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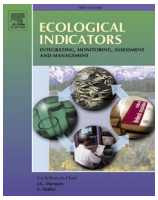

Seasonal periphyton response to low-level nutrient exposure in a least disturbed mountain stream, the Buffalo River, Arkansas

Billy G. Justus [a,*], Lucas J. Driver [a], David R.L. Burge [b]

[a] U.S. Geological Survey, Lower Mississippi-Gulf Water Science, Center, 401 Hardin Rd, Little Rock, AR 72211, USA
[b] Water Resources Science Graduate Program, University of Minnesota, St. Paul, MN 55108, USA

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ABSTRACT

Like most streams located in the Ozark Plateaus, the Buffalo River in Arkansas generally has excellent water quality. Water-quality conditions in Big Creek, however, a major tributary of the middle Buffalo River, have been less favorable than that of other Buffalo River tributaries. Concerns regarding the influence of water quality in Big Creek on the Buffalo River magnified in 2013 when a large confined animal feeding operation (CAFO) began operating in the watershed. In response to these concerns, the U.S. Geological Survey compared monthly nutrient concentrations and seasonal periphyton assemblage metrics of a site on Big Creek downstream of the CAFO, two Buffalo River control sites upstream of the confluence with Big Creek, and three Buffalo River test sites downstream of the confluence with Big Creek. In addition to identifying potential nutrient patterns and periphyton responses along a low-level nutrient exposure gradient, the study determined how nutrient contributions from Big Creek (and the CAFO) are affecting ecological conditions and consequent ecosystem services in the Buffalo River. Nutrient and periphyton data exhibited more temporal than spatial variability. Nutrient concentrations were generally highest of all sites at the Big Creek site. Concentrations at the five sites on the Buffalo River were typically low (near laboratory reporting limits), and concentrations at the three test sites rarely exceeded those of the two control sites. An index developed with three ecologically relevant periphyton metrics (oligotrophic taxa and *Homoeothrix* percent relative abundance and mesotrophic diatoms percent taxa richness) suggested that nutrient uptake at sites downstream of the Big Creek-Buffalo River confluence resulted in subtle shifts in downstream periphyton assemblages. The periphyton index of biological integrity at control sites was slightly and generally more favorable compared to test sites. Even so, when periphyton data were considered in conjunction with both hydrology and water-quality data, the negative consequences of antecedent high flows and associated scouring exceeded the potential positive effects that low-level nutrients had on algal productivity. These findings emphasize the importance of comparing biological and chemical data across extended temporal scales, particularly when working with low-level nutrient gradients.

1. Introduction

As the global human population increases and expands, previously undisturbed watersheds can be expected to experience increasing amounts of deforestation as natural landscapes are converted to agriculture or urban settings. With increasing land-use intensity, nutrient concentrations in least- or non-disturbed (i.e., reference) streams (also see Pardo et al., 2012; Hawkins et al., 2010; Nestler et al, 2010) can be expected to increase over time (Foley et al., 2005; Miserendino et al., 2011; Petersen et al., 2014; Fuß et al., 2017; Molina-Navarro et al., 2018). Thus, it is important to document the effects of nutrients on sensitive aquatic ecosystems and establish nutrient thresholds in relatively least-disturbed stream conditions. However, measuring and detecting biological changes associated with relatively minor changes in nutrient concentrations in least-disturbed streams with low background nutrient concentrations can be challenging for multiple reasons. Water-quality patterns may be catchment-specific (Burt, 1994; Neal et al., 1997; Soulsby et al., 2001), and the process of documenting the effects of low-level nutrient enrichment on streams can be confounded by temporal variability of nutrient concentrations associated with hydrology, timing and extent of nutrient sources, and the degree of nutrient attenuation (i.e., reduced nutrient concentrations) resulting from assimilation (i.e., absorption of nutrients) by bacteria and periphyton (Mulholland and Rosemond, 1992; Dent and Grimm, 1999; Cross et al.,

* Corresponding author.
E-mail addresses: bjustus@usgs.gov (B.G. Justus), ldriver@usgs.gov (L.J. Driver), dburge@smm.org (D.R.L. Burge).

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2006; Evans-White et al., 2009; Taylor et al., 2014; Cook et al., 2018).

In the United States (U.S.), water-quality standards are frequently developed for ecologically distinct areas known as ecoregions (Griffith et al., 1999). On a national scale, streams such as the Buffalo River (see Supplemental photos 1 – 4) in the Ozark Highlands Ecoregion (Woods et al., 2004; Ozarks, hereafter) have excellent water quality in their natural setting (Davis and Bell, 1998; Justus et al., 2010). The Buffalo River is designated as an Extraordinary Resource Water by the State of Arkansas, and its headwaters are federally designated Wild and Scenic (Arkansas Pollution Control and Ecology Commission, 2020; appendix D).

Monitoring conducted by the National Park Service (NPS) since the 1980s and other agencies more recently, indicates that water-quality conditions in Big Creek, a major tributary of the middle Buffalo River (Fig. 1), have been less favorable than in other Buffalo River tributaries (Watershed Conservation Resource Center, 2017). For example, quarterly water-quality sampling conducted by the NPS between 1998 and 2011 revealed that Big Creek ranked 3rd highest for orthophosphorus concentrations among 24 sampled Buffalo River tributaries.

Concerns over the water quality of Big Creek and its influence on water quality in the Buffalo River was further magnified when, in 2013, one of the largest confined animal feeding operations (CAFOs) in Arkansas began operating within the watershed. Based on the original (discharge) permit (Arkansas Department of Environmental Quality, 2012), this CAFO could house up to 6,500 swine and waste was to be stored temporarily in two adjacent ponds and later spread as fertilizer on 640 acres of hay fields and pastures in the Big Creek watershed.

Because aquatic assemblages are natural ecological endpoints in aquatic environments, biological assessments have been widely recognized as a primary tool for assessing water quality for some time (Katz and Gaufin, 1952; Hynes, 1960; Karr, 1981; Hilsenhoff, 1987; Davis et al., 1995; Norris and Morris, 1995). The response of biological assemblages to established nutrient gradients has been well documented in streams (Cole, 1973; Carr et al., 2005; Johnson et al., 2006; Justus et al., 2010; Smucker et al., 2013; Horwitz et al., 2016) and the deleterious effect of nutrient enrichment on species richness is well documented for multiple aquatic communities (Marcus, 1980; Evans-White et al., 2009; Rosset et al., 2014; Charles et al., 2019). However, there are few *in situ* studies measuring biological changes in response to small degrees of nutrification in small-to-moderate-sized, high-gradient, least-disturbed streams. Due to a direct relationship between nutrients and algae assemblage responses, benthic algae (periphyton, hereafter) are often the preferred biological assemblage when monitoring nutrient response in streams (Lowe, 1974; Lange-Bertalot, 1979; van Dam et al., 1994; Bahls, 1993; Wang et al., 2005; Lavoie et al., 2006; Potapova and Charles, 2007), particularly in low-nutrient settings (Justus et al., 2010). Periphyton responses to minor upticks in nutrient concentration are often subtle, however, and limited to the displacement of sensitive and/or rare periphyton species that can have a relatively narrow distribution or low detection (Porter, 2008).

1.1. Purpose and scope

Nutrient conditions at the Buffalo River were compared across local, regional, and national scales in support of the "least disturbed" (or "reference") stream designation. We evaluated periphyton assemblage metrics for their ability to differentiate between water-quality conditions across control and test sites on the Buffalo River and Big Creek for multiple seasons. Nutrient concentrations and periphyton assemblages at two Buffalo River sites upstream of the confluence with Big Creek (i.e., considered to be minimally disturbed 'control' sites) were compared to that of three Buffalo River sites downstream of the confluence with Big Creek ('test' sites) and one site on Big Creek. We compared the

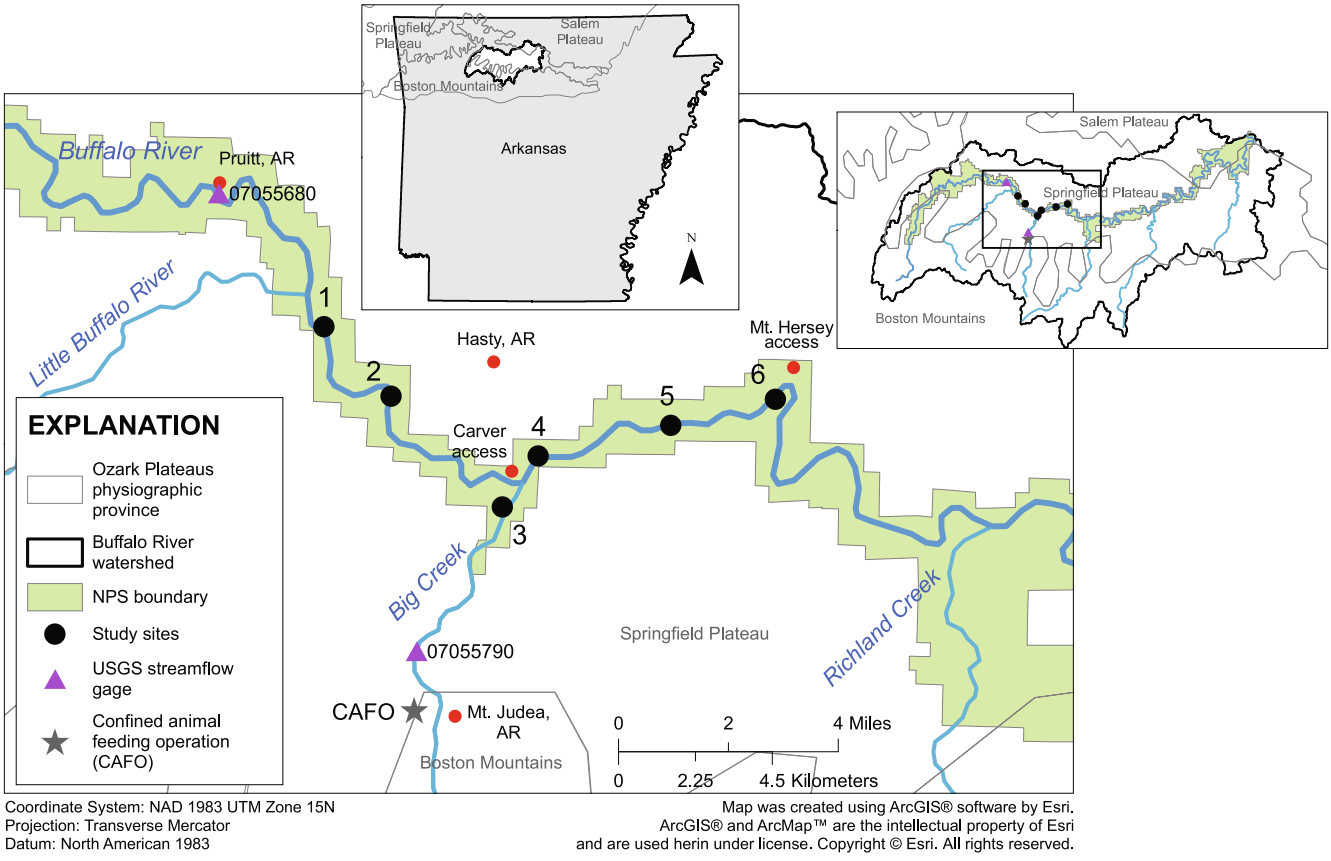

Fig. 1. Map showing the location of the study area and six USGS sampling sites within the Buffalo River watershed, Arkansas [NPS, National Park Service; USGS, U.S. Geological Survey].

degree of variability in nutrient and periphyton response occurring across space to the degree of variability occurring over time. Our hypotheses are that, (1) relative to upstream control sites, nutrient concentrations will be higher at Big Creek and at downstream test sites (Fig. 2a), (2) relative to upstream control sites, periphyton assemblages at sites downstream of Big Creek will exhibit changes in biovolume and trophic responses corresponding with higher nutrient exposure, and (3) the degree of spatial variability observed among upstream versus downstream periphyton assemblages will be related to seasonal changes in hydrology and associated nutrient water quality. Interpretations from this study will advance the science for monitoring reference streams with low-level nutrients and also provide a better understanding of the degree to which nutrient contributions from Big Creek (and the CAFO) are affecting ecological conditions and consequent ecosystem services in the Buffalo River.

