

# Nonlinear response of stream ecosystem structure to low-level phosphorus enrichment

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## SUMMARY

1. Anthropogenic inputs of nitrogen (N) and phosphorus (P) create environmental conditions that alter biological organisation and ecosystem functioning in fresh waters. We studied 38 wadeable streams spanning a N and P gradient to contrast responses of algal and fish assemblages to nutrient enrichment.
2. Surface water total P (TP) and total N (TN) concentrations represented a wide range (TP: 7–2380  $\mu\text{g L}^{-1}$ ; TN: 127–15 860  $\mu\text{g L}^{-1}$ ) and were correlated across our study sites. Total P explained significantly more variance in periphyton carbon (C)-to-nutrient (C : P, C : N) and N : P ratios than TN. Abrupt, nonlinear declines in these ratios were observed between 20 and 50  $\mu\text{g L}^{-1}$  TP and 500–1000  $\mu\text{g L}^{-1}$  TN; beyond these values, ratios exhibited minimal additional decline.
3. Algae assemblage structure was strongly linked to surface water TP, TN and catchment-scale nutrient sources (wastewater treatment plant (WWTP) discharges and % pasture cover). In particular, there were synchronous declines in frequency and cell densities of many alga species associated with TP concentrations  $>21 \mu\text{g L}^{-1}$  (90% CI of 18–48  $\mu\text{g L}^{-1}$ ) as well as simultaneous increases in tolerant species associated with increasing enrichment.
4. Fish assemblage structure was most strongly associated with % pasture, WWTP discharges and fine sediment cover, yet also showed significant but weaker correlations with surface water and periphyton nutrient variables. However, two benthic fish species, *Etheostoma spectabile* and *Camposotoma anomalum*, significantly declined with TP  $>28 \mu\text{g L}^{-1}$  (90% CI, 24–56  $\mu\text{g L}^{-1}$ ) and 34  $\mu\text{g L}^{-1}$  (90% CI, 21–56  $\mu\text{g L}^{-1}$ ), respectively. Conversely, the tolerant minnow *Cyprinella lutrensis* and invasive carp *Cyprinus carpio* increased nonlinearly with increasing surface water TP.
5. Our results provide new insights into interpretation and analysis of assemblage-level responses to nutrient enrichment. Our findings indicate that a numerical criterion for surface water TP of *c.* 20  $\mu\text{g L}^{-1}$  would be needed to maintain natural algae assemblages and at least two specialist fishes within our study region. Proliferation of weedy alga species and increased abundance of invasive fishes are also likely when surface water concentrations exceed these thresholds. While many streams likely exceed these thresholds, managers should consider potential low-level enrichment effects when developing criteria for ecosystems to protect existing nutrient-limited streams.

*Keywords:* attached algae, eutrophication, fish, running waters/rivers/streams, stoichiometry

## Introduction

Humans have altered the input of nitrogen (N) and phosphorus (P) to freshwater systems, and the resulting eutrophication is a major obstacle in protecting freshwa-

ter and coastal marine ecosystems (Carpenter *et al.*, 1998; Smith, Joye & Howarth, 2006). In response, regulatory authorities, such as the U.S. Environmental Protection Agency (US EPA) and European Union Water Framework Directive, have charged water resource managers

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with developing numerical criteria for nutrients that protect physical, chemical and biological integrity of aquatic ecosystems (US EPA, 1998; Hering *et al.*, 2010). Development of numerical nutrient criteria has progressed slowly, largely due to insufficient data on nutrients and biological endpoints, as well as inadequate statistical tools for quantifying levels of enrichment likely to cause biological impairments. In naturally nutrient-limited ecosystems, rapid ecological responses to relatively small changes in nutrient enrichment can occur and may be indicative of ecological thresholds. While observed nonlinear responses may not necessarily represent a system change in stable states, they can serve as useful benchmarks for protecting surface water from nutrient enrichment or other impacts, and more studies that explore techniques for detecting thresholds in freshwater ecosystems are needed (Dodds *et al.*, 2010).

Natural inputs of inorganic N and P to freshwater systems are influenced by catchment geology and vegetation. Whereas N requirements of freshwater organisms may be augmented by fixation of atmospheric N<sub>2</sub>, P is largely derived from the weathering of phosphate-bearing rocks that are patchily distributed (Notholt, Sheldon & Davidson, 1989). As a result, most freshwater species assemblages have evolved in low-P environments. For example, periphyton communities dominated by species with low P requirements are common in regions where biogeochemistry is driven by high Ca and low P availability (Noe, Childers & Jones, 2001). Relatively small increments of anthropogenic P can create novel environmental conditions for algae species that have evolved under low nutrient conditions, and often induce changes in species composition at relatively low levels of enrichment (Gaiser *et al.*, 2005; Richardson *et al.*, 2007). Shifts in primary producer species composition and biomass in response to P enrichment also can have substantial effects on ecosystem processes that influence higher trophic levels (Carpenter *et al.*, 1998; Miltner & Rankin, 1998; Smith *et al.*, 2006). For example, changes in relative and absolute abundances of primary producers associated with nutrient enrichment can influence consumer assemblages through altered consumer–resource stoichiometric relationships (Cross *et al.*, 2005; Evans-White *et al.*, 2009), habitat structure (Mittelbach, 1984) and production–respiration dynamics (Miltner & Rankin, 1998). All of these factors can influence resource availability and habitat suitability for higher consumers such as fishes.

Species-specific responses to nutrient enrichment through direct (species optima for growth) or indirect (ecosystem changes associated with enrichment) mecha-

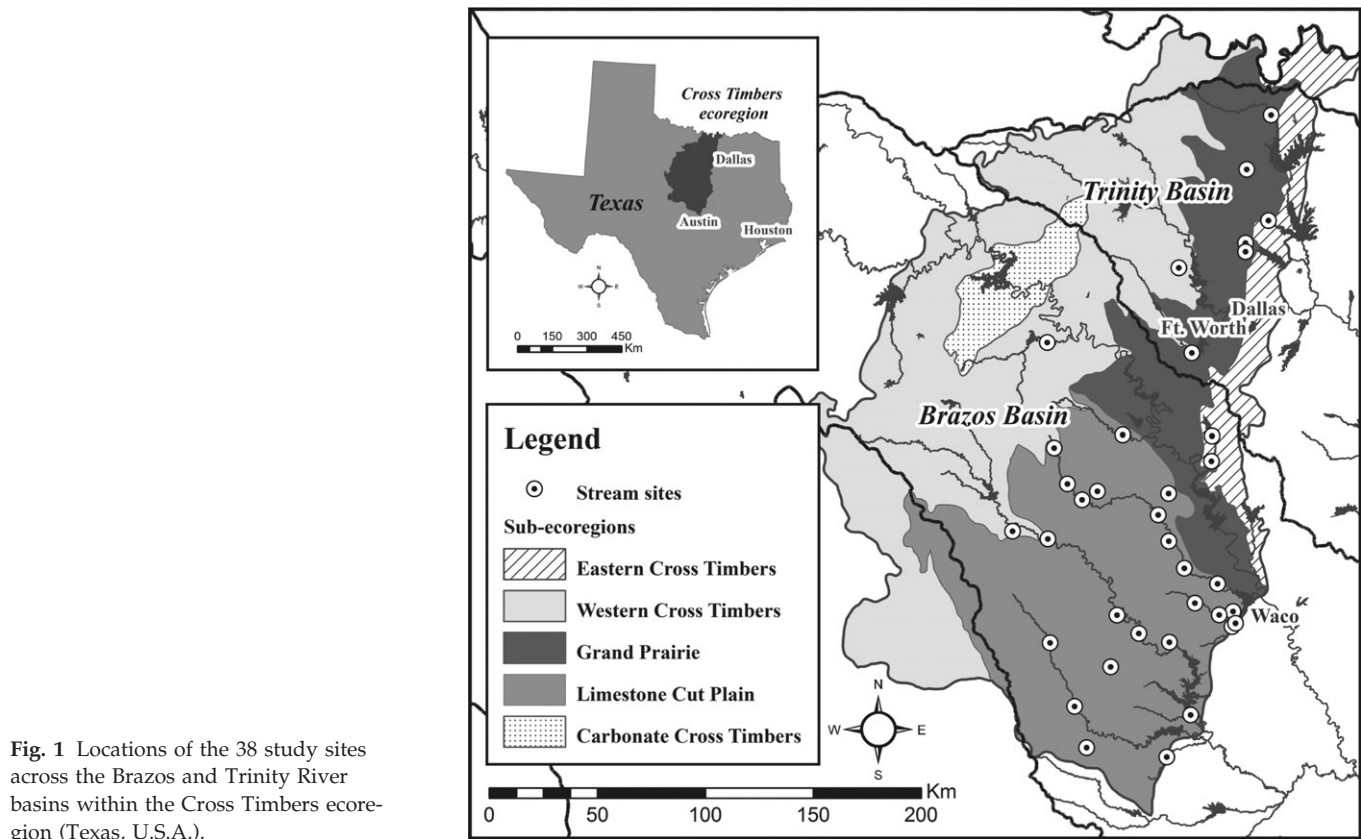
nisms likely culminate in shifts in species assemblage composition involving both producers and consumers. Quantifying these patterns will promote understanding of how nutrient subsidies alter stream ecosystems. In regions of the world that experience seasonal low-flow periods, understanding how nutrient subsidies influence biotic communities is particularly germane. In some catchments, wastewater discharges now account for >90% of instream flow during low-flow periods (Brooks, Riley & Taylor, 2006). Southern regions of North America are expected to experience reduced magnitude and increased frequency and duration of low stream flows due to climate change (Sun *et al.*, 2008). This emerging threat, combined with increased water consumption and nutrient enrichment associated with rising human population densities, will have significant impacts on freshwater biodiversity (Palmer *et al.*, 2009; Dudgeon, 2010).

