds are lower in late spring through summer (primary contact season) when more people are expected to be in contact with streams and lakes. More stringent limits also apply to Extraordinary Resource Waters (e.g., Buffalo River), Ecologically Sensitive Waterbodies, and Natural Scenic Waterways. During the primary contact season (May 1- Sept. 30), we do not want *E. coli* to exceed 298 MPN/100 mL, and during secondary contact season (Oct. 1 – Apr. 30) *E. coli* counts should not exceed 1490 MPN/100 mL in single samples.

III. How does the *E. coli* measurement “fit” into the context of the landscape?

While monitoring one bacterial species to assess the biological quality of water may seem simple, determining the actual ecological condition of a system is complicated. Measurement of *E. coli* is an indicator for potential fecal pollution and potential pathogen problems, but by itself, does not identify the source(s) of the bacteria.

E. coli is present in intestines (and feces) and is not supposed to grow in the environment; thus, the presence and abundance should indicate pollution and be directly related to human and animal sources. However, because *E. coli* is in many different animals (e.g. human, wildlife, agricultural) and because of the different pathways that bacteria may travel throughout the environment before ending up in water where we can measure it, the presence of *E. coli* does not identify the source of pollution.

IV. Why is *E. coli* monitoring important and why is it complicated?

There are many of factors that affect whether *E. coli* survives in the environment, for how long, and whether it moves to other locations. *E. coli* is adapted to living in intestines. After deposition from an animal, cells have to survive rapidly changing environmental conditions (e.g. temperature and moisture), exposure to harmful UV rays in sunlight, outcompete other organisms, and avoid predators. All these factors make it difficult to estimate how long *E. coli* will survive in lagoon, soil, and river environments. However, there is evidence that *E. coli* can persist in soil and sediments.

V. What are the numbers?

E. coli is measured weekly in Big Creek upstream and downstream of the C&H Farm, Mt. Judea, Newton County, Arkansas. Water sample collection for *E. coli* analysis began Sept 12, 2013, prior to manure from C&H Farm application to fields, either in fields adjacent to or distant from Big Creek. Manure application began in 2014 to fields distant from Big Creek, and then adjacent to Big Creek in March 2014. So far in the period following manure applications by C&H Farms (Jan 2 through May 19, 2014), no trends in *E. coli* with time or between sampling locations are apparent (Table 2).

The Table 2 data are detailed in the Big Creek Research and Extension Team Quarterly Reports and demonstrate the week-to-week variability in *E. coli* at upstream and downstream sites. The *E. coli* counts are expected to continue to be variable. Clearly, it is important to quantify the variability in *E. coli* concentrations long-term in order to determine if changes occur as a result of C&H operation.

The MPN is expected to increase with increases in flow, and the recent installation of a USGS gauge to measure flow will allow for the determination of the relationship between measured flow in

the stream and *E. coli* concentrations. Because of the dangers during high flows, most contact recreation is expected during base flow, and thus sampling during base flow may provide more meaningful data.

Table 2. Geometric mean of *E. coli* (and range of sample MPN) before (Sept 12 - Dec 17, 2013) and after manure applications began on the C&H Farm (Jan 2 - May 19, 2014).

C&H location	Before any manure application	After manure application
	Sept. 12 - Dec. 17, 2013	Jan. 2 - May 19, 2014
	(MPN/100mL)	
Upstream	82 (6 – 4080)	83 (ND ^a – 921)
Downstream	111 (5 – 3500)	39 (ND – 1553)

^aNot detected.

Regulation

Arkansas Pollution Control and Ecology Commission # 014.00-002 2014. Regulation Establishing Water Quality Standards for Surface Waters of the State of Arkansas as revised, effective March 24, 2014.

Arkansas Department of Environmental Quality (ADEQ). Available at

http://www.adeg.state.ar.us/regs/files/reg02_final_140324.pdf. Last accessed 20 Aug 2014.