1.2. Study area and site locations

The Buffalo River originates in the southern part of the Ozark Plateaus physio-geographic province in the Boston Mountains in north-central Arkansas (Fig. 1; Fenneman and Johnson, 1946) and flows through a scenic landscape in an easterly direction before its confluence with the White River. The study area, which is located approximately in the middle of the Buffalo River watershed, is also contained within the outcrop area of the Springfield Plateau sub-province (Fenneman and Johnson, 1946; Imes, 1990). The Springfield Plateau, one of the nation's largest karst regions (Mott et al., 2000), overlies and is part of a system that has fractured and dissolved over time to form an open network of caves, enlarged fractures, sinkholes, sinking streams, and springs (Aley and Aley, 2000). This open hydrologic network results in extensive interaction between groundwater and surface water, and many gaining and losing reaches of the Buffalo River have been identified (Moix and Galloway, 2003). Relatedly, streamflow in the Buffalo River does not always increase with downstream progression. In multiple locations, including one area downstream of the study area, streamflow is entirely lost during some summer baseflow periods.

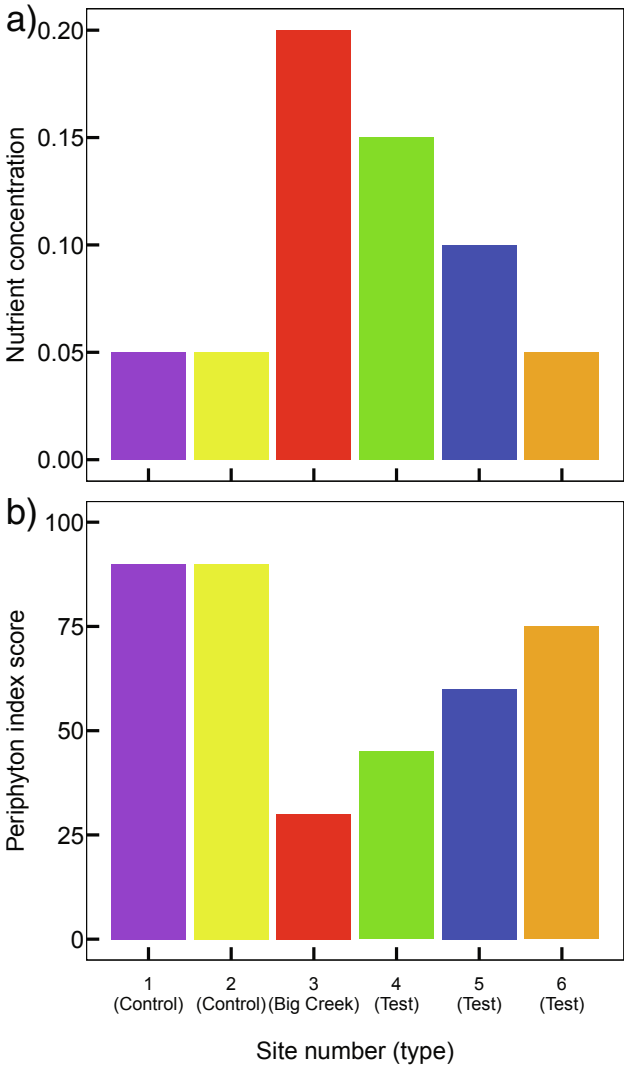

Fig. 2. a and 2b. Hypothetical *a priori* patterns of a) expected nutrient concentrations and b) periphyton index scores at two upstream control sites on the Buffalo River, three test sites on the Buffalo River downstream of the confluence with Big Creek, and a site on Big Creek downstream of a confined animal feeding operation (see Fig. 1).

While 217 km (km) of its 246-km length are contained within the Buffalo National River (BUFF), only 11 percent of the Buffalo River watershed is owned and managed by the NPS (Fig. 1); 29 percent is managed by other state or Federal entities, and 60 percent is private holdings. Approximately 80 percent and 14 percent of the Buffalo River watershed is forested and in pasture, respectively (FTN Associates Ltd, 2017). The NPS-managed corridor within the BUFF is relatively narrow, and small spans of riparian area buffer the river from pasture and associated surface runoff in many locations. Some hayfields located short distances from the river and maintained on BUFF property are fertilized by the private entities who rent them.

Although water-quality risks from the CAFO on Big Creek were concerning, other land- and river-use practices also place the Buffalo River at risk of declining water quality. Stream sections that drain pasture in the region have higher nutrient and bacteria concentrations than sections that drain forests (Mott, 1990; Petersen et al., 1998; Watershed Conservation Resource Center, 2017), and some tributaries to the Buffalo River have had 30 percent or more of their watersheds converted from forest to pasture (Watershed Conservation Resource Center, 2017). Human contact is another likely and growing source of nutrients to the Buffalo River. In 2015, the NPS estimated that more than 1.7 million people visited the BUFF (Thomas and Koontz, 2016), many of whom had direct contact with the river (i.e., through swimming, canoeing, kayaking) or lodged in nearby campgrounds or cabins with onsite wastewater (septic) systems.

The design for this study involved an upstream-downstream sampling strategy to investigate potential spatial variability for nutrient and periphyton samples collected at sites on the Buffalo River upstream of the confluence with Big Creek, downstream of the confluence, and on Big Creek. The CAFO within the Big Creek watershed is approximately 9.0 km upstream from the confluence of Big Creek with the Buffalo River. Biological and water-quality samples were collected at six sampling sites located near the confluence (Fig. 1, Table 1). Sites 1 and 2 were control sites on the Buffalo River that were approximately 3.2 and 6.4 km upstream, respectively, of the confluence. Site 3 was a sampling site on Big Creek approximately 8.1 km downstream of the CAFO and 0.8 km above the confluence with the Buffalo River. Sites 4, 5, and 6, were test sites that were approximately 1.6, 4.8, and 8.0 km (respectively) downstream of the confluence of Big Creek with the Buffalo River (Fig. 1).

2. Materials and methods

2.1. Nutrient and periphyton sampling methods, laboratory procedures, and data access

Because periphyton integrate nutrients over time and antecedent nutrient conditions affect periphyton assemblages, nutrient constituents were sampled more frequently than periphyton. Nutrient samples were collected monthly from May 2017 through February 2019 with two exceptions: (1) sample collection in March 2018 was delayed a month

Table 1
Water-quality station information and median field measurement values for six sites sampled on the Buffalo River and Big Creek from June 2017 to February 2019. [km^2, square kilometer; °C, degrees Celsius; mg/L, milligrams per liter; µS/cm, microseimens per centimeter]

Site number (Fig. 1)	USGS station name	USGS station number	Latitude	Longitude	Water-shed area km^2	Field measurements (median of 19 values)			
						Tempe-rature °C	Dissolved oxygen mg/L	pH	Specific conductance µS/cm
1	Buffalo River upstream of Hasty	07,055,760	36.024702°	93.103147°	940	22.3	9.1	7.7	214
2	Buffalo River near Hasty	07,055,770	36.005921°	93.080935°	984	21.4	9.5	7.8	214
3	Big Creek at Carver	07,055,814	35.978965°	93.043507°	233	18.6	10.3	7.8	240
4	Buffalo River downstream of Big Creek near Hasty	07,055,824	35.989982°	93.031891°	1,259	18.0	9.1	7.8	220
5	Buffalo River upstream of Mount Hersey	07,055,828	35.998975°	92.989674°	1,290	18.0	9.3	7.9	222
6	Buffalo River near Mount Hersey	07,055,832	36.005974°	92.955809°	1,300	18.4	10.4	8.0	219

because of safety concerns related to extreme flooding; and (2) the last sampling event was delayed 2 months because of freezing temperatures in December 2018 that prohibited periphyton sample processing and operational issues in January 2019.

Water-quality samples were collected in runs/glides just upstream of designated riffle habitats. Grab samples were collected at multiple points across a single transect at each site; however, when streamflow velocity exceeded 0.46 m/s, samples were collected using isokinetic flow-integration methods (USGS, 2006). Samples were processed according to U.S. Geological Survey (USGS) protocols (Wilde et al., 2004) and were stored on ice and shipped to the USGS National Water Quality Laboratory (NWQL) in Lakewood, Colorado, for analysis. Samples were analyzed for a suite of 17 nutrient constituents; 13 nitrogen constituents and 4 phosphorus constituents (Supplemental table 1).

Quality assurance (QA) samples (field-blank and replicate samples) were routinely collected to ensure field data and nutrient results were of acceptable quality. Nutrient concentrations in all blank samples were below the laboratory reporting limit (LRL), and concentrations in most replicate samples were within 10 percent of the original (sequential) water sample. Calibrated YSI™ multi-parameter meters were used to measure water temperature, dissolved oxygen, pH, and specific conductance at the time of water-quality sampling. Although streamflow gages were not located at any of the six sampling sites, continuous streamflow and precipitation data were obtained from the Buffalo River at Pruitt, Arkansas streamgage (USGS station no. 07055680; located approximately 8 km upstream of site 1; Fig. 1) and from the Big Creek at Mt. Judea, Arkansas streamgage (USGS station no. 07055790; located approximately 8.2 km upstream of site 3) and used to evaluate how nutrient concentrations were influenced by rainfall and changes in streamflow.

Periphyton samples were collected quarterly (a total of 7 samples) but were synchronized with water-quality sampling times (Fig. 3). Periphyton samples were collected from the same one to two riffles at each sample site by brushing algae from a quantifiable surface area of rock substrate (25 rocks ranging in size from large gravel to small cobble) (Moulton et al., 2002). A short cross section of polyvinyl chloride pipe with an outside diameter of 3.3 cm was placed on each rock; all algae outside of the pipe template was dislodged with a wire brush and rinsed from the rock with stream water. Next, any algae remaining inside the pipe template was dislodged with a wire brush and rinsed into a sample bottle. After the process was repeated for all rocks, the liquid slurry material from the 25 subsamples was composited. The area sampled from each rock was 8.55 cm^2 and the total area sampled at each site was approximately 213.8 cm^2.

Periphyton sample processing involved removing and filtering subaliquots for chlorophyll *a* (Chl *a*) and ash free dry mass (AFDM) analysis and preserving the remaining composite sample for taxonomic analysis. Once the algae slurry was homogenized, 10-mL aliquots were removed and filtered onto 47-mm glass microfiber filters. Filters containing the algal residue were frozen and shipped to the USGS NWQL for Chl *a* and AFDM analysis (Arar and Collins, 1997). The remaining composite slurry sample (typically ~ 150–300 mL) was preserved with a 4- to 5-percent solution of buffered formalin and shipped to Rithron Associates (Missoula, Montana) for algal taxonomic identification. Sample area, as well as volumes for the total sample, individual aliquots, and amount of formalin preservative were recorded on field forms. Sample processing and algal taxonomy for diatom and soft algae components were consistent with Charles et al. (2002).