In this study, we examined responses of benthic algae and fish species assemblages across 38 streams spanning a steep gradient of N and P in central Texas, U.S.A., in hopes of identifying nutrient levels protective of naturally nutrient-limited streams that might provide a basis for the development of numeric nutrient criteria. We hypothesised that benthic algal assemblages would show strong relationships with surface water nutrient concentrations and exhibit sharp, nonlinear responses to low levels of enrichment. This prediction was based on the assumption that relaxation of evolutionary constraints imposed on algae species in oligotrophic systems should result in rapid shifts in species assemblage structure (Gaiser *et al.*, 2006). We further hypothesised that due to indirect rather than direct mechanisms, nutrient enrichment effects on fish species distributions would be less predictable, resulting in weaker thresholds that are more difficult to detect.

## Methods

### *Study area and field sampling*

Study streams were located in the Cross Timbers ecoregion and Brazos and Trinity River basins in Texas (Fig. 1). Hydrology within this portion of the Southern Great Plains is highly variable due to seasonal precipitation patterns that include long, hot summers (Matthews *et al.*, 2005). Study sites were selected to provide broad geographical coverage and a range of landscape features that drive nutrient enrichment [pasture and wastewater treatment plant (WWTP) discharge] resulting in a wide range of stream nutrient conditions (Fig. 1, Table 1). At each sample site, we collected data on water chemistry,



**Fig. 1** Locations of the 38 study sites across the Brazos and Trinity River basins within the Cross Timbers ecoregion (Texas, U.S.A.).

**Table 1** Selected physical characteristics, water chemistry and benthic nutrient content for all 38 sampling sites used in the final analyses. Surface water TN is listed for reference despite being highly correlated with phosphorus variables

Variables	Min	Max	Mean	Median
Latitude DS	30.9	33.6	32.00	31.88
Catchment area (km <sup>2</sup> )	68	6112	845	404
WWTP ( $1 \times 10^6$ L d <sup>-1</sup> km <sup>-2</sup> )	0	0.45	0.04	0
% Shrub	0	56.6	12.6	4.9
% Pasture	0.1	18.6	5.6	3.3
Discharge (m <sup>3</sup> s <sup>-1</sup> )	0	24.6	3.5	1.3
% Mud/silt cover	0	48.3	8.4	2.2
Bank slope (%)	17.9	64.3	34.3	34.1
Specific conductivity ( $\mu$ S cm <sup>-1</sup> )	184	1225	646	604
Cl (mg L <sup>-1</sup> )	7	140	41	24
TSS (mg L <sup>-1</sup> )	1	105	14	7
Surface water TP ( $\mu$ g L <sup>-1</sup> )	7	2380	296	29
Surface water TN ( $\mu$ g L <sup>-1</sup> )	127	15860	1635	463

instream and riparian habitat variables, periphyton nutrient content and species composition and fish relative abundance following Texas Commission on Environmental Quality protocols (Texas Commission on Environmental Quality (TCEQ) (2005)) and protocols outlined in King *et al.* (2009).

#### Environmental variables

We selected a suite of environmental variables that were hypothesised to be associated with changes in species composition related to nutrient enrichment gradients. We considered five classes of variables: (i) catchment physiography, (ii) land cover, (iii) instream and riparian habitat, (iv) water chemistry and (v) periphyton biomass and tissue chemistry. We initially included a wide range of environmental variables including 23 landscape and 41 local habitat variables (Pease *et al.*, 2011), but narrowed these down to a few non-redundant variables within each broad category using scatterplot matrices and associated correlation coefficients between variables significantly correlated with periphyton or fish assemblage structure (see Table S1–S3 in Supporting Information) (see Data analysis section for more detail).

Surface water nutrient sampling consisted of triplicate surface water instantaneous grab samples. Surface water grab samples for total phosphorus (TP) and total nitrogen (TN) were analysed using the molybdate and cadmium reduction method, respectively, following persulphate digestion (APHA, 1998). Total alkalinity, chloride (Cl), total suspended solids (TSS), volatile suspended solids, sulphate, total dissolved solids (TDS) and fluoride were

sampled and analysed in accordance with TCEQ Surface Water Quality Monitoring Procedures (TCEQ, 2003).

#### *Periphyton sampling*

We collected reach-scale composite samples of epilithic periphyton for the analysis of nutrient content and assemblage structure by removing material from the surface of at least 25 rocks for a composite periphyton slurry following methods outlined in King *et al.* (2009). All samples were stored in Nalgene dark bottles and transported on ice (4 °C) to the laboratory within 24 h. Periphyton samples were homogenised, subsampled and filtered onto pre-weighed Whatman GF/F (pore size = 0.7 µm) filters for the quantification of chlorophyll a, dry mass and ash-free dry mass (AFDM) following Steinman, Lamberti and Leavitt (2006). We considered bulk periphyton samples for nutrient content analyses, because periphyton collectively includes autotrophic organisms and non-algal material, including heterotrophic microbes and detritus in the form of fine particulate organic matter (Frost, Hillebrand & Kahlert, 2005; Hillebrand, Frost & Liess, 2008), all of which can serve as a basal resource for consumers and potentially provide a reliable indicator of nutrient enrichment (Gaiser *et al.*, 2004). Previous work within the study region indicates little difference between bulk periphyton nutrient content and algae separated from fine sediments following centrifugation (Hamilton, Sippel & Bunn, 2005; King *et al.*, 2009). Subsamples of homogenised periphyton samples were dried at 60 °C for 48 h and pulverised into a fine powder using a Mini-Bead Beater 8 cell disrupter (Biospec Products, Inc., Bartlesville, OK, U.S.A.) for the analysis of nutrient content. We measured C and N contents of periphyton using a ThermoQuest Flash EA<sup>TM</sup> 1112 elemental analyser (Thermo Fisher Scientific, Waltham, MA, U.S.A.) following fuming with HCl to drive off inorganic carbonates (Hill & Middleton, 2006). Periphyton P content was analysed using the molybdate method following a 1-h digestion in 15 mL of distilled water with 1.8 mL of a mixture of peroxodisulphate (30 g L<sup>-1</sup> K<sub>2</sub>S<sub>2</sub>O<sub>8</sub>), boric acid (50 g L<sup>-1</sup> H<sub>3</sub>BO<sub>3</sub>) and sodium hydroxide (15 g L<sup>-1</sup> NaOH) at 121 °C (Faerøvig & Hessen, 2003). Soil (Thermo Finnigan 1.99% C) and peach leaf (SRM 1547, 0.137% P, 0.298% N) standards were analysed to assure that C, N and P recoveries met QA/QC standards (±10%) for each sample run.

#### *Species assemblage structure*

Additional aliquots of homogenised periphyton samples were preserved for species identifications in accordance

with taxonomic methods for soft algae and diatoms (TCEQ, 2005). One soft algae and one diatom sample were sorted and identified per stream, with at least 500 diatom and 300 soft algae cells identified per respective sample (TCEQ, 2005).