Nutrient Concentrations in Big Creek Correlate to Regional Watershed Land Use

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Core Ideas

- Nutrient concentrations are low at Big Creek relative to expected biological-response thresholds.
- Nutrient concentrations at Big Creek are typical of streams draining watersheds with similar land use.
- Flow-adjusted nutrient concentrations at Big Creek have not increased over the short-term.
- Nutrient concentrations in streams increase as watershed land area in pasture and urban uses increases.

Abstract: Nutrient concentrations in several streams of the Boston and Ozark Mountains region of Arkansas, including the Buffalo National River and its tributaries, have garnered tremendous interest. In particular, Big Creek has been the center of attention within the Buffalo River watershed because of a permitted concentrated animal feeding operation (CAFO). The objectives of this paper were to put nutrient concentrations of Big Creek into the context of the stream nutrient and watershed land-use relationship and develop a framework to evaluate regional land-use impacts on regional water quality. Nutrient concentrations in streams draining the Boston and Ozark Mountains region were related to the intensity of watershed land use. Concentrations in Big Creek were similar to other watersheds in the ecoregion with similar land use, suggesting limited impact of the CAFO on Big Creek at the present time. However, this does not preclude future impacts, and longer-term monitoring continues.

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NUTRIENT IMPAIRMENT of surface waters continues despite widespread conservation efforts to reduce losses from urban, rural, and agricultural land uses (Scavia et al., 2014). Land use within watersheds influences the quality and quantity of water in streams draining the landscape. As land disturbance increases and use intensifies, an increase in stormwater runoff and nutrient inputs that lead to a greater potential for transport to receiving water is generally observed (Dubrovsky et al., 2010; Rebich et al., 2011). This has led to efforts to identify and quantify nutrient sources within watersheds, strategically target, and apportion nutrient loss reduction (Reckhow et al., 2011).

Many factors influence the relationship between land use in a given watershed and nutrient transport downstream from that watershed. With an increase in percentage of the drainage area in pasture, row crop, and/or urban use, a general trend of increasing nutrient concentrations in storm and base flows will manifest (Buck et al., 2004; Giovannetti et al., 2013; Haggard et al., 2003; Migliaccio et al., 2007). Thus, nutrient concentrations in streams draining forested lands tend to be less than in watersheds with considerable anthropogenic land use.

For a range of reasons, great interest has been expressed in nutrient concentrations in several streams of the Boston and Ozark Mountains region of northwest Arkansas, including the Buffalo National River and its tributaries. In particular, Big Creek has been the center of attention within the Buffalo National River watershed (BRW) because of a permitted concentrated animal feeding operation (CAFO). The objectives of this letter are to put nutrient concentrations of Big Creek into the context of the stream nutrient and watershed land use relationship and assess whether stream nutrient concentrations have

Abbreviations: BRW, Buffalo River watershed; CAFO, concentrated animal feeding operation; LOESS, locally weighted regression; SRP, soluble reactive phosphorus; TN, total N; TP, total P; UIRW, Upper Illinois River watershed; UWRW, Upper White River watershed.

changed over the short term (3 yr of monitoring). The goal is to understand if, how, and why stream nutrient concentrations change downstream at Big Creek and whether the permitted swine CAFO has influenced water quality during the 3 yr since extensive monitoring began in September 2013.

Methods

Water samples have been collected over varying periods at the outlet of subwatersheds of the BRW, Upper Illinois River watershed (UIRW), and Upper White River watershed (UWRW; Fig. 1). Land use and cover (i.e., forest, pasture, and urban) for each subwatershed was obtained from high-resolution (4-m) imagery from the USGS National Elevation Dataset (USGS, 2015; Gesch et al., 2002), National Land Cover Dataset (USGS, 2017b), and National Hydrologic

Dataset (USGS, 2017a). In the UWRW, Giovannetti et al. (2013) monitored 20 sites monthly for 1 yr (June 2005–July 2006), collecting water samples during base-flow conditions. In the UIRW, Haggard et al. (2010) monitored 29 sites monthly during calendar year 2009, also collecting water samples during base-flow conditions.