All biological and chemical data collected in this study are publicly available from the USGS and searchable using the USGS station numbers in Table 1. Water-quality data (including Chl *a* and AFDM data) are available from the USGS National Water Information System database (USGS, 2016), and periphyton taxonomic data are available from the USGS BioData database (USGS, 2017).

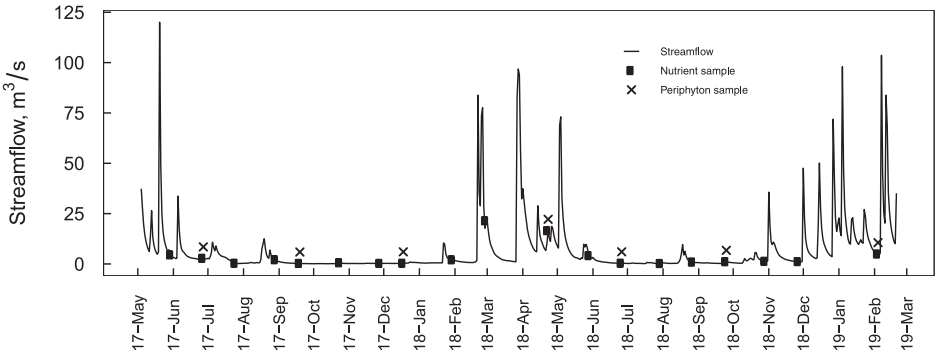

Fig. 3. Streamflow at Buffalo River at Pruitt, Arkansas (USGS station 07055680) in relation to the monthly timing of 19 nutrient water-quality samples (dots) and 7 periphyton samples (x). With exception of three occasions—September 2017, December 2017, and February 2019—samples were collected in the last week of each month (e.g. samples for Jun-17 were collected June 27–28 of 2017).

2.2. Selection of nutrient constituents for data analysis

Because background nutrient levels in the Buffalo River are naturally low, a high percentage of measured concentrations for some nutrient constituents were below LRLs. Therefore, only a subset of nutrient constituents was selected for final analysis. The subset of constituents was selected based on a combination of the following criteria: the number of times the constituent was detected above the LRL (i.e., constituent robustness) (Supplemental table 1), the collinearity of similar constituents, and the number of times the constituent was used in the scientific literature (e.g., NO_3 (as N), NO_3 (as NO_3), and NO_3 plus nitrite (as N) all were detected above the LRL in 80 percent of samples (Supplemental table 1) but only NO_3 (as N) was retained). Of the 17 nutrient constituents measured, two nitrogen and two phosphorus constituents—dissolved nitrate as nitrogen (NO_3 (as N) (NO_3 hereafter)), total ammonia plus organic nitrogen as nitrogen (total Kjeldahl nitrogen, TKN), total phosphorus as phosphorus (TP), and total dissolved phosphorus as phosphorus (TDP)—were retained for final data analysis.

2.3. Spatial and temporal comparisons of nutrient concentrations

Statistical analyses of nutrient data comparing concentrations among upstream control sites to downstream test sites were performed using methods that account for concentrations below the LRL (i.e., "censored" data) (Helsel, 2012). Regression on ordered statistics (ROS) methods were used to estimate the distributions of censored concentrations in relation to concentrations above the LRL (Helsel, 2012). In addition, censored regression analysis of variance (ANOVA; 1-way), which involved estimation of censored data using maximum likelihood estimation (MLE) techniques (Helsel, 2012), was used to statistically compare concentrations among sites and over time. Prior to statistical analysis, nutrient data were log-transformed, and the assumption of log-normal distributions was checked by examining histograms and probability plots of the residuals (Helsel, 2012). For tests of censored regression ANOVAs on data sets that were found to be significant, a series of individual pairwise tests between groups were performed. Significance of overall tests was evaluated at alpha = 0.05, whereas the alpha levels of pairwise tests were adjusted to account for inflation of type I error rates by dividing 0.05 by the number of individual comparisons among groups (i.e., Bonferroni's equation) (Helsel, 2012). ROS methods and censored regression ANOVA were performed using functions within the Nondetects and Data Analysis (NADA) package (Lee, 2017) using R statistical software (R Core Team, 2019).

Bar plots for NO_3, TKN, TP, and TDP were used to compare temporal patterns and variability across different sample months. Bar plots were constructed using nutrient concentrations at or above the LRL for each constituent, and censored values were plotted at the LRL. Boxplots, by contrast, were used to compare the spatial variability of nutrient concentrations among sample sites and were constructed using the distribution of concentration values at or above the LRL as well as the estimated distribution of censored values below the LRL using ROS methods (Helsel, 2012). It is important to note that the identity of individual sample concentrations is lost when censored values are estimated with ROS methods (i.e., estimates of nutrient concentrations cannot be assigned to a specific site or sample date).

2.3.1. Comparisons of nutrient data across the six sites and to previous reference site studies

Nutrient concentrations can vary to large extents across ecoregions, even within small state or regional areas (Morgan et al., 2013) and some comparisons were deemed necessary to verify that sampling sites on the Buffalo were appropriately classified as "least disturbed". Two nutrient indices (local and regional) were calculated using methods described in Minns et al. (1994), Justus et al. (2010), and Justus et al. (2016) and detailed in section 2.5 of this paper. The regional nutrient index consisted of combining TN and TP scores from summer samples at Big Creek and each of the five Buffalo River sites and comparing nutrient conditions to published TN and TP nutrient-index scores from 30 Ozark streams (i.e., a 32-site comparison) across a regional land-use gradient (Justus et al., 2010). The local nutrient index consisted of combining NO_3, TKN, TP, and TDP scores among sites and over time. In addition to providing baseline information for what is implied by "least-disturbed" condition, the process above facilitated a comparison of nutrient conditions between our six sample sites, and also put nutrient conditions of the Buffalo River and Big Creek within a regional context.

2.4. Periphyton metric selection, scoring, and analytical procedures

We calculated a large number of periphyton metrics (Supplemental tables 2a and b) using R-scripts and methodology developed as part of other USGS studies conducted in the southeast and northwest U.S. (Spaulding et al., 2019; USGS, pers. commun.) and assigned expected ecological responses of many metrics in relation to nutrient exposure according to previous literature (Lowe, 1974; Lange-Bertalot, 1979; van Dam et al., 1994; Bahls, 1993; Kociolek and Spaulding, 2003; Wang et al., 2005; Lavoie et al., 2006; Potapova and Charles, 2007; and Porter, 2008). Additionally, common taxa-based metrics (taxa groups occurring in 50 percent or more of samples) were also calculated and considered for inclusion in the periphyton index of biotic integrity (PIBI) (Supplemental table 2b).

Periphyton metric values for each sample were scored (i.e., standardized) using a centering method where scores ranged from 0 to 100 (Minns et al., 1994; Justus et al., 2010; and Justus et al., 2016). For this scoring approach, if a high metric value indicated least-disturbed conditions (e.g., high taxa richness or number of intolerant organisms) the metric value was first divided by the maximum metric value (for 6 sites in this instance), and the quotient was multiplied by 100. If a low metric value indicated least-disturbed conditions (e.g., a low Chl *a* concentration or number of tolerant taxa), the metric value was divided by the maximum metric value, but the resulting quotient was subtracted from 1 before being multiplied by 100. Scores from multiple metrics were then combined into an index by averaging scores across metrics (Minns et al., 1994).

A subset of ecologically relevant periphyton metrics that discriminated between upstream test sites and downstream control sites was identified for final inclusion into the PIBI. Metrics were selected for the PIBI based on three considerations: 1) the robustness (i.e., occurrence in 50 percent or more of samples) of measurable metrics among samples, 2) statistical significance (ANOVA; p-values ≤ 0.1) of metrics between test and control sites, and 3) reduction of redundant metrics. A liberal p-value of 0.1 was used to signify statistical significance due to the relatively small sample sizes and relatively few metrics available for comparing among the two control and three test sites for the seven periphyton sampling dates (i.e., 14 control values compared to 21 test values). When redundant metrics were suspected, we relied on metric relevancy to nutrient enrichment (e.g., increasing biomass, a decrease in organisms intolerant of organic pollution, an increase in organisms tolerant of organic pollution) to determine which metric was retained for further analysis. The subset of retained metrics were then evaluated with bar charts to determine performance relative to their expected response to nutrients (i.e., ecological relevance) (Lowe, 1974; Lange-Bertalot, 1979; van Dam et al., 1994; Bahls, 1993; Lavoie et al., 2006; Potapova and Charles, 2007). Specifically, we evaluated how metric scores at upstream control sites and at site 3 (which had the highest nutrient concentrations) related to each other and to downstream test sites.

PIBI scores were calculated by site on each sampling date and by site for all sampling dates, which involved two steps. First, scores for all retained metrics and for each sampling date were averaged to obtain a PIBI value for each site on each sampling date. Second, PIBI values determined for all seven sampling dates for each site were then averaged to form a mean PIBI value that indicated average overall conditions of

Table 2
a-2d Nitrate, total Kjeldhal nitrogen, total phosphorus, and total dissolved phosphorus concentrations for water samples collected at five sites on the Buffalo River and one site on Big Creek, Arkansas, from May 2017 to Feb 2019.

Site type	Control	Control		Test	Test	Test
Sample date	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
30-05-2017	0.097	0.105	0.131	**0.115**	0.105	0.097
27-06-2017	0.123	0.099	0.14	0.075	0.051	0.044
25-07-2017	0.117	0.071	0.121	0.084	0.052	0.077
29-08-2017	<0.040	<0.040	0.083	**0.039**	<0.040	**0.039**
19-09-2017	0.066	<0.040	0.086	0.073	<0.040	0.05
24-10-2017	0.109	0.120	0.712	**0.195**	**0.183**	**0.185**
28-11-2017	0.059	<0.040	0.094	<0.040	<0.040	**0.103**
18-12-2017	0.076	0.052	0.109	<0.040	<0.040	<0.040
30-01-2018	0.157	0.170	0.183	0.146	0.138	0.12
28-02-2018	0.373	0.390	0.406	0.369	0.371	0.377
23-04-2018	0.047	0.055	0.062	**0.057**	0.049	0.047
29-05-2018	0.070	0.063	0.138	**0.076**	0.06	0.049
26-06-2018	0.102	0.073	0.125	0.091	0.061	0.074
30-07-2018	0.080	<0.040	0.08	<0.040	<0.040	<0.040
27-08-2018	0.046	0.046	0.101	<0.040	<0.040	<0.040
25-09-2018	0.091	0.057	0.275	**0.105**	0.086	0.056
29-10-2018	0.072	0.065	0.281	**0.114**	**0.103**	**0.088**
27-11-2018	0.088	0.095	0.277	**0.162**	**0.134**	**0.134**
04-02-2019	0.222	0.221	0.217	**0.252**	**0.242**	**0.226**
Mean (HLDL)	0.106	0.093	0.191	0.107	0.092	0.096
Mean (ROS)	0.105	0.093	0.189	0.110	0.088	0.095