We estimated fish assemblage structure by sampling all obvious habitat components (e.g. open pools or runs, undercut banks, brush piles, rocks, riffles) within each survey reach with a backpack electrofisher (Smith-Root Model LR-24) and seine net (4.6 × 1.8 m or 1.8 × 1.8 m). Reach length was determined based upon 40 times the average wetted width. Crews of three to four people electrofished each study reach in a single upstream pass with a minimum effort of 900 s. The reach was then sampled with the seine net with a minimum of six 10-m hauls that covered all habitat components within the study reach. If the sixth haul yielded species not previously collected, additional seine hauls were made until no additional species were captured (TCEQ, 2005). Fish specimens were identified, counted and either released unharmed into the habitat or euthanised by ice-bath immersion and then preserved in 10% buffered formalin for later identification according to Hubbs, Edwards and Garrett (1991) and Thomas, Bonner and Whiteside (2007). Numerical abundance of each algae and fish species was recorded for each study reach for analyses of species assemblage structure. Fish and instream habitat data were part of a previous larger-scale study examining fish–habitat relationships across several ecoregions over multiple years (Pease *et al.*, 2011).

#### *Data analysis*

We used a combination of generalised additive models (GAM), non-metric multidimensional scaling (nMDS) ordinations and threshold indicator taxa analysis (TITAN), to analyse associations between ecosystem structure and water nutrients and other catchment and reach-scale variables hypothesised to be important drivers of stream communities in this region.

*Generalised Additive Modelling (GAM).* We used GAM to model relationships between bulk periphyton nutrient content and surface water nutrients (TP and TN) because graphical evaluation of scatterplots revealed nonlinear patterns. GAMs are well suited for fitting nonlinear response relationships where the precise form between the independent and dependent variables is not known *a priori* (Zuur *et al.*, 2009). We fit responses of periphyton nutrient ratios to surface water nutrients using GAMs with the mgcv package in R 2.11.2 (Wood,

2006). We fit GAMs using the gamma distribution because periphyton nutrient content data were positively skewed. Cross-validation was used to determine the optimal amount of smoothing. We limited the number of knots ( $k$ ) to three to avoid model overfitting due to small sample size (Wood, 2006).  $P$  values obtained from GAM for smoothing splines are approximate; therefore, we interpreted ecological significance for GAM responses at  $P \leq 0.001$  (Zuur *et al.*, 2009).

*Ordination and environmental vector fitting.* We ordinated stream locations according to periphyton and fish species structure, separately, using nMDS (Minchin, 1987; Clarke, 1993). Prior to nMDS analysis, we  $\log_{10}(x)$ -transformed abundance of periphyton and fish species to down-weight the contribution of numerically dominant species to the ordinations (McCune & Grace, 2002). We used Bray–Curtis dissimilarity (BCD) as the distance measure. We performed nMDS and related analyses in the vegan package in R 2.11.2 (Oksanen *et al.*, 2010; R Core Development Team 2010). Ordination plots were rotated to have the strongest correlation with the TP along axis 1 because TP was the strongest correlate of periphyton nutrient ratios and algal species composition (see Results). We used the function ‘envfit’ to examine linear correlations between assemblages (nMDS axes) and environmental factors associated with nutrient enrichment, and assessed significance of the fitted environmental vectors using 1000 random permutations (Oksanen *et al.*, 2010). All significant environmental vectors were assessed for collinearity within the broad categories of catchment physiography, land cover, stream reach, water chemistry and periphyton stoichiometry using scatterplot matrices and associated correlation coefficients. A subset of significant environmental variables were retained for plotting in nMDS that did not have strong correlations ( $r > 0.7$ ) with each other within broad environmental categories (landscape, instream habitat, water quality) (see Tables S1–S3) (Zuur *et al.*, 2009). We included TN and Cl despite strong relationships with surface water TP because we wanted to contrast assemblage responses to both nutrients, whereas Cl provides a conservative tracer of the relative contribution of wastewater discharges to stream flow (see Table S3). Additionally, we overlaid TP concentrations within site ordination plots to confirm any observed relationships with nutrient enrichment. We also explored non-linearity in nutrient–species assemblage relationships by fitting GAM responses curves of nMDS axis 1 to surface water nutrients using methods presented above but with the Gaussian distribution.

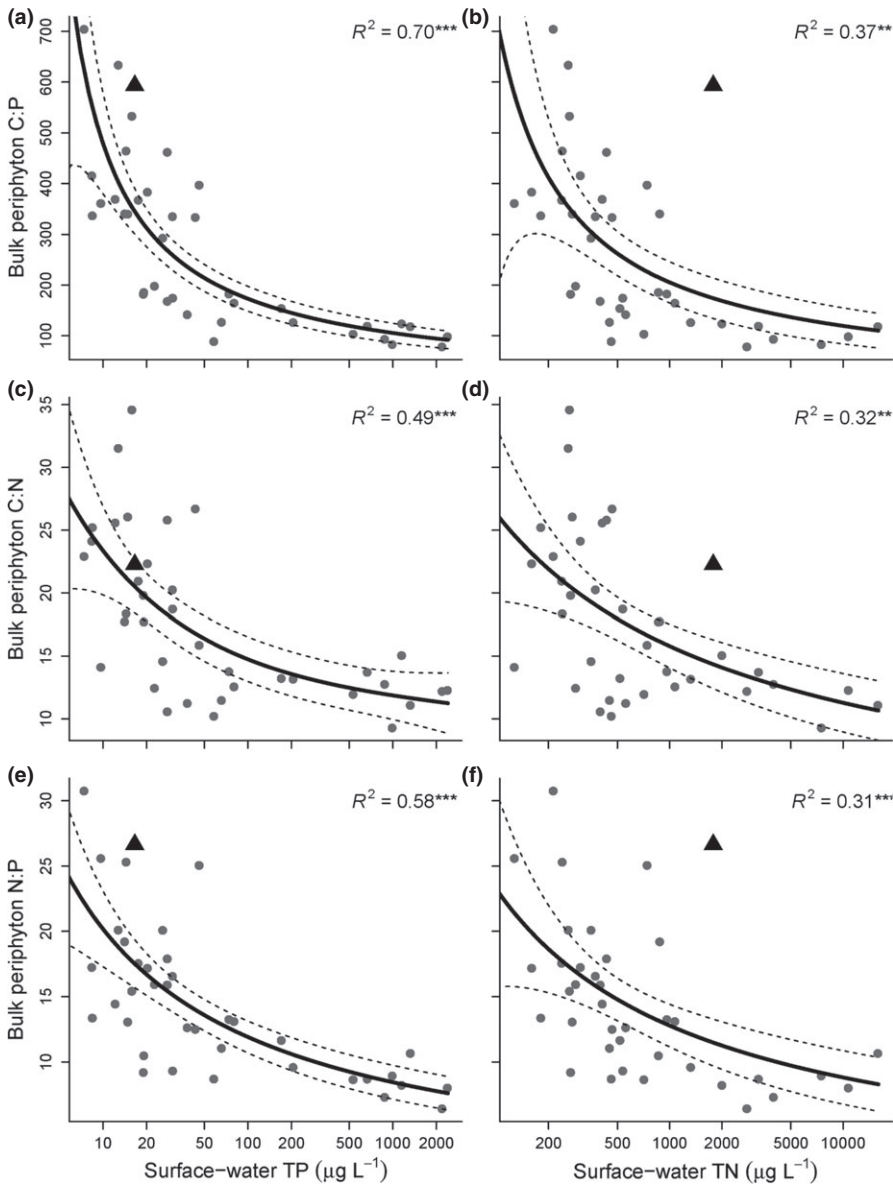
Finally, to assess the potential confounding effect of river basin (Trinity versus Brazos, the two major basins samples in this study) on patterns of assemblage structure, we estimated the central tendency and dispersion of assemblage composition within ordination space by river basin using permutational multivariate analysis of variance (PERMANOVA) and permutational analysis of multivariate dispersion (PERMDISP) with the functions ‘adonis’ and ‘betadisper’ (Oksanen *et al.*, 2010).