In the BRW, the National Park Service in partnership with the Arkansas Department of Environmental Quality periodically collected water samples and measured nutrient concentrations at 20 stream sites from 1985 through 2015. Nitrate-nitrogen ($\text{NO}_3\text{-N}$), total N (TN), soluble reactive phosphorus (SRP), and total P (TP) concentrations were obtained directly from these data. Forest, pasture, and urban land-use areas were determined from 2006 high-resolution (4-m) land use–land cover imagery.

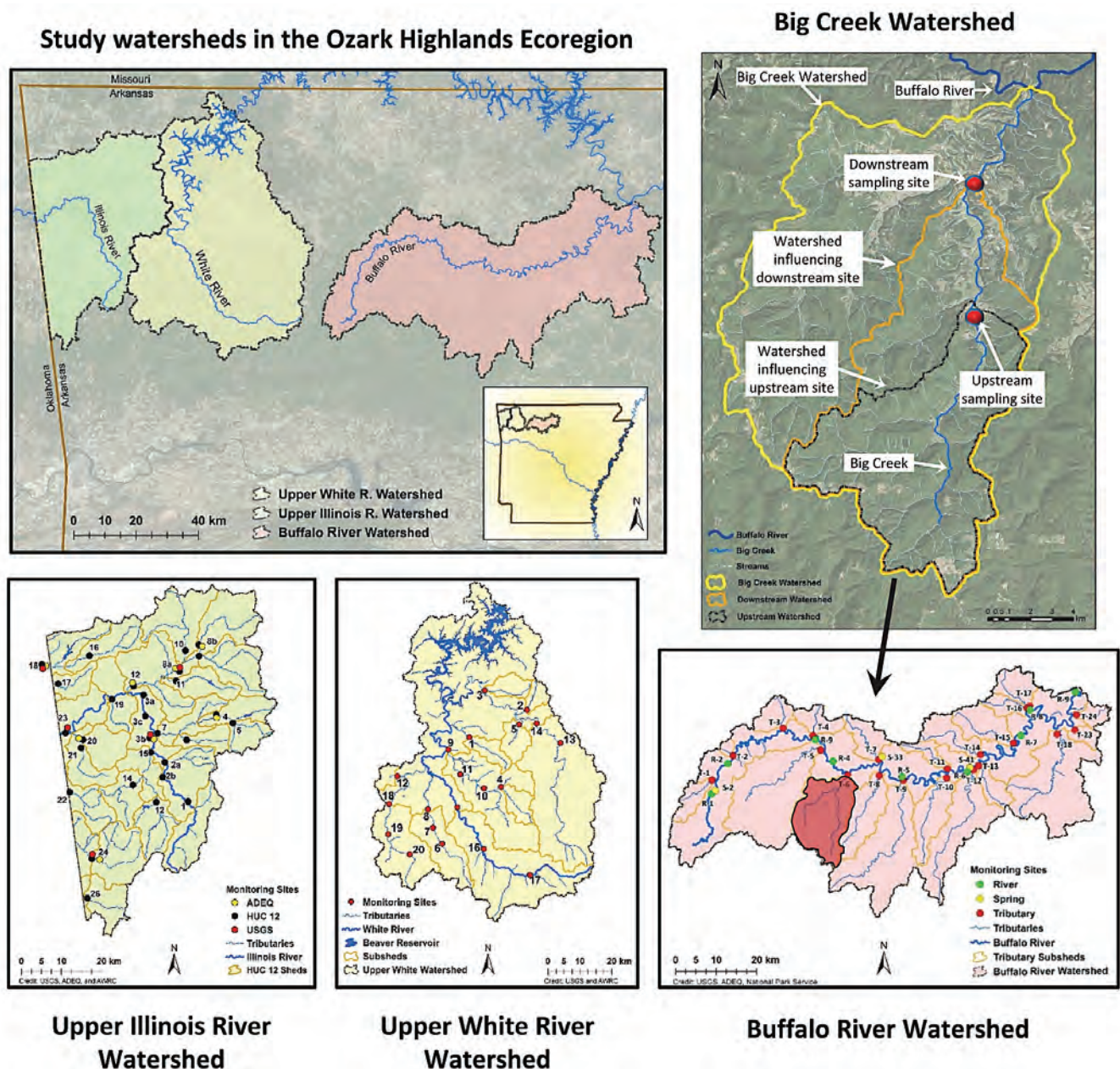


Fig. 1. Location of the Big Creek, Buffalo River, Upper Illinois River and Upper White River watersheds in the Boston Mountains and Ozark Highlands ecoregion. Information from USGS, Environmental Systems Research Institute (ESRI), and NASA.

Big Creek is monitored by the Big Creek Research and Extension Team, a partnership between the University of Arkansas System's Division of Agriculture and USGS. Water samples have been collected upstream and downstream of the swine CAFO on a near-weekly basis since September 2013 (Fig. 1). The water samples were analyzed at an Arkansas Department of Environmental Quality certified water quality laboratory within the Arkansas Water Resources Center (<http://arkansas-water-center.uark.edu/water-quality-lab.php>), according to methods detailed in Table 1. The data collected is made publicly available at <https://bigcreekresearch.org/>.

The geometric mean of nutrient concentrations of base-flow samples collected between September 2013 and April 2017 were determined in order to compare with base-flow nutrient concentrations available for BRW, UIRW, and UWRW. Base-flow conditions in Big Creek were classified from hydrograph inspection when flow had not increased or decreased within 3 d of sample collection. McCarty and Haggard (2016) suggested that stream nutrient concentrations under base flow can be used to identify nonpoint sources and target remedial measures in Boston Mountains and Ozark Highland watersheds.