Site type	Control	Control		Test	Test	Test
Sample date	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
30-05-2017	0.19	0.09	<0.07	0.09	<0.07	<0.07
27-06-2017	<0.07	<0.07	<0.07	**0.11**	**0.10**	<0.07
25-07-2017	0.10	0.09	0.11	**0.14**	**0.12**	**0.12**
29-08-2017	0.09	0.11	0.17	0.11	**0.16**	0.10
19-09-2017	0.12	0.11	0.15	<0.07	0.09	0.09
24-10-2017	<0.07	<0.07	0.15	**0.10**	**0.10**	**0.09**
28-11-2017	0.08	<0.07	<0.07	<0.07	<0.07	0.07
18-12-2017	<0.07	<0.07	<0.07	**0.09**	**0.09**	<0.07
30-01-2018	<0.07	<0.07	<0.07	**0.08**	**0.08**	**0.07**
28-02-2018	0.14	0.14	0.15	**0.17**	0.14	**0.15**
23-04-2018	0.13	0.15	0.11	0.11	0.11	0.13
29-05-2018	0.13	0.12	0.10	**0.17**	0.11	0.08
26-06-2018	0.11	0.09	0.14	**0.12**	0.11	0.10
30-07-2018	0.11	0.11	0.11	0.07	**0.16**	0.10
27-08-2018	0.13	0.11	0.17	0.12	**0.14**	**0.14**
25-09-2018	0.11	0.08	0.14	0.11	0.10	0.08
29-10-2018	0.11	0.08	0.09	0.10	**0.13**	0.08
27-11-2018	<0.07	<0.07	0.09	<0.07	**0.14**	<0.07
04-02-2019	0.10	<0.07	<0.07	0.08	<0.07	0.08
Mean (HLDL)	0.10	0.08	0.10	0.10	0.10	0.09
Mean (ROS)	0.11	0.09	0.11	0.10	0.11	0.09

Site type	Control	Control		Test	Test	Test
Sample date	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
30-05-2017	0.007	0.010	0.009	0.007	0.007	0.006
27-06-2017	0.008	0.007	0.011	0.006	0.007	0.007
25-07-2017	0.009	0.008	0.017	**0.013**	**0.014**	**0.013**
29-08-2017	0.008	0.009	0.027	0.009	0.009	0.008
19-09-2017	0.012	0.015	0.024	0.015	0.015	0.015
24-10-2017	0.009	0.008	0.027	**0.011**	**0.010**	**0.010**
28-11-2017	0.007	0.006	0.010	0.007	0.006	0.006
18-12-2017	0.007	0.007	0.008	0.005	0.004	0.004
30-01-2018	0.007	0.008	0.010	0.007	0.006	0.007
28-02-2018	0.027	0.038	0.047	0.031	**0.040**	0.036
23-04-2018	0.017	0.015	0.012	0.010	0.012	0.013
29-05-2018	0.009	0.009	0.017	0.009	0.009	0.008
26-06-2018	0.008	0.008	0.018	**0.010**	**0.009**	**0.009**
30-07-2018	0.007	0.006	0.016	0.007	**0.008**	0.007
27-08-2018	0.006	0.005	0.015	**0.007**	**0.009**	**0.008**
25-09-2018	0.008	0.006	0.018	0.008	0.008	0.007
29-10-2018	<0.004	0.005	0.013	0.005	0.005	<0.004
27-11-2018	<0.004	0.004	0.010	0.005	0.005	<0.004
04-02-2019	<0.004	<0.004	0.009	<0.004	<0.004	0.004
Mean (HLDL)	0.008	0.009	0.017	0.008	0.009	0.008
Mean (ROS)	0.009	0.009	0.017	0.009	0.010	0.009

Site type	Control	Control		Test	Test	Test
Sample date	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
30-05-2017	<0.003	0.004	0.006	<0.003	<0.003	<0.003
27-06-2017	<0.003	<0.003	0.004	**0.004**	<0.003	<0.003
25-07-2017	0.003	0.004	0.009	0.004	0.003	<0.003
29-08-2017	0.003	<0.003	0.011	<0.003	<0.003	<0.003
19-09-2017	0.004	<0.003	0.009	**0.005**	0.003	0.004
24-10-2017	0.007	0.004	0.022	0.007	0.006	0.004
28-11-2017	0.003	<0.003	0.005	0.003	<0.003	<0.003
18-12-2017	0.005	0.003	0.006	0.004	<0.003	0.004
30-01-2018	<0.003	<0.003	0.004	<0.003	<0.003	<0.003
28-02-2018	0.008	0.010	0.023	0.010	**0.011**	0.010
23-04-2018	0.003	<0.003	0.003	<0.003	<0.003	<0.003
29-05-2018	<0.003	<0.003	0.009	**0.003**	<0.003	<0.003
26-06-2018	0.004	<0.003	0.009	0.004	<0.003	<0.003
30-07-2018	<0.003	<0.003	0.006	<0.003	<0.003	**0.003**
27-08-2018	<0.003	<0.003	0.006	<0.003	<0.003	<0.003
25-09-2018	<0.003	0.003	0.009	<0.003	<0.003	<0.003
29-10-2018	<0.003	0.003	0.010	**0.004**	**0.004**	<0.003
27-11-2018	<0.003	<0.003	0.008	**0.004**	**0.004**	<0.003
04-02-2019	<0.003	<0.003	0.005	<0.003	<0.003	<0.003
Mean (HLDL)	0.003	0.003	0.009	0.003	0.003	0.002
Mean (ROS)	0.003	0.002	0.009	0.004	0.003	0.002

[Mean (HLDL), mean values were calculated using one half of the laboratory detection level; Mean (ROS), mean values were calculated using Regression on Ordered Statistics methods (Helsel 2012)]

the periphyton assemblage for all dates by site. Mean PIBI scores for all sites across all dates (the latter step) were compared to the nutrient index (calculated with mean concentrations for four constituents).

2.5. Temporal and spatial comparisons of nutrient data and biological metrics

Two-way ANOVA (SigmaPlot version 13, Systat Software, Inc. San Jose, California) was used to test for (1) spatial differences in nutrient concentration or periphyton metric values between upstream and downstream site groups, (2) temporal differences among sample date (i.e., seasonal) groupings, and (3) the interaction of spatial and temporal sample groups. A combination of index and multivariate approaches also facilitated comparisons of biological and chemical variability over time and across space. Mean concentrations of NO_3, TKN, TP, and TDP (calculated for the 1 or 2 months prior and the month of each of the seven periphyton samples) were used to perform a cluster analysis to compare the relative spatial and temporal similarity (i.e., distance) of nutrient samples. Non-metric multidimensional (NMDS) scaling ordination was similarly used to evaluate the similarity of periphyton assemblages among sites and seasonal samples in multivariate space. The cluster analysis and NMDS were performed using Primer (version 6) software (Clarke and Warwick, 2001).

3. Results

3.1. Temporal and spatial comparisons of nutrient data

Highest nutrient concentrations were generally measured at site 3 on Big Creek and were often associated with high streamflow. The highest NO_3 concentration of all sites was measured at site 3 in the October 2017 sample (0.712 mg/L, Table 2a), which was collected 2 days after a small, localized rain event occurred in the Big Creek watershed (USGS, 2016). The second highest NO_3 concentration (0.406 mg/L) and the highest TP concentration (0.047 mg/L) were measured at site 3 in late February 2018 (Table 2a and c) when streamflow across all sites was the highest of any sampling event (Fig. 3). Relatively high TP concentrations at site 3 in both the August and October 2017 samples (0.027 mg/L) also likely resulted from localized rain events in the Big Creek watershed (USGS, 2016) prior to sampling. Concentrations of NO_3 at all sites were relatively high in February 2019 when streamflow was relatively low and comparable to streamflow for most other baseflow sampling events (Fig. 3).

Nutrient concentrations measured at the five mainstem sites were typically very low (i.e. near the LRL) and comparable (Tables 2a-d, Fig. 4a-d, also see Supplemental document 1). Concentrations of NO_3, TKN, TP, and TDP at the three downstream test sites on the Buffalo River exceeded those of the two upstream control sites in less than half of the samples collected (Tables 2a-d). Concentrations at the three downstream test sites exceeded concentrations at both upstream control sites most often for TKN (23 of 57) and least for TDP (9 of 57 samples, Tables 2b and 2d). No statistical differences existed for NO_3, TKN, TP, or TDP concentrations between the two upstream control sites compared to concentrations at the three downstream test sites (Fig. 5, Table 3). Compared to the two control sites, least square mean values (Table 3) indicated overall concentrations were only slightly higher (i.e., <0.002 mg/L different) at the three downstream test sites for TKN, TP, and TDP and were only slightly lower (i.e., ~0.002 mg/L different) at test sites for NO_3.

Nutrient concentrations at the six sites varied across sampling months and flow conditions (also see Supplemental document related to gaining and loosing assessment). With the possible exception of some nutrient samples collected at site 3, temporal variation of the nutrient data from the five Buffalo River sites was greater than spatial variation (Table 3, Fig. 6). Concentrations of NO_3, TKN, TP, and TDP at all five Buffalo River sites were statistically different overall when compared among the seven (seasonal) periphyton sampling events (by season p-values in Table 3), and least square means among seasonal samples were variable. Cluster analysis further indicated that nutrient concentrations were often grouped more closely according to seasonal sample dates rather than by stream site (Fig. 6), suggesting seasonal/temporal influences (e.g., hydrology) were greater than spatial influences (e.g., control sites relative to test sites).

3.2. Temporal and spatial comparisons of biological variability

Consistent with water-quality data, the periphyton data exhibited more variability across time than space and patterns exhibited by some periphyton taxa seem to have a strong association with rainfall events. A multidimensional scaling plot comparing the similarity of periphyton assemblage data reveals that, regardless of which of the five Buffalo River sites are compared to each other, periphyton samples collected

on the same dates (and in the same years) had more taxa in common than samples collected at the same sites but on different dates (Fig. 7). As an example of the degree of biological variation exhibited across time – even though some blue-green algae (BGA), particularly *Homoethrix*, were common most of the time – diatoms had the highest relative abundance during baseflow periods that followed high-flow periods when some BGA taxa such as *Calothrix* were susceptible to scouring. Consequently, BGA:diatom ratios were highest after hydrologically stable periods and were much lower for samples collected shortly after storm events (Fig. 8a). Among types of BGA, the genus *Calothrix* was dominant in samples collected in summer and fall baseflow periods (Fig. 8b and 8d), while the genus *Homoeothrix* was relatively stable across six of the seven periphyton sampling events (Fig. 8c).

3.2.1. Periphyton metrics selected for the PIBI

Of the 144 potential periphyton metrics tested for the ability to distinguish differences between test and control sites, statistical differences were detected for only 10 metrics (Table 4). Of those 10 periphyton metrics, four metrics were dropped from consideration for the PIBI because of (1) taxonomic similarity (a high probability of redundancy) with other metrics that were more statistically significant, (2) a direct association between the metric and nutrients was not found in the literature (Table 4), or (3) the metric did not distinguish between (a) the site on Big Creek (which had the highest nutrient concentrations of the six sampling sites) and the remaining five sites and (b) the control sites and test sites (Fig. 9a-f). Regarding reason (3a), an evaluation of individual metric performance revealed that three of the six metrics with $p \leq 0.1$—oligotrophic taxa percent relative abundance, *Homoeothrix* percent relative abundance, and mesotrophic diatoms percent taxa richness—distinguished the site on Big Creek from the remaining five sites (Fig. 9a, 9c, and 9e, respectively). Also, with the exception of BGA biovolume (which was statistically different across sites (p value of $<$ 0.001)), those same three metrics were more statistically significant across sampling sites ($p \leq 0.05$) than the remaining two metrics (Table 4). Further, the metric *Achnanthidium* percent relative abundance did not indicate biological conditions at the control sites and test sites were different (Fig. 9b), and the two metrics BGA biovolume and AFDM, indicated that ecological conditions at site 3 were more favorable than those of the five remaining sites.