*Threshold indicator taxa analysis (TITAN).* We analysed the magnitude, direction and uncertainty of responses of individual taxa to nutrient enrichment gradients (TP, TN) using TITAN (Baker & King, 2010). TITAN identifies the value of a predictor variable that maximises association of individual taxa with either the negative or positive side of the partition. Association is measured by IndVal, computed as the product of the percentage of sample units in which a taxon occurred and the percentage of the total number of individuals captured by each partition (Dufrene & Legendre, 1997). Bootstrapping is used to identify reliable threshold indicator taxa. A taxon is determined to respond positively or negatively to the gradient of interest if (i) the change in frequency and abundance of the taxon is in the same direction for at least 95% of the 1000 bootstrapped runs = ‘high purity’ and (ii) at least 95% of 1000 bootstrapped runs are significantly different from a random distribution (at  $P < 0.05$ ) = ‘high reliability’. The sum of IndVal  $z$  scores can also be used as an indicator of assemblage-level thresholds by identifying peaks in sums of all taxa  $z$  scores along the gradient associated with the maximum decline in all negative responders ( $z^-$ ) or increase in frequency and abundance of all positive responders ( $z^+$ ). We performed TITAN on  $\log_{10}(x)$ -transformed abundances of periphyton and fish taxa occurring in at least three sites to down-weight very large values. TITAN was run with the TITAN 2.0 package (Baker & King, 2010, 2013) in R.2.11.2.

## Results

### *Surface water nutrient concentrations*

Surface water nutrient concentrations ranged between 7 and 2380  $\mu\text{g L}^{-1}$  for TP and 127–15860  $\mu\text{g L}^{-1}$  for TN (Table 1). Simple correlation analysis suggests that the observed nutrient gradient was driven primarily by differences in wastewater effluent contributions to stream flow. Both TP and TN were positively correlated with Cl concentrations (see Table S3). In general, sites with high TP also had high Cl, indicating a wastewater effluent



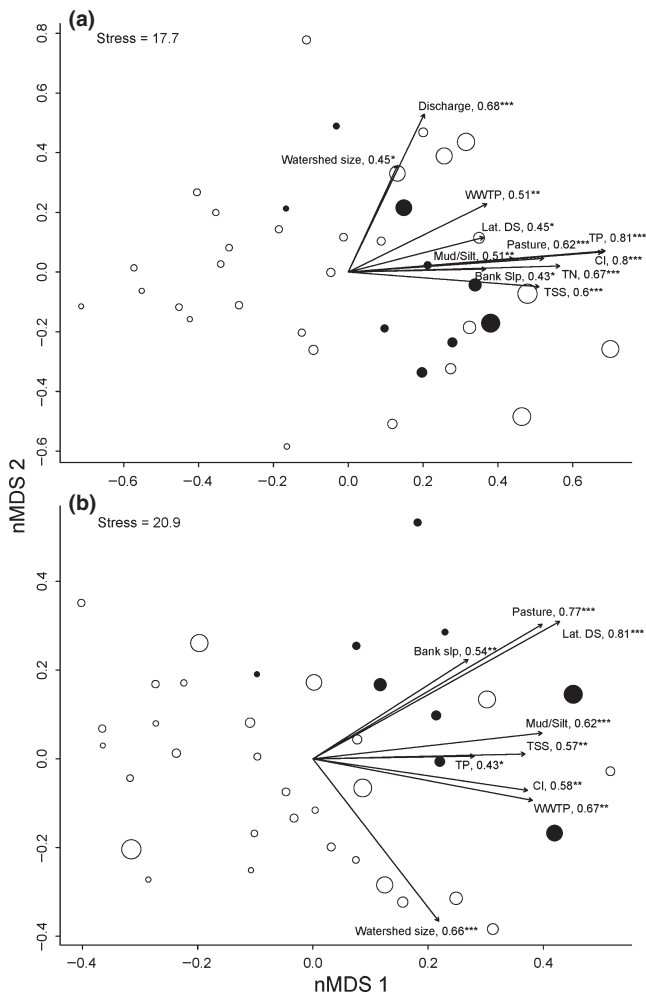
**Fig. 2** GAM smoothers for periphyton C : P (a, b), C : N (c, d) and N : P (e, f) in response to surface water TP and TN. Solid lines represent predicted ratios with 95% CI (dashed lines).  $R^2$  = null deviance – residual deviance/null deviance, and  $P < 0.001$  is indicated by \*\*\*. Black triangle represents sample site with low surface water TP and high TN. Note that x axis is presented on log scale for ease of interpretation.

influence on TP concentrations (see Figure S1a in Supporting Information). Similar trends were observed for TN, but were more variable (see Figure S1d). Many sites with high nutrient concentrations also had high amounts of pasture within the catchment (see Figure S1b,e), but those sites generally also had high Cl concentrations, and several sites with high% pasture had relatively low nutrient concentrations. Additionally, surface water TP and TN concentrations were positively correlated (Pearson's product-moment correlation,  $r = 0.86$ ,  $P < 0.001$ ) (see Table S3, Figure S1c).

#### Periphyton nutrient content

Periphyton C : P, C : N and N : P ratios declined sharply with low levels of nutrient enrichment (Fig. 2). We

observed the strongest relationship between periphyton C : P and surface water TP. Periphyton C : P ranged from 300 to 700 below  $20 \mu\text{g L}^{-1}$  TP, but rapidly approached 100–200 above  $20 \mu\text{g L}^{-1}$  TP and showed little additional decline with increasing TP up to  $2000 \mu\text{g L}^{-1}$  (Fig. 2a). Carbon : P ratios were always below 200 once surface water TP exceeded  $50 \mu\text{g L}^{-1}$ . While we observed similar patterns between periphyton nutrient ratios and surface water TN, TP always explained more variation (Fig. 2). This was due primarily to sites that appeared to be outliers for TN relationships. For example, periphyton nutrient ratios for one site (plotted as a triangle in Fig. 2) characterised by high surface water TN ( $1783 \mu\text{g L}^{-1}$ ) but low TP ( $16.6 \mu\text{g L}^{-1}$ ) followed observed patterns expected for a low TP site but was an outlier for the TN relation-



**Fig. 3** Non-metric multidimensional scaling (NMS) ordination of sites based on periphyton (a) and fish (b) assemblage data. Symbol shading indicates membership among the Brazos (open) and Trinity (filled) river basins. Environmental vectors show the direction and magnitude of significant correlations between environmental factors and assemblage structure within the ordination space and associated correlation coefficients (\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ). Ordinations are rotated on axis 1 to surface water TP ( $\mu\text{g L}^{-1}$ ), which is represented by size of circles. Lat. DS, latitude in decimal degrees; WWTP = total upstream wastewater effluent per  $\text{km}^2$  ( $1 \times 10^6 \text{ L d}^{-1}$ ), Bank Slp, bank slope (%); TSS, total suspended solids ( $\text{mg L}^{-1}$ ), Cl, chloride ( $\text{mg L}^{-1}$ ); TP, total phosphorus ( $\mu\text{g L}^{-1}$ ); TN, total nitrogen ( $\mu\text{g L}^{-1}$ ).