Using all above-listed data sources, the geometric means of nutrient concentrations for streams in the BRW, UIRW, and UWRW were used to develop a relationship with human development within the watershed. Human development is defined as the percentage of pasture plus urban land use within the watershed. Exponential relationships with 95% confidence bands around the observations were developed for $\text{NO}_3\text{-N}$, TN, SRP, and TP concentrations to put nutrient concentration at Big Creek into the context of regional stream nutrients and watershed land use.

Data from Big Creek were paired with discharge available from a gaging station just downstream from the swine CAFO, where the USGS developed the rating curve; discharge information was only available from May 2014 through April 2017. The data were then used in a simple three-step process (White et al., 2004) to look at monotonic changes in the nutrients at Big Creek: (i) log-transform concentration (mg L^{-1}) and associated instantaneous discharge ($\text{m}^3 \text{s}^{-1}$); (ii) use locally weighted regression (LOESS) to smooth the data with a sampling proportion (n) of 0.5; and (iii) plot the residuals from LOESS (i.e., the flow-adjusted concentrations) over time and use linear regression to evaluate monotonic trends.

Results and Discussion

Putting Stream Nutrient Concentrations into Context at Big Creek

In Big Creek, upstream of the swine CAFO, the geometric mean concentrations of base flow sampled at weekly intervals from September 2013 for $\text{NO}_3\text{-N}$, TN, SRP and TP were 0.098, 0.205, 0.009, and 0.030 mg L^{-1} , respectively. Directly downstream of the CAFO, geometric mean concentrations at Big Creek during base flow conditions during the same period were 0.242, 0.356, 0.011, and 0.031 mg L^{-1} for $\text{NO}_3\text{-N}$, TN, SRP and TP, respectively. Arkansas has narrative criteria for nutrient concentrations in streams (Arkansas Pollution Control and Ecology Commission, 2016), but its proposed assessment methodology has numeric screening concentrations for TN (0.450–2.430 mg L^{-1}) and TP (0.040–0.100 mg L^{-1}) in the Boston Mountains and Ozark Highlands. The geometric mean concentrations at Big Creek upstream and downstream from the CAFO were below these values for the Boston Mountains and Ozark Highlands ecoregion.