Based on the above considerations, the three metrics—oligotrophic taxa percent relative abundance, *Homoeothrix* percent relative abundance, and mesotrophic diatoms percent taxa richness—were selected for the PIBI (Fig. 10). It should also be noted, however, that although they were the best of the large number of metrics that were tested for differentiating water quality between test and control sites, scores for two of the three metrics—oligotrophic taxa percent relative abundance and *Homoeothrix* percent relative abundance—were skewed high (i.e., extended over a limited but high range).

A comparison of the PIBI and the nutrient index suggests that a weak association exists between the periphyton assemblage and low-level nutrient exposure (Fig. 10). For five of seven periphyton sampling events, when nutrients were highest (nutrient index scores were lowest), the PIBI had an associated, albeit subtle, negative response.

4. Discussion

4.1. Influence of a priori expectations on the study design

The study design was based on the assumption that, regardless of the level and timing of nutrient exposure (i.e., sporadically high TP runoff in storms versus stable, baseflow NO_3 exposure), periphyton metrics would indicate differences in nutrient availability and the associated degree of assimilation and attenuation by the periphyton assemblage. For example, a decrease in overall algal biovolume in progression from sites 4 to 6 would indicate a higher degree of nutrient assimilation was occurring at sites nearest the Big Creek and Buffalo River confluence, such as would be expected during a baseflow assimilation scenario. Conversely, an increase in overall algal biovolume in progression from sites 4 to 6 might indicate nutrients were made available by storm runoff (i.e., when high turbidity inhibited sunlight and photosynthesis or when sediment-bound phosphorus inhibited assimilation at sites nearest to Big Creek).

Although we expected nutrient concentrations to be lower at the two upstream control sites compared to the site on Big Creek and the three downstream test sites (Fig. 2a), we also anticipated that increases in assimilation capacity of periphyton at downstream test sites could diminish downstream nutrient concentrations, which would result in weak differences in both nutrient concentrations and periphyton metrics across control and test sites. Because nutrient concentrations for this study were lower than those of most previous *in situ* studies, we also anticipated that metrics pertaining to periphyton taxa or taxa groups capable of tolerating high nutrient concentrations would not be useful. Related to biological index scores, we anticipated that scores for a PIBI would have a negative association to nutrient concentrations and to the nutrient assimilating capacity of periphyton assemblages at different sites and across sampling dates (Fig. 2b).

4.2. Temporal and spatial variability of nutrient data

The degree of temporal variability observed in nutrient concentrations in this study reflects the degree that water quality of the six sampling sites fluctuated in response to changes in hydrology and to differing land-use practices and intensity in the Big Creek and Buffalo River watersheds. Slightly higher TP concentrations in the spring/summer (April – September) compared to fall/winter (October – February) timeframes are likely associated with hydrology (increases in concentration associated with spring rains) and land use (i.e., fertilizer application or spreading of manure prior to or early in the growing season). However, the degree of seasonal fluctuations in TP concentrations at all six sites seemed to be in direct association with storms and periods of surface runoff near the time of sampling.

Concentrations of NO_3 also fluctuated in response to hydrologic variability to some degree; however, some samples with relatively high NO_3 concentrations were collected when streamflow was relatively low. Rather than having a strong association to hydrology, land use (e.g., fertilizer applications), and surface runoff, high NO_3 concentrations in samples collected in February 2019 (Fig. 4a), for instance, likely resulted from low primary production during the nongrowing season (and associated reduced nutrient-assimilation and consequential attenuation capacity) and contribution of high NO_3 concentrations from groundwater in this region. During stable baseflow periods, flow is composed predominantly of groundwater, which in this study area, frequently has a higher NO_3 concentration than surface runoff (Kresse et al., 2014).

Higher nutrient concentrations and loads at site 3 relative to the five Buffalo River sites reflect the more intense land use (in terms of pasture and CAFO activities) in the Big Creek watershed than in the remaining part of the Buffalo River watershed. Even so, nutrient concentrations at the three sites on the Buffalo River downstream of the Big Creek confluence were low and comparable to those of the two upstream control sites for most sampling occasions (Figs. 4 and 5), thus, substantial spatial variability between test and control sites was not evident.

4.3. Periphyton response

Before selecting biological metrics for biological indices, it is important that ecological relevance of the metrics be considered. Oligotrophic taxa are adapted to nutrient-poor aquatic environments (van Dam et al., 1994) and would be expected to be more successful in the presence of very low nutrient concentrations relative to higher nutrient concentrations. Mesotrophic diatoms (van Dam et al., 1994) and the species within the genus *Homoeothrix* (Potapova, 2005) would

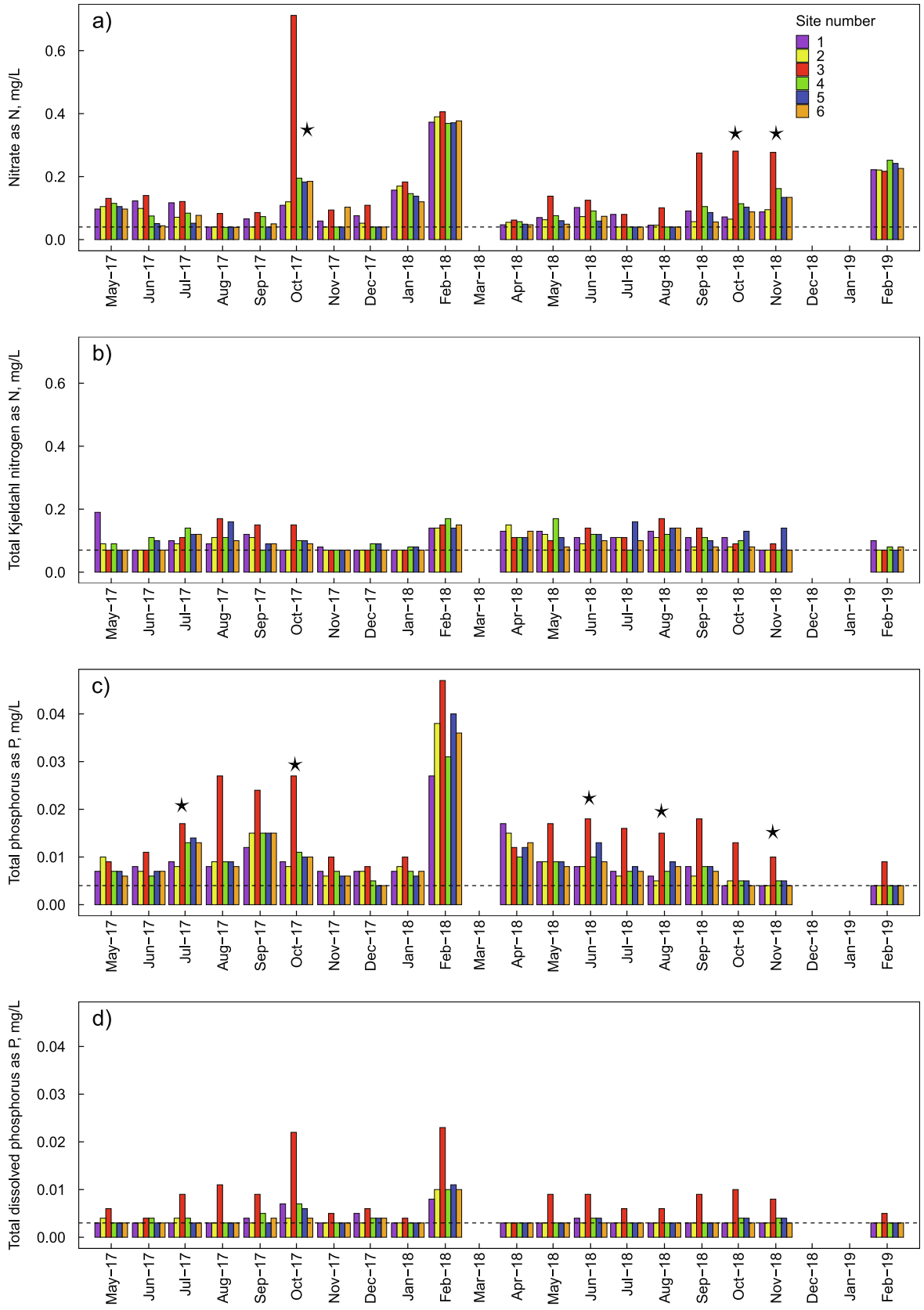

Fig. 4. a-d Concentrations of nitrate as nitrogen (NO_3) (4a), total Kjeldahl nitrogen as N (TKN) (4b), total phosphorus as phosphorus (TP) (4c), and total dissolved phosphorus as phosphorus (TDP) (4d), measured at each study site. Dashed lines represent the laboratory reporting limit for concentrations of each constituent. Stars denote sampling events when patterns of nutrient concentrations generally met *a priori* expectations (see Fig. 2a) for a) NO_3 and c) TP.

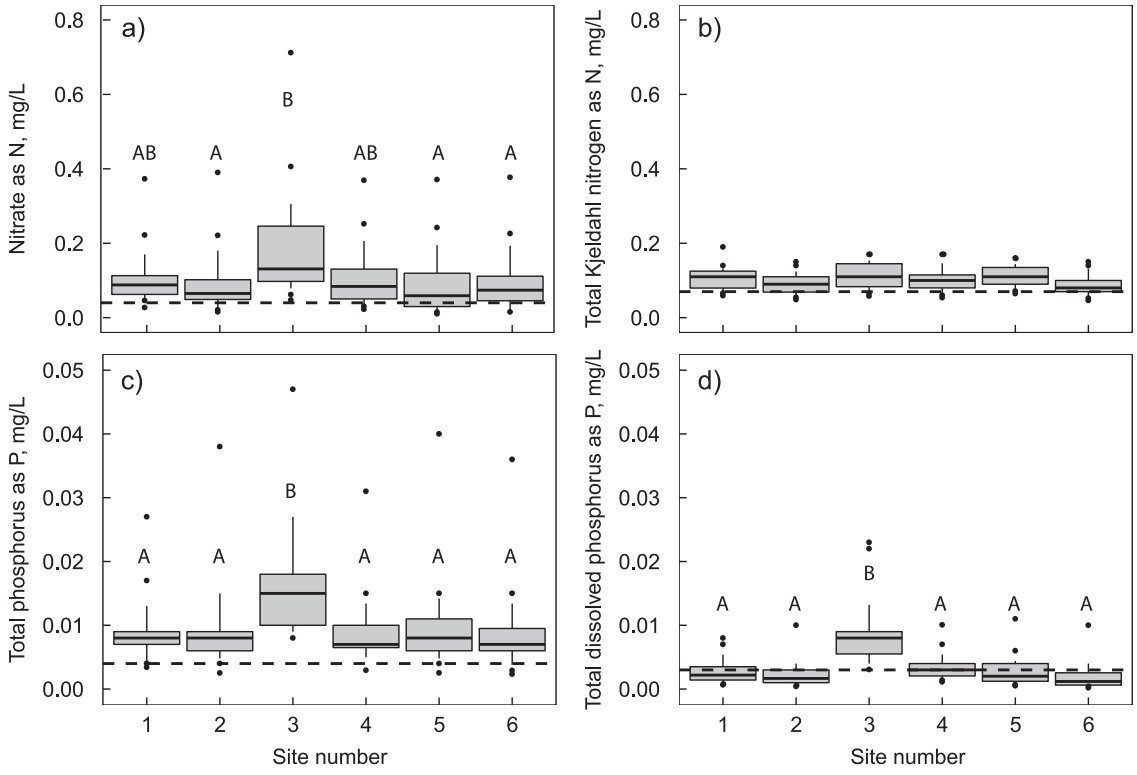

Fig. 5. a-d Boxplots of nitrate as nitrogen (NO_3) (5a), total Kjeldahl nitrogen as N (TKN) (5b), total phosphorus as phosphorus (TP) (5c), and total dissolved phosphorus as phosphorus (TDP) (5d), at each study site across 19 monthly samples. Dashed lines represent the laboratory reporting limit (LRL) for concentrations of each constituent. Point values depicted below the LRL were estimated using regression on ordered statistics (ROS) methods (Helsel, 2012). Letter notation indicates pairwise statistical relations between sites (ANOVA and Bonferroni adjusted pairwise tests). Groups that do not share a letter were statistically different.