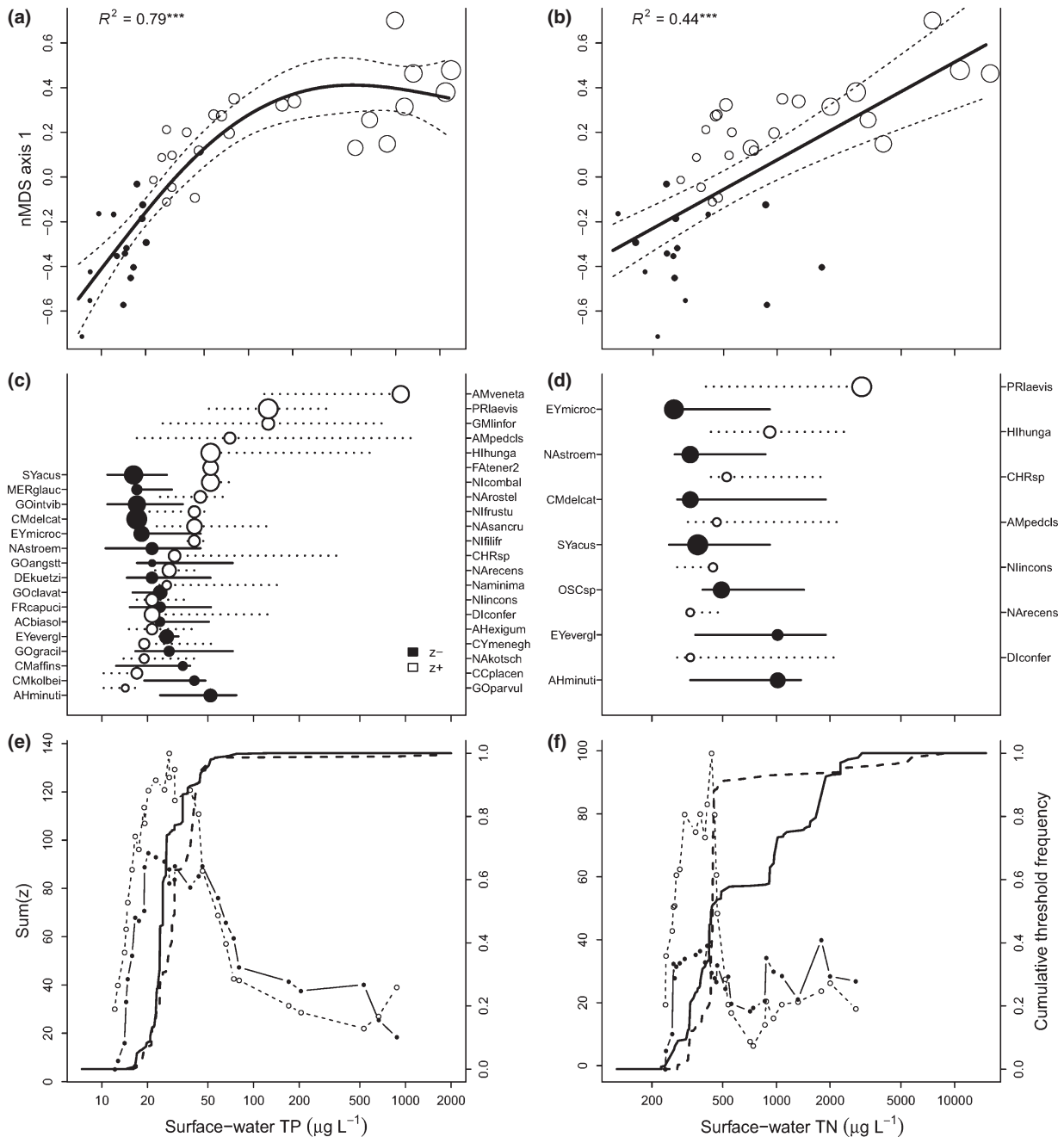
ship, indicating that P was the most likely driver of the observed patterns. Overall, GAM-fitted relationships identified large reductions (34–61%) in nutrient content across a small range of TP (7–50  $\mu\text{g L}^{-1}$ ) and TN (127–1000  $\mu\text{g L}^{-1}$ ) concentrations (Fig. 2).

#### Environmental variables and assemblage structure

Ordination of periphyton assemblage structure sorted streams along two major axes that explained 79% of the

variance among original distances in  $n$ -dimensional space (2-D stress = 17.7). The first axis represented a gradient primarily defined by nutrient enrichment variables, whereas the second represented a shorter gradient associated with stream discharge (Fig. 3a). Variation in periphyton assemblage structure was most strongly linked to measures of nutrient enrichment defined by TP, TN and Cl. A graphical overlay of TP on the nMDS plot helped validate the statistical relationship between TP and periphyton assemblage structure, illustrating that assemblages in sites with high TP are more similar to each other than those in sites with lower TP (Fig. 3a). Catchment pasture and accompanying environmental stressors (mud/silt, TSS) were closely associated with the nutrient enrichment gradient along axis 1, and WWTP density was associated with both nutrient enrichment and discharge on axis 2 (Fig. 3a).

Fish assemblage composition arranged sites along two major axes that explained 73% of the original distances in  $n$ -dimensional space (2-D stress = 20.9). In contrast to patterns in periphyton assemblages, composition of fish species assemblages was most strongly associated with latitudinal gradients on axis 1 and a stream size gradient on axis 2 (Fig. 3b). Percentage pasture in the catchment and bank slope had significant associations with fish assemblage structure along the same trajectory as latitudinal gradients (Fig. 3b). Fish assemblage structure also was related to measures of nutrient enrichment on axis 1, with TP increasing in conjunction with increased mud/silt substrata, TSS, Cl and WWTP density. However, the relationship between fish assemblage structure and nutrient enrichment was weaker than that observed for periphyton assemblage structure (Fig. 3b). While most sites with high TP plotted on the right side of the ordination plot (consistent with the TP vector), two sites with high TP and WWTP influence had fish species structure more similar to sites on the left side that had low values for pasture, mud/silt substrata and nutrients (Fig. 3b). Latitudinal gradients in fish species structure among river basins were indicated by separation of fish assemblages in ordination space based on river basin (PERMANOVA,  $F_{1,36} = 9.51$ ,  $P = 0.009$ ) (Fig. 3b) and PERMDISP results ( $F_{1,36} = 0.91$ ,  $P = 0.36$ ), which indicates no significant heterogeneity of variance for assemblage composition in the two basins. However, latitude was correlated with catchment % pasture (Pearson's product-moment correlation,  $r = 0.76$ ,  $P < 0.001$ ) (Table S1), and this pattern was driven by higher median % pasture values for sites within the Trinity Basin (Wilcoxon rank-sum test,  $W = 251$ ,  $n_1 = 9$ ,  $n_2 = 26$ ,  $P < 0.001$ ).



**Fig. 4** Change in periphyton assemblage structure (nMDS axis 1 from Fig. 3a) across surface water TP and TN ( $\mu\text{g L}^{-1}$ ) gradients spanning 38 sample sites (a, b). Solid lines represent predicted abundance with 95% CI (dashed lines). Solid symbols represent sites below the surface water TP ( $\mu\text{g L}^{-1}$ ), assemblage-level threshold identified by TITAN. Dot sizes represent the distribution of surface water TP ( $\mu\text{g L}^{-1}$ ) concentrations among sites.  $R^2 = \text{null deviance} - \text{residual deviance} / \text{null deviance}$ , and  $P < 0.001$  is indicated by \*\*\*. Note that x axis is presented on log scale for ease of interpretation. Significant periphyton indicator taxa identified in threshold indicator taxa analysis (TITAN) across surface water TP and TN ( $\mu\text{g L}^{-1}$ ) gradients spanning 38 sample sites (c, d). Significant (purity  $\geq 0.95$ , reliability  $\geq 0.95$ ,  $P \leq 0.05$ ) indicator taxa are plotted in increasing order with respect to 90% confidence in their observed change point. Solid symbols correspond to negative ( $z^-$ ) indicator taxa, whereas open symbols correspond to positive ( $z^+$ ) indicator taxa. Symbols are sized in proportion to magnitude of the response ( $z$  scores). Horizontal lines overlapping each symbol represent 5th and 95th percentiles among 1000 bootstrap replicates. TITAN sum ( $z^-$ ) (aggregate response of negative indicator taxa, black symbols) and sum ( $z^+$ ) (positive indicator taxa, open symbols) are shown in response to TP and TN enrichment (e, f). Peak sum ( $z$ ) scores correspond to the nutrient value resulting in the largest synchronous change among negative and positive indicator taxa, respectively. Solid ( $z^-$ , negative indicator taxa) and dashed ( $z^+$ , positive indicator taxa) lines represent the cumulative threshold frequency distribution of the peak sum( $z$ ) value obtained among 1000 bootstrap replicates for negative and positive indicator taxa, respectively (right  $y$  axis).



**Table 2** Nutrient thresholds estimated by TITAN sum ( $z^-$ ) (negative indicator taxa) and sum ( $z^+$ ) (positive indicator taxa). The estimated community threshold (Obs.) is shown for each nutrient with lower (5%), mid (50%) and upper (95%) cumulative probabilities that correspond to change point quantiles of 1000 bootstrap replicates

Nutrient	Sum ( $z^-$ )	Sum ( $z^+$ )
Periphyton		
TP	21.43 (17.73, 25.20, 48.26)	27.77 (19.05, 30.18, 48.13)
TN	1891.67 (266.0, 434.0, 2285.0)	440.83 (328.17, 440.83, 2393.33)
Fish		
TP	34.18 (26.78, 52.08, 598.33)	34.18 (13.42, 27.28, 52.08)
TN	–	238.83 (238.83, 370.58, 491.16)

Observed thresholds are the maximum sum ( $z$ ). Values in parentheses are the 5th, 50th and 95th percentiles corresponding to the frequency distribution of thresholds from 1000 bootstrap replicates. TP, surface water total phosphorus ( $\mu\text{g L}^{-1}$ ) and TN, surface water total nitrogen ( $\mu\text{g L}^{-1}$ ).