Nutrient concentrations in Big Creek upstream and downstream from the CAFO are low with respect to nutrient–biological response thresholds for algae, macroinvertebrates and fish. Evans-White et al. (2014) reviewed the literature, summarizing nutrient–biological response thresholds across the United States:

- *Algal metric responses.* TN: 0.38–1.79 mg L^{-1} ; TP: 0.011–0.28 mg L^{-1}
- *Macroinvertebrate metric responses.* TN: 0.61–1.92 mg L^{-1} ; TP: 0.04–0.15 mg L^{-1}
- *Fish metric responses.* TN: 0.54–1.83 mg L^{-1} ; TP: 0.06–0.14 mg L^{-1}

Total N concentrations at Big Creek upstream and downstream of the swine CAFO were well below thresholds that result in some expected biological response, whereas TP concentrations were below thresholds for expected macroinvertebrate and fish response and on the low end of the range for expected algal response. However, these lower TP thresholds (0.006–0.026 mg L^{-1} ; Stevenson et al., 2008) were focused on shifts in diatom species and metrics rather than nuisance algal biomass. A recent study on the Illinois River Watershed showed that stream TP thresholds with *Cladophora* biovolume and nuisance taxa proportion of biovolume were observed between 0.032 and 0.058 mg L^{-1} (Joint Study

Table 1. Minimum detection limits for each chemical and biological constituent.

Constituent	Analytical method†	Minimum detection limit‡	Reporting limit§
Soluble reactive P, mg L^{-1}	EPA 365.2	0.002	0.010
Total P, mg L^{-1}	APHA 4500-P J; EPA 365.2	0.012	0.020
Nitrate-N, mg L^{-1}	EPA 300.0	0.004	0.050
Total N, mg L^{-1}	APHA 4500-P J; EPA 353.2	0.006	0.050
Total suspended solids, mg L^{-1}	EPA 160.2	No detection limit	4.0

† EPA = Approved CWA Chemical Test Methods (USEPA, 2017); APHA = American Public Health Association from the *Wadeable Streams Assessment, Water Chemistry Laboratory Manual* (USEPA, 2004).

‡ The minimum detection limit of an analyte is the value, which can be measured and reported with 99% confidence that the analyte concentration is greater than zero. Further information is available at USGS (1999).

§ The reporting limit is the least (non-zero) calibrated standard used in analysis, or as defined by method for total suspended solids.

Committee, 2017). Thus, TP concentrations at Big Creek upstream and downstream of the CAFO were in the range in which the natural assemblage of algae is shifting, but these concentrations would likely not be indicative of problematic nuisance algae in this ecoregion.

Geometric mean nutrient concentrations varied upstream and downstream of the swine CAFO at Big Creek, and Kosič et al. (2015) used the publicly available data to allude to the N increase being from human activities on the landscape, such as the CAFO. However, the historic land use and how stream nutrient concentrations during base-flow conditions increase with human development within the Boston Mountain and Ozark Highland watersheds need to be considered (e.g., see Giovannetti et al., 2013; Haggard et al., 2003; Migliaccio et al., 2007). In the Big Creek watershed, the percentage of land influenced by human activities (i.e., pasture plus urban) doubles from ~10 to ~20% in the drainage area upstream and downstream of the CAFO. Nutrient concentrations in Big Creek upstream and downstream of the CAFO are within the range typical of streams draining similar land uses (Fig. 2).

At this time, nutrient concentrations in Big Creek upstream and downstream from the swine CAFO are consistent with the range in concentrations for other watersheds with similar pasture and urban land use characteristics (Fig. 2), as well as less than most nutrient thresholds for nuisance water-quality conditions (Omernik and Griffith, 2014). However, this does not preclude the possibility that nutrient concentrations at Big Creek may increase over time, especially if human development and activity in the drainage areas increase. The most important observation is that nutrient concentrations were low in Big Creek, providing the ability to detect changes over time.

Have Nutrient Concentrations Changed in the Short Term at Big Creek?