Table 3
Least square means (LSM; reported as mg/L) and associated p-values determined with 2-way analysis of variance comparing (1) nutrient constituent results measured at control and test sites and (2) mean concentrations compiled by season according to periphyton sample dates. For example, LSMs for Sep-2017 were computed from nutrient data collected in the two months preceding and including the September periphyton sample (Jul-Sep 2017). [TKN, total kjeldahl nitrogen; TP, total phosphorus; TDP, total dissolved phosphorus].

	LSM by Site		LSM by Season							p-values		
Nutrient	Control	Test	17-Jun	17-Sep	17-Dec	18-Apr	18-Jun	18-Sep	19-Feb	By site	By Season	Site X Season
Nitrate	0.0977	0.0956	0.094	0.049	0.079	0.192	0.073	0.045	0.144	0.893	<0.001	0.964
TKN	0.0955	0.0972	0.097	0.099	0.068	0.101	0.122	0.112	0.076	0.838	0.007	0.131
TP	0.0082	0.0085	0.005	0.011	0.006	0.018	0.009	0.006	0.003	0.786	<0.001	0.988
TDP	0.0027	0.0028	0.002	0.003	0.004	0.004	0.002	0.002	0.002	0.779	0.009	0.991

be expected to have a positive response to moderately high nutrient concentrations. Because oligotrophic taxa percent relative abundance and *Homoeothrix* percent relative abundance were skewed high, we can assume that the two metrics may not reliably differentiate between ecological conditions at the six sites on all sampling occasions.

Although comparisons of these three periphyton metrics between upstream control sites and downstream test sites indicate slight spatial differences, spatial variability of periphyton metrics was much less pronounced than temporal variability. Across all sites, successive seasonal periphyton samples were more taxonomically similar than samples collected across multiple seasons, and periphyton samples collected in the same years were more taxonomically similar than samples collected in different years (Fig. 7). Thus, both biological data and nutrient data indicated substantial temporal variability while spatial variability of both data sets was negligible.

When periphyton data from this study are considered in conjunction with nutrient data and hydrology, it is apparent that the negative consequences of antecedent high flows and associated scouring on some algae exceeded the potential positive effects that low-level nutrient enrichment had on algal productivity. BGA, for instance, are capable of fixing nitrogen, particularly the genus *Calothrix,* which has been positively associated with low levels of nutrients (Renuka et al., 2013; Douterelo et al., 2004) and are well adapted to highly variable concentrations of ambient phosphorus (Perona and Mateo, 2006). On a more local scale, Petersen and Femmer (2003) suggested a positive association between *Calothrix* and agricultural land use when investigating periphyton communities in Ozarks streams. *Calothrix* was common in our study, and on some sampling occasions, composed most of the biovolume for all diatom and BGA taxa (Fig. 8b). Although nutrient concentrations detected in samples in the months preceding (and including) the April 2018 periphyton sample were higher than most other times, *Calothrix* biovolume was extremely low in that sample. Further, a comparison of *Calothrix* biovolume to streamflow for the gage at the Buffalo River at Pruitt, Arkansas, (approximately 8 km upstream of site 1) (Fig. 8b, and 8d) seems to indicate a negative association between *Calothrix* and high streamflow. In terms of algal productivity, it

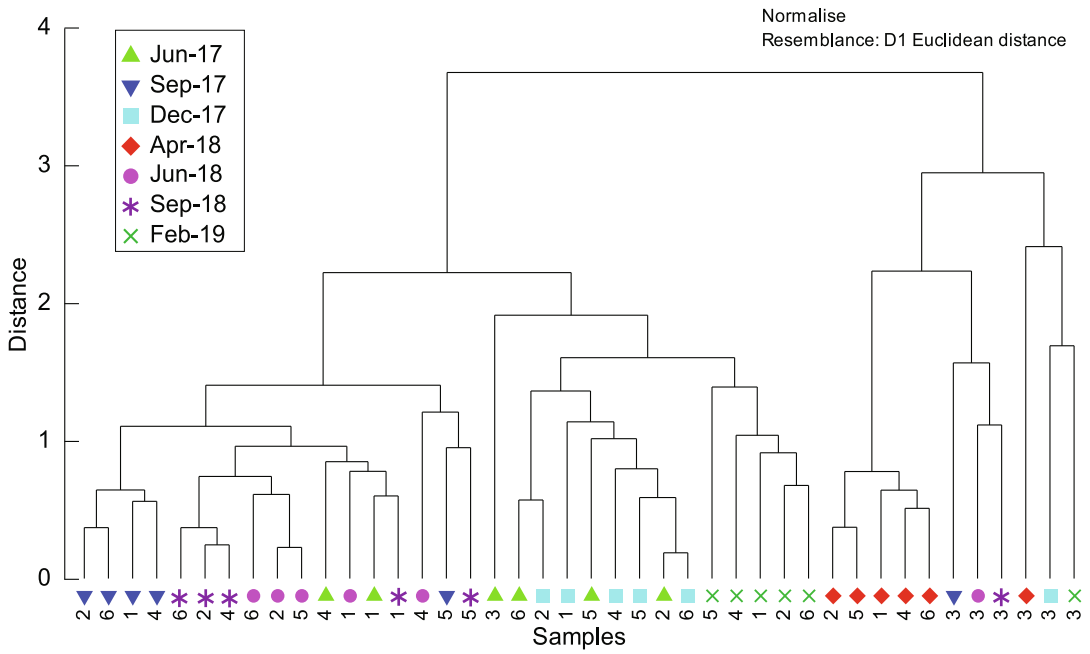

Fig. 6. Cluster analysis showing Euclidean distances among combined mean concentrations of nitrate as nitrogen (NO_3), total Kjeldahl nitrogen as N (TKN), total phosphorus as phosphorus (TP), and total dissolved phosphorus as phosphorus (TDP) for seven periphyton sampling dates at six sites.

seems that the negative consequences of scouring on *Calothrix* exceeded any stimulation capacity resulting from high nutrient concentrations and loads associated with storms that occurred short periods prior to periphyton sampling (Fig. 8d).

The BGA with the second highest biovolume in our samples, *Homoeothrix*, was more abundant than *Calothrix* in samples that were collected after large flood events, indicating that *Homoeothrix* may be less susceptible to scouring than *Calothrix*. However, Potapova (2005) reported that *Homoeothrix* has a low optima for TP but has a moderately high optima for NO_3 and TN and suggested the preference for moderate to high nitrogen content by species of *Homoeothrix* might be due partly to the absence of heterocytes and its inability to fix free nitrogen. Bar charts comparing BGA:diatom ratios to *Calothrix* and *Homoeothrix* percent relative abundance (Fig. 8a-8c) suggest that diatoms and *Homoeothrix* seem to flourish when *Calothrix* percent relative abundance was low. *Homoeothrix* was more common at site 3 (which had statistically higher nutrient concentrations than the five sites on the Buffalo River) relative to the control sites, and in four of seven events, *Homoeothrix* relative abundance was lower at both control sites relative to all three test sites.

The PIBI (Table 5, Fig. 10) performed much like the *a priori* expectation for the periphyton assemblage among sample sites (Fig. 2b). More specifically, the index indicated that biological integrity at the two control sites was generally higher than the three test sites and that the biological integrity at site 3 was least favorable of all sites. However, it should be noted that the PIBI performed differently than our *a priori* site by site expectation (Fig. 2b) in that, (1) the biological integrity at control site 2 was comparable and slightly lower than biological conditions at test site 5, and (2) the biological integrity at test site 6 did not improve relative to test site 5. Hence, it is not possible to make inferences regarding nutrient transport and assimilation processes (if a higher degree of nutrient assimilation was occurring at sites nearest the Big Creek and Buffalo River confluence, or the degree that periphyton responded to nutrients in storm runoff) or if ecological conditions were improving at sites with distance downstream.

A comparison of the PIBI to a nutrient index developed with mean NO_3, TKN, TP, and TDP concentrations indicated that for five of seven periphyton sampling events, when mean concentrations for the four nutrient constituents were highest (nutrient index scores were lowest), the PIBI had a similar negative response (Fig. 8). The most notable exception occurred for the September 2017 comparison, when we observed the most favorable (highest) periphyton condition and when nutrient index scores were second lowest (least favorable). Stable hydrologic conditions prior to the September 2017 sampling effort seemed to give *Calothrix* a competitive advantage (see Fig. 8b and 9d) resulting in *Homoeothrix* percent relative abundance being lowest in the September 2017 sample compared to all other sampling dates (Fig. 8c). Because *Homoeothrix* percent relative abundance was one of only three metrics in the PIBI, low values for this metric had a negative effect on the PIBI for the September 2017 sample. Consequently, we are reminded that biological indices with a restricted and small number of metrics are more susceptible to variation or noise in the data set than biological indices developed with larger numbers of metrics.

Although nutrient concentrations were not different across control and test sites, differences in periphyton biovolume and relative abundance measured at the three test sites downstream of Big Creek compared to upstream controls sites suggest that ecological conditions at the three test sites downstream changed in response to nutrient assimilation by some periphyton taxa. Consequently, our first hypothesis— relative to upstream control sites, nutrient concentrations will be higher at Big Creek and at test sites downstream (Fig. 2a)— was only partially true. Nutrient concentrations were higher in Big Creek, however, concentrations at sites downstream were generally not higher than concentrations at control sites. Our second hypothesis—periphyton assemblages at sites downstream of Big Creek will exhibit changes in biovolume and trophic responses corresponding with higher nutrient exposure (Fig. 2b)—seems to have been supported. With regards to our third hypothesis—the degree of spatial variability observed among upstream versus downstream periphyton assemblages will change over time related to seasonal changes in hydrology and associated nutrient water quality—our multivariate analysis indicated that nutrient concentrations and periphyton assemblages did vary over time, likely in relation to seasonal changes in hydrology and associated nutrient water quality. Our data did not indicate, however, that the relations between periphyton assemblages at upstream control sites and downstream test sites demonstrated different degrees of similarity over time, thus our third hypothesis was not supported.