#### Taxon and assemblage responses to nutrients

**Periphyton.** Generalised additive model-fitted relationships identified a large change in assemblage structure characterised by changes in nMDS axis 1, over a small range of TP values (10–50  $\mu\text{g L}^{-1}$ ) that was consistent with shifts in assemblage structure identified by TITAN (Fig. 4a). Trends in assemblage structure summarised by nMDS axis 1 associated with TN were less clear than those observed for TP. A potential nonlinear relationship between TN and periphyton assemblage structure was confounded by multiple sites that had high TN concentrations, but also had TP concentrations below the observed TITAN threshold and periphyton assemblages consistent with low-P sites (Fig. 4b).

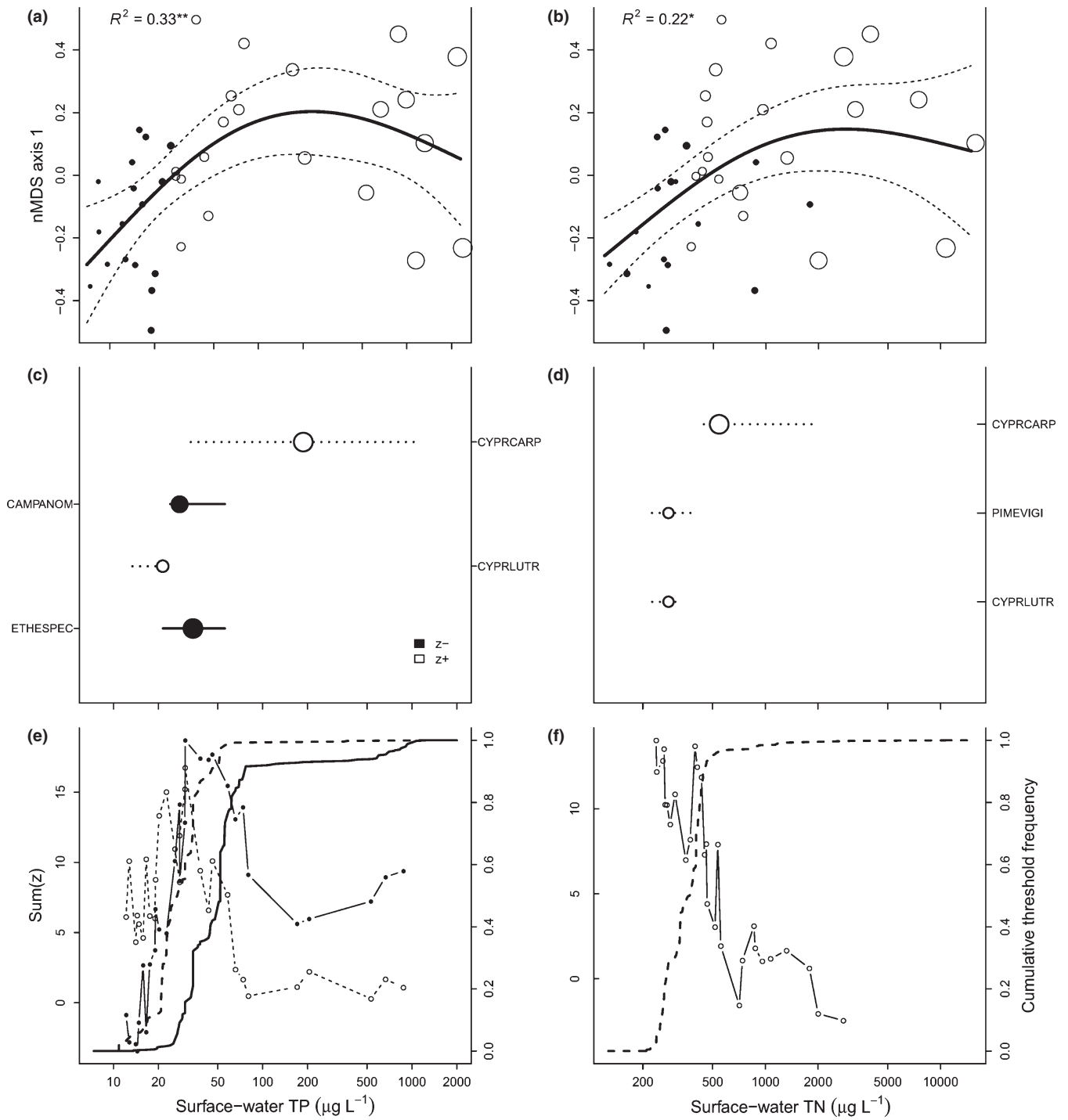
Threshold indicator taxa analysis identified 16 of the 148 alga taxa as pure and reliable indicator taxa that declined sharply with increasing surface water TP between 16 and 52  $\mu\text{g L}^{-1}$  (Fig. 4c, See Table S4 in Supporting Information). Synchronous declines detected by TITAN resulted in an assemblage-level threshold estimate for sensitive taxa of 21  $\mu\text{g L}^{-1}$  TP (90% CI, 18–48  $\mu\text{g L}^{-1}$ ) (Table 2, Fig. 4e). There was also an assemblage-level threshold identified at 28  $\mu\text{g L}^{-1}$  (90% CI, 19 and 48  $\mu\text{g L}^{-1}$ ) for the taxa that sharply increased in response to TP (Table 2, Fig. 4e). This assemblage-level threshold aligned closely with the synchronous increases in the frequency and cell density of the majority of 21 positive responding alga taxa (Fig. 4c, see Table S4).

Only seven alga taxa declined in response to increasing surface water TN (Fig. 4d, See Table S5 in Supporting Information). Of these, six also had significant negative responses to TP. *Oscillatoria*, a genus capable of N fixation in low-N environments, was the only taxon not previously associated with increasing P enrichment. Likewise, seven taxa that increased with N enrichment also exhibited significant positive associations with increasing P enrichment (Fig. 4, see Table S5). Strong synchronous changes consistent with assemblage-level thresholds were not observed for taxa either negatively or positively associated with surface water TN (Table 2, Fig. 4f).

**Fish.** Trends in fish assemblage structure (represented by nMDS axis 1, Fig. 3b) associated with surface water TP were less clear than trends observed for periphyton assemblage structure. There was a weaker nonlinear trend in assemblage structure in response to P enrichment, but this trend was not significant (GAM,  $P > 0.001$ ). Assemblage change appeared to be associated with low to moderate P enrichment; thereafter, assemblages became highly variable regardless of TP concentrations (Fig. 5a). GAM response curves were not significant, and there was also no clear trend between overall fish assemblage structure (nMDS axis 1) and surface water TN (Fig. 5b).

Threshold indicator taxa analysis only identified significant declines in two of 31 fish taxa associated with TP. Central stonerollers (*Camptostoma anomalum* Rafinesque) and orangethroat darters (*Etheostoma spectabile* Agassiz) had markedly lower occurrence and abundance at TP concentrations  $>28 \mu\text{g L}^{-1}$  (90% CI, 24–56  $\mu\text{g L}^{-1}$ ) and 34  $\mu\text{g L}^{-1}$  (90% CI, 21–56  $\mu\text{g L}^{-1}$ ), respectively (Fig. 5c, See Table S6 in Supporting Information). In contrast, TITAN revealed a significant increase in occurrence and abundance of red shiners (*Cyprinella lutrensis* Baird and Girard) that was associated with relatively low TP value of 21  $\mu\text{g L}^{-1}$  (90% CI, 13–24  $\mu\text{g L}^{-1}$ ). TITAN also identified higher threshold concentrations for increasing common carp (*Cyprinus carpio* Linnaeus) at higher TP concentrations of 187  $\mu\text{g L}^{-1}$  (90% CI of 33–1069  $\mu\text{g L}^{-1}$ ) (Fig. 5c, see Table S6). TITAN estimated an assemblage-level threshold of 34  $\mu\text{g L}^{-1}$  for both negative and positive responding fish, but there was more uncertainty around the declining species component (90% CI of 27–598  $\mu\text{g L}^{-1}$ ) than the increasing species component (90% CI of 13–52  $\mu\text{g L}^{-1}$ ) (Table 2, Fig. 5e).