Understanding that long-term (e.g., decadal-scale) water-quality data are needed to reliably assess how stream nutrient concentrations have changed in response to watershed management and climate variations is of critical importance (Hirsch et al., 2015). The literature shows that stream nutrient concentrations can change relatively quickly in response to effluent management (e.g., Haggard, 2010; Scott et al., 2011), but seeing a response (i.e., decrease in concentrations) from landscape management can take decades or more (Green et al., 2014; Sharpley et al., 2013). A myriad of factors may influence observed nutrient concentrations in streams, including discharge (Petersen et al., 1998), biological processes and climactic conditions (i.e., drought and floods; Jones and Stanley, 2016), and dominant transport pathways (Sharpley et al., 2013). Thus, we need to use caution when

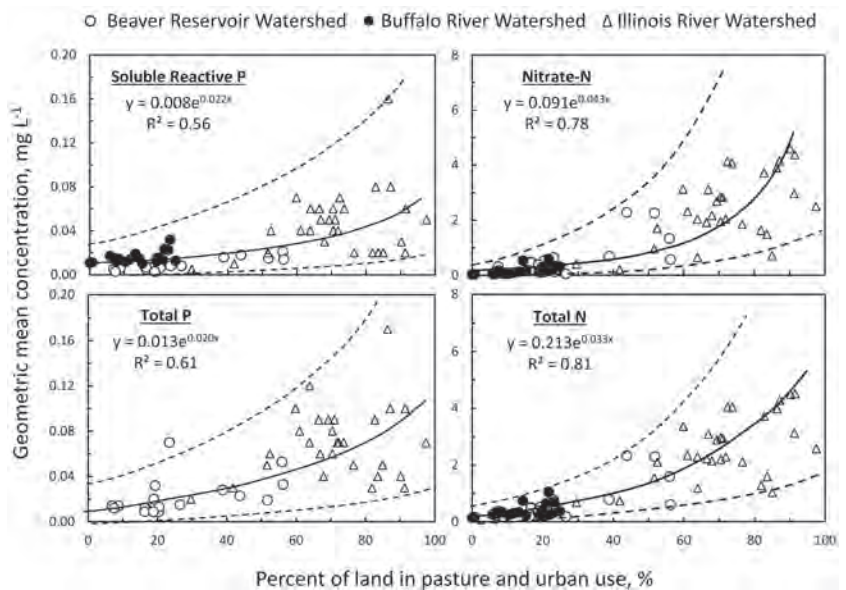


Fig. 2. Relationship between land use and the geometric mean N and P concentrations (mg L^{-1}) in the Buffalo, Upper Illinois, and Upper White River watersheds (no total P data available for the Buffalo River watershed). Dashed lines represent the 95% confidence intervals for the estimated mean (solid line).

interpreting trends in water quality over databases that only cover a limited timeframe.

Three years of flow-adjusted nutrient concentration data at Big Creek downstream from the swine CAFO (May 2014–April 2017) show different relationships with flow for the various constituents:

- Nitrate-nitrogen was greatest ($\sim 0.5 \text{ mg L}^{-1}$) during the lowest flows sampled, and concentrations decreased with increasing flow;
- Total N generally decreased with increasing flow until a minimal value occurred; then TN increased with increasing flow;
- Soluble reactive P concentrations did not change much during base-flow conditions, and the greater concentrations ($\sim 0.100 \text{ mg L}^{-1}$) sporadically occurred at larger flows, indicating that enrichment from stormflow may have been influenced by availability of source or other nontransport factors; and
- Total P concentrations were also relatively stable during base-flow conditions and then increased in association with rainfall-runoff events, with only a few samples having concentrations $> 0.100 \text{ mg L}^{-1}$, indicating relatively small enrichment from the landscape.

Flow-adjusted concentrations (White et al., 2004), showed no monotonic (i.e., increasing or decreasing) trends in SRP, TP, or TN ($P > 0.16$) over the current monitoring period (Fig. 3). However, flow-adjusted $\text{NO}_3\text{-N}$ concentrations decreased over time ($R^2 = 0.05$, $P = 0.01$) by $7\% \text{ yr}^{-1}$ (Fig. 3c).