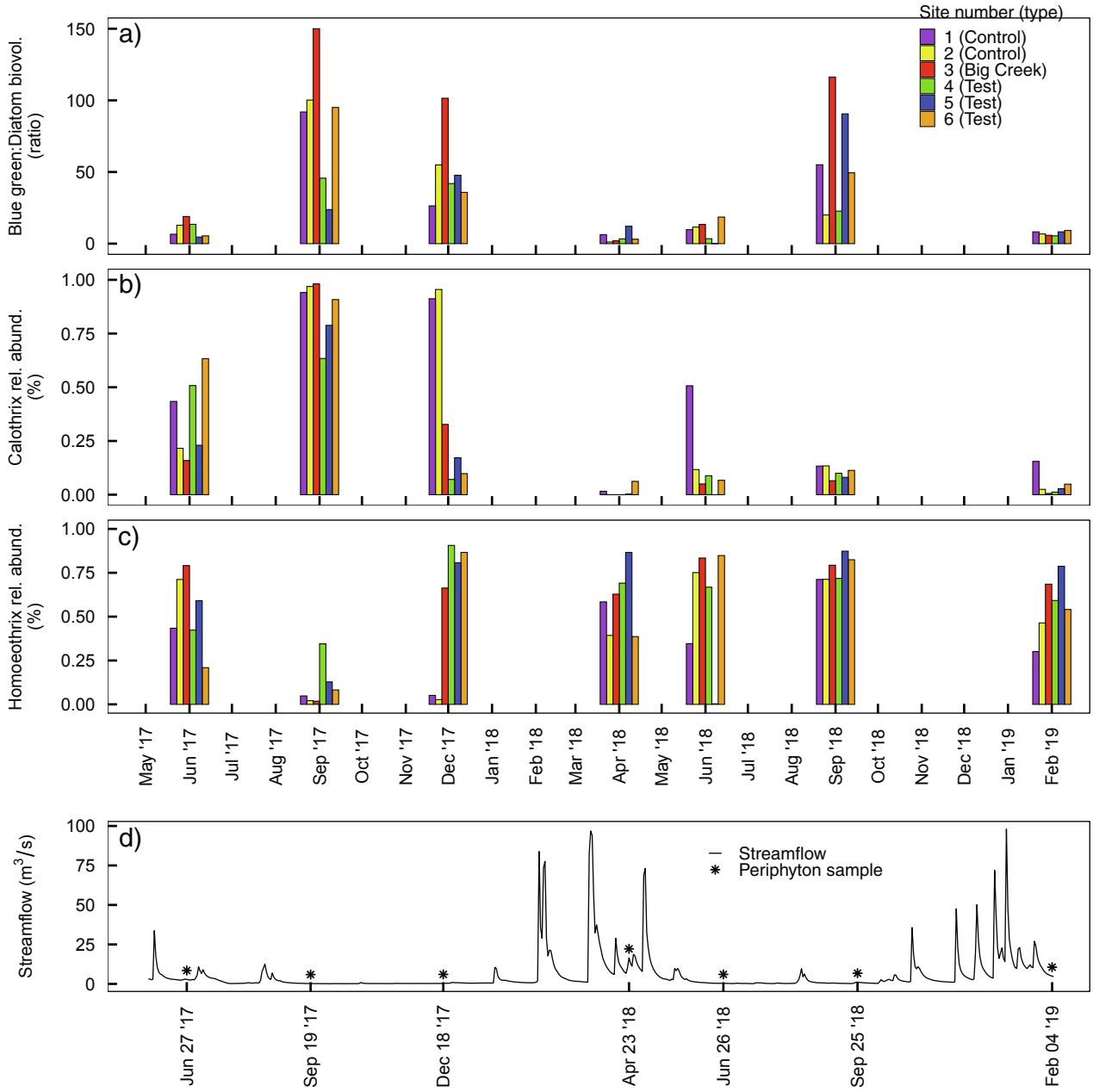

Fig. 7. a-d. Periphyton metric scores for BGA:diatom ratios (8a), *Calothrix* relative abundance (8b), and *Homoeothrix* relative abundance (8c) demonstrating patterns among sites and the seven seasonal periphyton samples in relation to streamflow (8d) at Buffalo River at Pruitt, Arkansas (USGS station no. 07055680). Asterisks on 8d indicate the specific date of periphyton sampling and are approximately aligned with the discrete monthly temporal scale in 8a-8c.

4.4. Implications and considerations

Statistical variability and resolution can be less than ideal in least-disturbed systems with naturally low levels of background nutrient concentrations near laboratory detection limits. To appropriately account for temporal variability in least-disturbed systems, it is important that water-quality data be collected at a higher frequency and that biological and chemical data are collected for longer periods than studies designed for sites across a large range of nutrient gradients. Previous studies by Soulsby et al., (2001), Lavoie et al. (2008), Reisinger et al. (2016), and Cook et al. (2018) have highlighted the importance of comparing biological and chemical data across extended temporal scales. The relatively subtle differences that we observed in ecological conditions across seasons likely could not have been recognized with less frequent (annual or semiannual) biological sampling.

Our results emphasize the degree of influence that hydrology can have on biological assessments conducted using periphyton. While general observations suggest that hydrology for our study period was not grossly different from recent periods of the same duration, it is possible that if periphyton and nutrient data had been collected under more stable hydrological conditions, the magnitude of biological responses to nutrient exposure would have been greater.

Sampling other biological assemblages, particularly macroinvertebrates and fish, could have expanded our ability to explain subtle fluctuations in periphyton and nutrient data. More specifically, quantifying grazing intensity by herbivorous (aquatic) macroinvertebrates and vertebrates might have facilitated detection of changes in the periphyton assemblage that were not apparent using periphyton as a sole indicator (Power, 1992; Rosemond et al., 1993).

Because autecology for some North American algal species

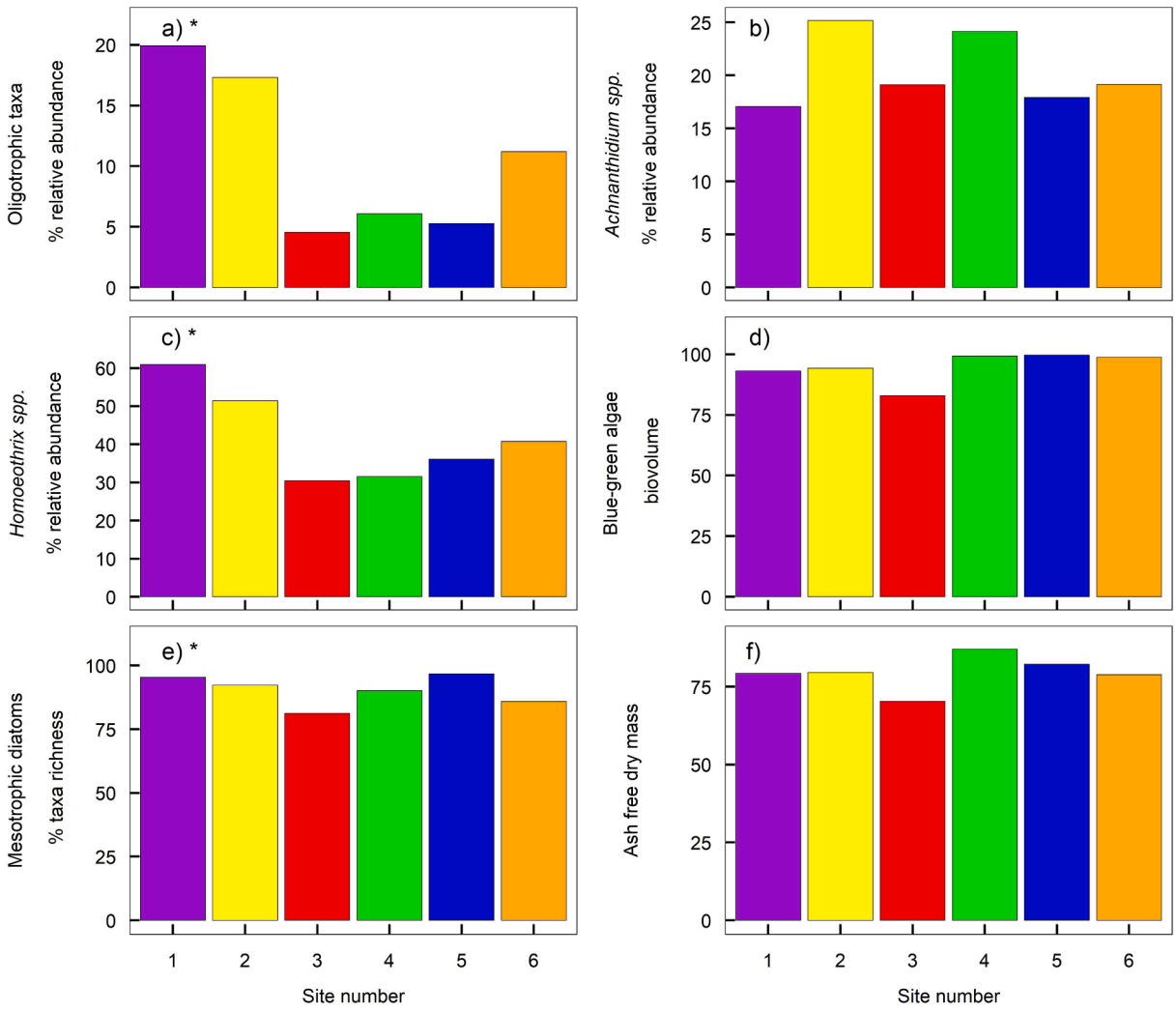

Fig. 8. a-f. Metric scores of six statistically significant periphyton test metrics among sites: oligotrophic taxa percent relative abundance (9a), *Achnanthidium* percent relative abundance (9b), *Homoeothrix* percent relative abundance (9c), BGA biovolume (9d), Mesotrophic diatoms (9e), and AFDM (9f). Periphyton metric scores were calculated and centered such that they have a negative association to nutrient enrichment, where higher metric scores indicate more favorable ecological conditions. The three metrics retained for use in the periphyton index of biotic integrity (PIBI) are indicated with an asterisk (*).

(particularly soft algae) is poorly understood, our knowledge for how some periphyton genera and many species respond to nutrients remains relatively poor (Porter, 2008). Likely because some species within rather large genera (e.g., *Achnanthidium, Nitzschia, Navicula*) have different nutrient responses, there seem to be instances of conflicting nutrient tolerance at the genus level. Identification to the species level would benefit low-level nutrient studies where subtle biological responses are anticipated.

Nutrient data collected in this study suggest that least-disturbed streams that have very low nutrient concentrations may also have an associated high nutrient demand, and thus a relatively strong ability to assimilate small increases in nutrient concentrations. The indication that reference quality streams have a relatively strong ability to assimilate nutrients seems to be supported by findings of Greenwood and Rosemond (2005) who suggested that chronic nutrient enrichment at moderate concentrations may have little effect on benthic algal composition or periphyton biovolume, albeit in headwater streams. However, contrary to our findings and perhaps those of Greenwood and Rosemond (2005), Lavoie et al. (2008) found that diatom communities in oligotrophic streams were directly altered by increasing nutrients, while diatom communities of eutrophic rivers were less sensitive to increasing nutrients. Some of the disparity regarding the extent of biological response for selected periphyton taxa to low-level nutrient conditions may be attributed to differences in opinion for the general nutrient setting (and associated concentrations) that constitute reference conditions in different geographic areas, timing of nutrient exposure, extent of hydrologic variability, and the degree that nutrient concentrations fluctuate in one study area versus another.