None of the fish species revealed significant declines in association with N enrichment. Red shiners and bullhead minnows (*Pimephales vigilax* Baird and Girard)



**Fig. 5** Change in fish assemblage structure (nMDS axis 1 from Fig. 3a) across surface water TP and TN ( $\mu\text{g L}^{-1}$ ) gradients spanning 38 sample sites (a, b). Significant fish indicator taxa identified in threshold indicator taxa analysis (TITAN) across surface water TP and TN ( $\mu\text{g L}^{-1}$ ) gradients spanning 38 sample sites (c, d). TITAN sum (z-) (aggregate response of negative indicator taxa, black symbols) and sum (z+) (positive indicator taxa, open symbols) are shown in response to TP and TN enrichment (e, f). See Fig. 4 legend for explanation of figure symbols.

were significantly higher in occurrence and abundance when TN concentrations were  $>280 \mu\text{g L}^{-1}$ , and carp increased significantly at sites where TN concentrations were  $>546 \mu\text{g L}^{-1}$  ( $446\text{--}1892 \mu\text{g L}^{-1}$ ) (Fig. 5b, See Table

S7 in Supporting Information). TITAN identified a TN threshold beyond which tolerant species such as red shiner and carp are expected to increase within the assemblage (Table 2), but this value should be treated with

caution as there was no clear peak in the sum(z) score (Fig. 5d).

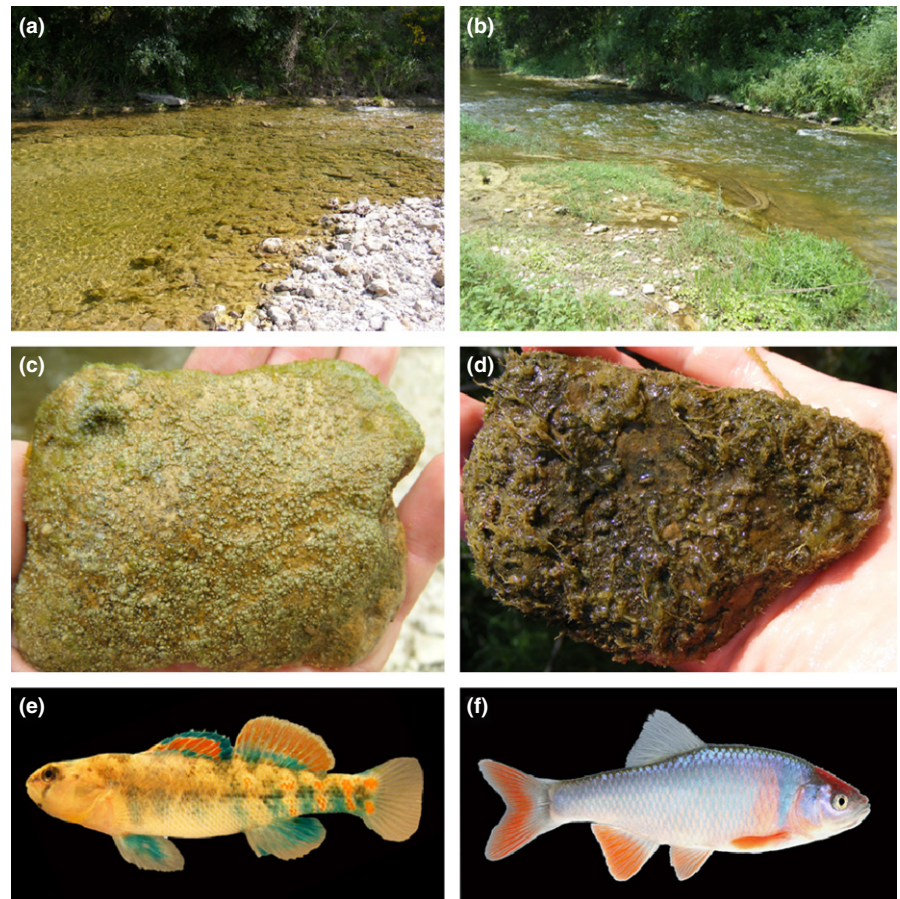
## Discussion

Low levels of nutrient enrichment, particularly P, corresponded with significant nonlinear responses of periphyton nutrient content and markedly lower abundances of many alga taxa and two benthic fish species that represent two distinct functional groups (central stoneroller = periphyton grazer; orangethroat darters = riffle-dwelling, benthic invertivore). Previous studies have reported changes in periphyton assemblages based on biotic indices at relatively low levels of P enrichment (Gaiser *et al.*, 2005; Richardson *et al.*, 2007; Stevenson *et al.*, 2008a). Our study provides new insights into periphyton assemblage responses to nutrient enrichment by demonstrating rapid nonlinear change in bulk periphyton nutrient content and abrupt, negative changes in abundance for many taxa with small increases in P. This, combined with positive changes in the abundance of several tolerant taxa associated with increasing P enrichment, contributed to overall changes

in periphyton assemblages. Two fish species revealed significant, nonlinear declines in association with low levels of P enrichment. In contrast, a tolerant native minnow (red shiners) that is invasive in many regions of North America, and an invasive exotic species (common carp), occurred more often and in greater abundance at sites with higher P. Our results suggest that large changes in assemblage structure of primary producers, as well as distributions of native and non-native fish species involved in strong interactions within stream food webs, are associated with small changes in nutrient status of streams (Fig. 6). These results can be used to help water resource managers identify ecologically relevant criteria that are protective of naturally P-limited streams.

### *Phosphorus as a driver of change in stream ecosystem structure*

Nitrogen covaried with P across our study sites, and in every case of elevated P, N was also elevated above background. However, almost all of the species that had significant negative responses associated with the



**Fig. 6** Representative differences in stream habitat (a, b), periphyton assemblages (c, d) and fish indicator species (e, f) between low nutrient (TP =  $7 \mu\text{g L}^{-1}$ ; TN =  $213 \mu\text{g L}^{-1}$ ) (a, c, e) and slightly enriched (TP =  $66 \mu\text{g L}^{-1}$ ; TN =  $450 \mu\text{g L}^{-1}$ ) (b, d, f) streams from central Texas. Fish indicator species are (e) orangethroat darter (*Ettheostoma spectabile*) and (f) red shiner (*Cyprinella lutrensis*).

N-enrichment gradient were a subset of species that also had negative responses associated with P enrichment. Uncertainty around assemblage-level thresholds was much higher based on TN versus TP, and trends in overall assemblage structure (nMDS axis 1) in response to nutrient enrichment indicated that sites with high TN but low TP were associated with structures typically associated with low-P ecosystems.

Additionally, patterns of association between periphyton nutrient content and N enrichment were always weaker than those between periphyton nutrient content and P enrichment. Uncertainty in periphyton nutrient content models based on surface water TN was driven primarily by the fact that certain streams had high N but relatively low P, and always had nutrient ratios consistent with low-P environments rather than high N. These sites were in catchments draining row-crop agriculture which can be a substantial non-point source of both N and P (Carpenter *et al.*, 1998). However, croplands in our study area appeared to contribute mostly N and little P to streams, and under those conditions, stream biota appeared to be relatively unresponsive. Negative associations between periphyton C : N and N : P and P enrichment demonstrate that N is important in central Texas streams, but it is unlikely that there would have been a strong response to N alone under very low P. Virtually, all sources of P in our study area as well as in most parts of the world are also sources of N, whereas not all sources of N, particularly row-crop agriculture, are sources of P. P enrichment appears to be the primary driver of ecological responses to nutrient gradients in our study area.