Nutrient concentrations at Big Creek upstream and downstream of the swine CAFO, and indeed most tributaries of the Buffalo River, are low relative to other watersheds in this ecoregion (Fig. 2). This provides a starting point to build a framework to evaluate changes in nutrient concentrations of streams as a function of land use and management. The

evaluation of flow-adjusted concentrations over time showed that nutrients in Big Creek were not increasing over the short duration of monitoring for which concentration and discharge data were available (May 2014–April 2017). At this point in time (April 2014–April 2017), it is evident that nutrient concentrations in Big Creek have not increased at the monitored site. However, flow and nutrient concentration data over a longer period are needed to reliably quantify water-quality trends and characterize sources, and monitoring needs to continue for at least a decade to evaluate how discharge, season, and time influence nutrient fluxes (Hirsch et al., 2010).

This research details a process by which regional monitoring networks can be developed to establish baseline, in-stream nutrient concentrations and by which time and/or land use and management impacts can be determined.

Acknowledgments

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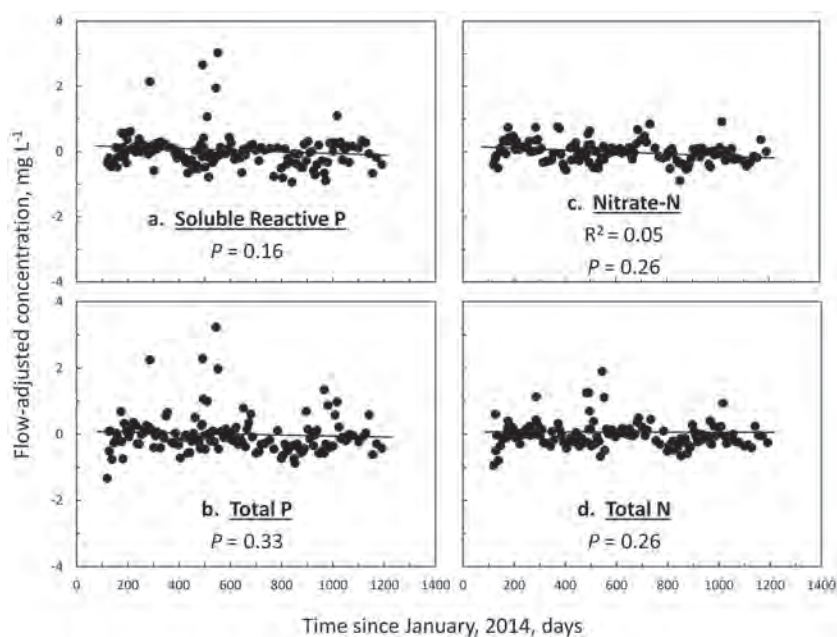


Fig. 3. Flow-adjusted concentration of (a) soluble reactive P, (b) total P, (c) nitrate-N, and (d) total N over time since January 2014, when monitoring in Big Creek started.

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Sampling Liquid Manure

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Liquid animal manure sampling can be an important management tool. Proper sampling provides the producer with nutrient analysis results that can be used in a sound farm fertilization program. Nutrient analysis of manure, in conjunction with soil sampling, helps determine how much manure should be applied to fields to maintain adequate fertility while minimizing potential environmental problems such as ground and surface water pollution. However manure applications should not exceed the maximum application rates in a manure management plan until sufficient data can be collected to justify revising the plan.

When to Sample

Liquid animal manure should be sampled for nutrient analysis as close to land application time as possible. This helps ensure that the reported nutrient content accurately reflects what is being applied to the land. If the manure is sampled as it is being land applied, the results will not be available to govern present application rates. It does, however, provide information for future land applications of animal manure, if the manure management remains fairly constant over time.

How to Collect a Liquid Manure Sample During Land Application

The easiest way to collect liquid animal manure samples is to collect the manure as it is being land applied. This approach ensures what is sample reflects what is applied. Randomly place catch pans in the field to collect the liquid manure as it is land applied by an irrigation system or honey wagon. Flexible rubber feed pans work well. Immediately after the manure has been applied, collect the manure from the catch pans, combine in a bucket to make one composite sample and mix well by stirring. This bucket will be the source of the manure sent for analysis.