Although the association between increasing phosphorus concentrations and loads with stormflow runoff is well documented (Hopmans et al., 1987; Kim et al., 2006; Ramos et al., 2015), we found little literature regarding the ability of periphyton assemblages to use phosphorus suspended during turbid stormflow in least-disturbed (or other) streams. When storms occur in this study area, turbidity can range from near 0 to greater than 1,000 formazin turbidity units (USGS, 2016). In other words, the two streams can range from near 100 percent clarity to highly turbid water where sunlight is prevented from reaching benthic periphyton. The limited amount of work conducted on the effects of light limitation on nutrient processing by algal communities suggests that nutrient retention by algae in turbid conditions could be much reduced over ambient light conditions if it occurs at all (Hill et al., 1995; Mosisch et al., 2001; Pratt et al., 2014; Howard-Parker et al., 2020). Extreme turbidity prevents nutrient assimilation by benthic and suspended algae, and so, we would expect that high phosphorus concentrations encountered under stormflow conditions (relative to baseflow conditions) would have little influence on periphyton richness and diversity

Table 4
Two-way ANOVA results indicating the association of periphyton metrics to nutrients at test and control sites relative to the expected nutrient association in the literature.

	Expected nutrient association (reference)	Hypothesis	Control LSM	Test LSM	Hypothesis supported?	p-values By site	By Season	Site X Season
Percent relative abundance metrics								
Oligotrophic taxa[1]	**Negative (van Dam et al. 1994)**	**Control > Test**	**0.040**	**0.017**	**Yes**	**<0.001**	**<0.001**	**<0.001**
***Homoeothrix* sp.**[1]	**Positive (Potapova 2005)**	**Control < Test**	**0.397**	**0.579**	**Yes**	**0.015**	**0.004**	**0.028**
***Achnanthidium* sp.**	**Negative (Ponader and Potapova 2007)**	**Control < Test**	**0.760**	**0.800**	**Yes**	**0.100**	**<0.001**	**0.199**
Percent taxa richness metrics								
Mesotrophic diatoms[1]	**Positive (van Dam et al. 1994)**	**Control < Test**	**0.05**	**0.09**	**Yes**	**0.037**	**0.412**	**0.666**
Richness metrics								
Mesotrophic diatoms[2]	Positive (van Dam et al. 1994)	Control < Test	0.64	1.10	Yes	0.073	0.099	0.536
Biovolume metrics								
Total biovolume[3]	Positive (Various)	Control < Test	1.13E + 10	1.40E + 09	No	<0.001	<0.001	<0.001
Blue-green algae biovolume[3]	**Positive (Petersen and Femmer 2003)**	**Control < Test**	**4.81E + 03**	**911**	**No**	**<0.001**	**<0.001**	**<0.001**
Calothrix[2]	Positive (Petersen and Femmer 2003)	Control < Test	1.11E + 10	1.05E + 09	No	<0.001	<0.001	<0.001
Bluegreen algae - *Calothrix*[4]	Positive (None)	Control < Test	1.61E + 08	3.46E + 08	Yes	0.006	<0.001	<0.001
Algal production metrics								
Ash free dry mass (grams per square meter)	**Positive (Various)**	**Control < Test**	**32**	**27**	**No**	**0.053**	**<0.001**	**0.645**

The six metrics in bold font were statistically different ($p \leq 0.1$) between control and test sites. The probability of metric values being different across sites (by site) are also compared to the probability of metric values being different across the seven dates (by season) when periphyton were sampled, and by site and date (site X season). [LSM, least square means; <, less than; the six metrics in bold were selected for further screening for a periphyton index]

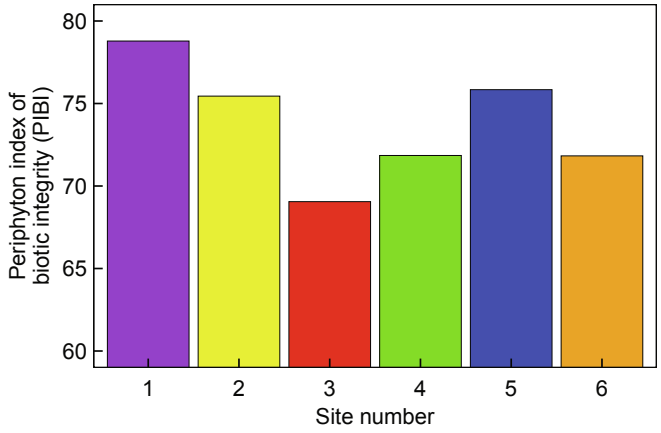

Fig. 9. a-d. Periphyton index of biological integrity (PIBI) scores for each site averaged across the seven seasonal periphyton samples. PIBI scores are a composite of three periphyton metrics—Oligotrophic taxa percent relative abundance, *Homoeothrix* percent relative abundance, and Mesotrophic diatoms percent taxa richness. Higher index scores indicate more favorable ecological conditions.

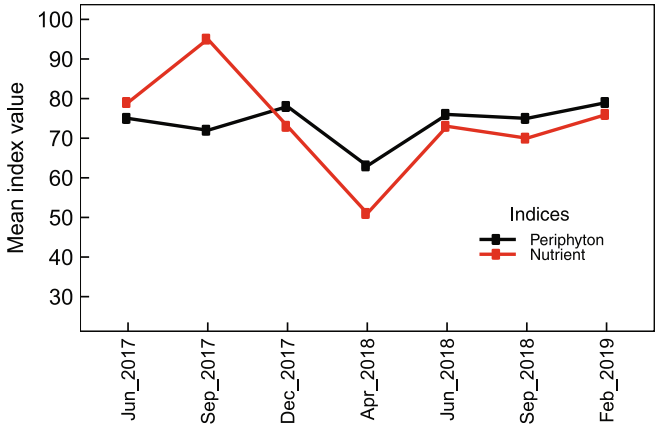

Fig. 10. Comparison of mean periphyton index of biological integrity scores to nutrient index scores (calculated with mean concentrations for four nutrient constituents) across seven seasonal sampling events.

measures over short, downstream distances. Based on the above considerations, our sampling design was based on the premise that biological response to baseflow nutrient concentrations would be stronger than response to stormflow concentrations. Studies are needed, however, that more thoroughly investigate the effects of storm runoff on primary production over time and distance, especially as it relates to least-disturbed streams that are nutrient limited and may have a substantial ability to assimilate and attenuate nutrients.

As land-use intensity increases in response to a growing population, ecological degradation of aquatic environments with the most least-disturbed conditions can be anticipated. Because the chronic degradation of least-disturbed streams can be extremely challenging to measure, biological monitoring techniques capable of documenting subtle biological changes over extended periods will be needed. Unfortunately, current monitoring resources are focused on waterbodies where water-quality and ecological conditions can be improved the most. The findings from our study, however, can help guide water-resource

Table 5
Metric scores for seven periphyton samples and resulting periphyton index of biological integrity (PIBI) scores at 5 sites located on the Buffalo River and one site on Big Creek Arkansas. Metric scores were determined by a centering approach (see Minns et al., 1994). PIBI scores by site and each date are an average of the scores for the three metrics selected for the index. Mean PIBI scores by site for all dates are an average of all PIBI scores at each site and for all seven periphyton sampling dates [%, percent].

		Index metics							
Site number (type; fig. 1)	Collection Date	Oligotrophic taxa		Mesotrophic diatoms		Homoeothrix		PIBI scores by site	Mean PIBI scores by site for all dates
		Relative abundance (%)	Metric score	Taxa richness (%)	Metric score	Relative abundance (%)	Metric score		
1 (Control)	2017-06-27	0.0000	100	0.0100	94	0.4338	52	82.05	78.78
2 (Control)	2017-06-27	0.0000	100	0.0033	98	0.7122	21	73.14	75.45
3	2017-06-27	0.0000	100	0.0184	89	0.7907	13	67.26	69.05
4 (Test)	2017-06-28	0.0017	99	0.0100	94	0.4231	53	82.19	71.85
5 (Test)	2017-06-28	0.0000	100	0.0017	99	0.5913	35	77.91	75.84
6 (Test)	2017-06-28	0.0083	96	0.0067	96	0.2090	77	89.72	71.83
1 (Control)	2017-09-19	0.0050	98	0.0000	100	0.0478	95	97.48	
2 (Control)	2017-09-19	0.0000	100	0.0034	98	0.0212	98	98.55	
3	2017-09-19	0.0000	100	0.0033	98	0.0180	98	98.68	
4 (Test)	2017-09-20	0.0000	100	0.0000	100	0.3450	62	87.31	
5 (Test)	2017-09-20	0.0034	98	0.0017	99	0.1279	86	94.45	
6 (Test)	2017-09-20	0.0054	98	0.0036	98	0.0816	91	95.46	
1 (Control)	2017-12-18	0.0641	71	0.0000	100	0.0512	94	88.35	
2 (Control)	2017-12-18	0.0399	82	0.0000	100	0.0267	97	92.94	
3	2017-12-18	0.0400	82	0.0017	99	0.6626	27	69.20	
4 (Test)	2017-12-19	0.0200	91	0.0000	100	0.9059	0	63.61	
5 (Test)	2017-12-19	0.0224	90	0.0052	97	0.8066	11	65.88	
6 (Test)	2017-12-20	0.0608	72	0.0018	99	0.8663	4	58.50	
1 (Control)	2018-04-23	0.1944	11	0.0000	100	0.5841	36	48.88	
2 (Control)	2018-04-23	0.2187	0	0.0781	54	0.3934	57	36.72	
3	2018-04-23	0.0297	86	0.1683	0	0.6277	31	39.05	
4 (Test)	2018-04-24	0.0606	72	0.0379	77	0.6906	24	57.85	
5 (Test)	2018-04-24	0.0476	78	0.0079	95	0.8656	4	59.32	
6 (Test)	2018-04-24	0.0575	74	0.0690	59	0.3860	57	63.38	
1 (Control)	2018-06-26	0.0417	81	0.0000	100	0.3457	62	80.93	
2 (Control)	2018-06-26	0.0000	100	0.0000	100	0.7514	17	72.35	
3	2018-06-26	0.0000	100	0.0200	88	0.8338	8	65.36	
4 (Test)	2018-06-27	0.0000	100	0.0357	79	0.6680	26	68.35	
5 (Test)	2018-06-27	0.0000	100	0.0000	100	0.0026	100	99.90	
6 (Test)	2018-06-27	0.0396	82	0.0693	59	0.8482	6	49.03	
1 (Control)	2018-09-25	0.0000	100	0.0130	92	0.7120	21	71.23	
2 (Control)	2018-09-25	0.0000	100	0.0000	100	0.7132	21	73.76	
3	2018-09-25	0.0000	100	0.0043	97	0.7929	12	69.97	
4 (Test)	2018-09-26	0.0000	100	0.0057	97	0.7177	21	72.46	
5 (Test)	2018-09-26	0.0073	97	0.0146	91	0.8735	4	63.86	
6 (Test)	2018-09-26	0.0000	100	0.0046	97	0.8241	9	68.77	
1 (Control)	2019-02-04	0.0000	100	0.0320	81	0.3013	67	82.58	
2 (Control)	2019-02-04	0.0065	97	0.0065	96	0.4641	49	80.66	
3	2019-02-04	0.0000	100	0.0047	97	0.6854	24	73.85	
4 (Test)	2019-02-05	0.0109	95	0.0272	84	0.5924	35	71.17	
5 (Test)	2019-02-05	0.0000	100	0.0075	96	0.7872	13	69.55	
6 (Test)	2019-02-05	0.0000	100	0.0109	94	0.5409	40	77.93	

management decisions and policies related to maintaining healthy aquatic ecosystems (U.S. Environmental Protection Agency, 2012) to ensure that the ecological well-being, recreational uses, and aesthetic values vital to the Buffalo National River can be sustained.

CRediT authorship contribution statement

Billy G. Justus: Funding acquisition, Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - original draft, Project administration. **Lucas J. Driver:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing - review & editing, Visualization. **David R.L. Burge:** Resources, Writing -

review & editing, Data curation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2020.107150.

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