#### *Stream assemblage patterns*

Our results contribute to growing evidence for nonlinear responses of aquatic assemblages to nutrient enrichment (King & Richardson, 2003; Richardson *et al.*, 2007; Stevenson *et al.*, 2008b; Evans-White *et al.*, 2009). Many studies have reported linear responses (Miltner & Rankin, 1998; Pan *et al.*, 2000; Johnson & Hering, 2009; Justus *et al.*, 2010) or subsidy-stress relationships (King & Richardson, 2007) between aggregate measures of stream assemblage structure (metrics, ordination axes) and increasing nutrient enrichment. Whereas linear responses provide evidence of linkages between nutrient enrichment and changes in biotic assemblages in streams, these are limited in application to nutrient criteria development and can be an artefact of univariate measures and associated analyses techniques that simplify complex responses to novel environmental gradi-

ents (Baker & King, 2010; King & Baker, 2010). We observed a nonlinear trend in periphyton assemblage structure associated with increasing P based on an aggregate measure (nMDS axis 1) (Fig. 4a). This trend may be interpreted differently in terms of management thresholds, depending on different threshold analyses (Dodds *et al.*, 2010). For example, univariate threshold detection techniques that identify assemblage-level change based on a change in slope (piecewise regression) would likely identify a threshold at higher concentrations than assemblage-level thresholds identified by TITAN. The cumulative distribution of assemblage-level change points estimated by bootstrapping in TITAN represents a zone of rapid change that corresponded well with the zone of greatest change in nMDS axis 1 in the GAM (Fig. 4a). Observed TITAN assemblage-level thresholds corresponded with the lower end of that distribution, where assemblage-level change starts to happen. Combining more traditional data analysis approaches with TITAN or other approaches that separate negative and positive responses within assemblages will help resource managers to identify dominant negative and positive responders within the assemblage, and estimate (with confidence intervals) where major shifts in assemblage structure occur. Current studies are starting to utilise this approach in assessing assemblage-level responses to novel environmental gradients in both freshwater (King *et al.*, 2011; Kail, Arle & Jähnig, 2012; Smucker, Detenbeck & Morrison, 2013) and terrestrial (Cardoso *et al.*, 2013; Payne *et al.*, 2013; Suarez-Rubio *et al.*, 2013) ecosystems. Such an approach may lead to more proactive nutrient criteria for high-quality streams by identifying TP concentrations where change starts to happen, versus concentrations where changes in assemblage structure has already occurred.

Threshold responses in periphyton assemblage structure can be used to help managers identify ecological relevant criteria that are protective of naturally P-limited streams and prevent shifts from periphyton assemblages adapted to low-P conditions to those that flourish in eutrophic conditions. Additionally, our results may have applicability outside of our study region, as many indicator species for low-P and high-P conditions identified in the current study have been identified in other regional studies and at the national scale. Several of the alga taxa that had negative associations with P enrichment in our study have been shown to have optima at relatively low levels of TP (Stevenson *et al.*, 2008a; Wang *et al.*, 2009; Justus *et al.*, 2010), and all corresponded particularly well with the low-P indicator taxa identified by Potapova and Charles (2007) for streams in the United

States. In every case, species with high abundance associated with low TP concentrations in our study had previously been identified as significant indicator species of low-P conditions. Species that had higher abundances in association with greater concentrations of P also agreed well with high-P indicator taxa identified by Potapova and Charles (2007) and those used by others in diatom indices that measure responses of tolerant species to nutrient enrichment (Stevenson *et al.*, 2008a,b).

Fish assemblages showed stronger relationships with pasture coverage than P enrichment or related variables, suggesting that other pasture-related stressors, such as increased bank erosion and mud/silt substrata, may have contributed to fish patterns (Fig. 3b). However, widespread application of manure on pasturelands in our study region can elevate P in shallow, subsurface ground water that flows into stream bank sediments during low-flow periods, and this can result in substantial inputs of sediment-bound P to streams via bank erosion (Thompson & McFarland, 2010). Observed latitudinal gradients and basin differences in fish assemblage structure may be caused, at least in part, by inter-basin differences in pasture coverage.

Despite a relatively weak correlation between P enrichment and fish assemblage structure (Figs 3b & 5a), TITAN identified species that had negative and positive associations with increasing P. In particular, two benthic species, orangethroat darters and central stonerollers, showed markedly lower abundance as surface water TP increased beyond relatively low concentrations. In contrast, red shiners and carp were more abundant in sites with greater nutrient concentrations. Negative fish responses to nutrient enrichment may be due to intolerance to environmental conditions associated with increased primary production, particularly in streams with natural summer low flows. Valenti *et al.* (2011) observed that low-flow periods and P enrichment interact to increase the magnitude of diel fluctuations in dissolved oxygen and pH across streams within our region. Dissolved oxygen fluctuations in enriched streams could have exceeded the physiological limits of orangethroat darters and central stonerollers within isolated pools during low-flow conditions (Miltner & Rankin, 1998; Miltner, 2010). Dissolved oxygen fluctuations may have been ameliorated by flow augmentation from effluent discharge in some high-P sites, contributing to increased variation in fish assemblage structure at high-P sites (Fig. 5a). Source-sink dynamics associated with variation in toxic  $\text{NH}_4$  releases coupled with recolonisation dynamics from adjacent low-nutrient habitats (Waits *et al.*, 2008) could contribute to higher variation in fish

assemblage structure at effluent-dominated sites with high P.

#### *What are the implications for nutrient management in streams?*

This study presents multiple lines of evidence that support the development of nutrient criteria at low levels of P (*c.*  $20 \mu\text{g L}^{-1}$ ) to maintain natural periphyton and fish assemblages in our study region. First, we observed nonlinear changes in bulk periphyton elemental ratios at low levels of P enrichment, particularly for C : P ratios which never fell below 300 until TP approached  $20 \mu\text{g L}^{-1}$ . Second, there was a cumulative decline of sensitive species within periphyton assemblages around  $21 \mu\text{g L}^{-1}$  TP (90% CI of  $18\text{--}48 \mu\text{g L}^{-1}$ ) that corresponded with an overall nonlinear shift in assemblage structure (depicted by nMDS axis 1). Third, we observed significant declines in two functionally important benthic fish species (central stonerollers and orangethroat darters) associated with similar levels of P enrichment,  $28 \mu\text{g L}^{-1}$  (90% CI:  $24\text{--}56$ ) and  $34 \mu\text{g L}^{-1}$  (90% CI:  $21\text{--}56$ ), respectively. These findings, combined with positive changes in abundance of colonising algae species as well as tolerant minnows and invasive carp associated with increasing concentrations of TP (*c.*  $21\text{--}187 \mu\text{g L}^{-1}$ ), support our assertion that major shifts in stream ecosystem structure occur at low levels of P enrichment in streams (Fig. 6). However, it is important to recognise that observed responses are limited to our data set and confidence intervals represent the expected range where shifts are likely to occur 90% of the time. Managers should apply a precautionary approach and consider the lower portion of these distributions when developing nutrient criteria to ensure adequate protection for nutrient-limited stream ecosystems. While meeting such criteria may be infeasible for many streams, particularly those that receive significant effluent discharge, such criteria will provide benchmarks for preventing impacts to existing low-nutrient stream ecosystems.

This approach moves beyond setting management standards for eutrophic systems by recognising that significant changes in stream assemblages that have evolved under low nutrient conditions can occur with slight enrichment (Fig. 6). However, managing sources of P should also include best management practices for N reduction. N enrichment covaried with P across our study sites, and Lang, King and Scott (2012) demonstrated that stream periphyton in this region can be co-limited by both N and P. While assemblage and species-level thresholds identified in the current study may serve as starting

points for establishing criteria, managers also should consider the influence of N enrichment on downstream waterbodies with different nutrient limitation status when developing nutrient criteria, because N generally is the nutrient that most directly impacts estuaries and associated coastal ecosystems (Smith *et al.*, 2006).

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### Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Table S1.** Correlation coefficients between landscape variables.

**Table S2.** Correlation coefficients between instream variables.

**Table S3.** Correlation coefficients between water quality variables.

**Table S4.** Declining ( $z^-$ ) and increasing ( $z^+$ ) taxa results from Threshold Indicator Taxa Analysis (TITAN) of periphyton species composition in response to surface water total phosphorus (TP,  $\mu\text{g L}^{-1}$ ) in central Texas streams.

**Table S5.** Declining ( $z^-$ ) and increasing ( $z^+$ ) taxa results from Threshold Indicator Taxa Analysis (TITAN) of periphyton species composition in response to surface water total nitrogen (TN,  $\mu\text{g L}^{-1}$ ) in central Texas streams.

**Table S6.** Declining ( $z^-$ ) and increasing ( $z^+$ ) taxa results from Threshold Indicator Taxa Analysis (TITAN) of fish species composition in response to surface water total phosphorus (TP,  $\mu\text{g L}^{-1}$ ) in central Texas streams.

**Table S7.** Declining ( $z^-$ ) and increasing ( $z^+$ ) taxa results from Threshold Indicator Taxa Analysis (TITAN) of fish species composition in response to surface water total nitrogen (TN,  $\mu\text{g L}^{-1}$ ) in central Texas streams.

**Figure S1.** Scatterplots of nutrient measures and dominant sources.

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