From a Storage Facility

If collecting liquid animal manure samples during land application is not possible, collect the samples from the storage facility. Liquid animal manure storage facilities have a tendency for the manure to stratify with the solids settling to the bottom and the liquids remaining on top. It is also not uncommon for some solids to form a floating crust. This stratification affects the manure nutrient concentrations in the storage facility. The nitrogen and potassium will be more concentrated in the top liquid, while the phosphorus will be more concentrated in the settled solids. This stratification of nutrient concentrations increases the challenge of getting samples that represent what will be applied to a particular field. If the liquids from the top and middle of the profile will be applied, only this material should be sampled. If the settled solids will be applied, then they should be sampled. However, if the manure is to be agitated before pumping, as has been the traditional recommendation, the sample should contain representative proportions of manure from the top, middle, and bottom. The idea is to collect a sample of an entire column of manure to represent the manure after agitation.

If agitating the manure prior to sampling is not possible, an alternative approach is to make a sampler to collect the required sub-samples. The sub-samples are then mixed to represent the manure after agitation. The easiest to construct is simply a container such as a cup, attached to the end of a pole. Liquids from the manure surface can be simply scooped up. To collect liquids from the middle depths, or settled solids, the container is held up side down, trapping air, until the desired sampling depth is reached. Then the container is rotated, releasing the air and collecting the sample. When collecting a sample of the entire profile of the manure, sub-samples are collected and mixed in a bucket.

A sampler design that automatically collects a sample of the entire profile uses 10 foot, 1 ½ inch PVC pipe with a PVC ball valve at the bottom. The handle of the ball valve is replaced with a lever arm about 2 feet long. The free end of the lever arm is attached to the end of a 10 foot, 1 inch PVC pipe. The lever arm and smaller pipe allow the ball valve to be operated while holding to top of the sampler. To use the sampler the valve is opened and the sampler is inserted (in a line, not an arc) into the manure. When the foot of the valve is at the bottom of the settled solids, it is closed. Then the sample of the entire manure profile can be removed from the manure and placed in a bucket.



Sketch of Cup Sampler



Sketch of Foot Valve Sampler

Whichever sampler is used, at least 8 locations around the manure storage unit should be sampled and mixed in a bucket to serve as a final composite sample. This bucket will be the source of the manure sent for analysis

Getting the Sample Analyzed

After thoroughly mixing the final composite sample, fill a one liter plastic bottle half full. These bottles may be obtained from your county Extension office. Never fill the bottle more than half full to allow for gas expansion of the sample and to prevent the bottle from exploding. Keep the samples as cool as possible until you can take them to your county Extensions office for shipping to the University of Arkansas lab for analysis. There you will get assistance in filling out an information sheet on your manure sample. There is a fee to have the sample analyzed. While the sample can be sent to a private lab, the fees are often higher. If you are required by the Arkansas Department of Environmental Equality (ADEQ) to sample your liquid animal manure as part of your Regulation No. 5 permit, make sure that you inform the individual helping you with the paperwork so the correct set of analyses can be performed. In addition to the analyses to determine the fertilizer value of manure, it is recommended to analyze for the amount of phosphorus in the manure that is water soluble. Water soluble phosphorus is needed to evaluate the potential environmental risk associated with phosphorus application rates specified in manure management plans. Having good farm based information should help planners develop plans tailored to and individual farm.

Key Points to Remember

The important things to remember in collecting a liquid animal manure sample are:

- Collect a sample that best represents the nutrient content of the manure in that storage facility and what will be applied. If only the top water is to be applied it should be sampled. If the storage unit will be agitated prior to application the sample should contain material from the entire depth profile.
- Only fill the sample bottle ½ full.
- Keep the sample cool prior to shipping.
- Ship the sample to the lab as soon as possible.

Handout, April 27, 2010