



August 26, 2019

Office of the Chief Clerk
Texas Commission on Environmental Quality
P.O. Box 13087
Austin, Texas 78711-3087

VIA ELECTRONIC FILING

**RE: Comments on RR 417's application for draft permit for
TPDES Permit No. WQ0015713001**

Dear Chief Clerk:

Save Our Springs Alliance (SOS) submits these comments on the draft permit issued to RR 417, LLC (RR 417 or Applicant) for Texas Pollution Discharge Elimination System Permit (TPDES) No. WQ0015713001. If issued, this permit would authorize the construction of a new wastewater treatment facility and discharge of up to 49,000 gallons of treated wastewater per day into Commissioners Creek, then Hondo Creek (in Segment No. 2114 of the Nueces River Basin), then the Frio River, then to the Nueces River.

SOS is a non-profit organization that works to protect the Edwards Aquifer, its springs and contributing streams, and the natural and cultural heritage of the Hill Country region and its watersheds. SOS has members who own property downstream of the proposed discharge point and who own property adjacent to the site of the proposed wastewater treatment facility. These members would be adversely affected by RR 417's application. SOS also has members who recreate in Hondo Creek downstream of its confluence with Commissioners Creek, and who enjoy recreating and observing wildlife in the Hill Country State Natural Area, adjacent to the WWTP property.

SOS strongly objects to direct discharge of sewage effluent into Commissioners Creek and Hondo Creek due to the high potential of negative impacts to this crystal clear water. TCEQ should deny this permit, and applicant should pursue a Texas Land Application Permit (TLAP) and/or alternative means to dispose of all of its wastewater.

Specifically, SOS objects to this draft permit for the reasons below.

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1. The Effluent Limits in the Draft Permit Do Not Sufficiently Protect the Edwards Aquifer and the Receiving Surface Waters for the People and Wildlife that Rely on Them.

a) Impact on the Edwards Aquifer. There are numerous human-health risks associated with the contamination of the Edwards Aquifer due to wastewater plant effluent. The proposed wastewater treatment plant (WWTP) and discharge point are within the Contributing Zone of the Southern segment of the Edwards Aquifer, just upstream of the Recharge Zone. The Edwards Aquifer provides drinking water for the San Antonio in addition to a range of recreational, agricultural, and ecological uses. The discharge authorized by the draft permit will put these water sources, and the people who rely on them, at risk.

The proposed effluent discharge point is in a flow route less than five miles from the Edwards Aquifer recharge zone. However, because the discharge point is in Bandera County (very close to the Medina County line), it is not subject to the Edwards Aquifer Rules' as a result of the regulatory definition of "Edwards Aquifer." These rules require that discharges of treated wastewater within zero to five miles upstream from the recharge zone achieve effluent levels of (based on a 30-day average):

- 5 mg/L carbonaceous biochemical oxygen demand (CBOD)
- 5mg/L Total Suspended Solids (TSS)
- 2 mg/L Ammonia nitrogen (NH₄)
- 1 mg/L phosphorus

30 TAC § 213.6(c). The effluent limits in the draft permit for the first three pollutants are multiples higher than this at 10 mg/L CBOD, 15 mg/L TSS, and 3 mg/L NH₄.

The fact that the discharge point is just outside of the regulated area of the Edwards Aquifer does not preclude or exempt TCEQ from establishing effluent limits that protect the Edwards Aquifer. The stated purpose of the Edwards Rules declares that "Nothing in this chapter is intended to restrict the powers of the commission... to prevent, correct, or curtail activities that result or may result in pollution of the Edwards Aquifer or hydrologically connected surface waters." 30 TAC § 213.1(2). The scientific basis for requiring the higher effluent standards still applies regardless of whether the discharge point is in a county explicitly regulated by the Edwards Aquifer rules. And the discharge point's proximity to the Edwards Aquifer Recharge Zone incurs a significant risk that sewage spills would contaminate both surface water and groundwater.

Moreover, the path through the contributing zone would often consist primarily of effluent, as Commissioners Creek and Hondo Creek are dry or low-flow during much of the year along these creek segments. There is little soil or vegetation in Commissioners Creek or Hondo Creek to absorb the excess nutrients and contaminants associated with the effluent, prior to entry into the recharge zone and the Edwards Aquifer. At the beginning of the Edwards Aquifer recharge zone, the effluent would seep from Hondo Creek into the Edwards Aquifer. Therefore, introducing a stream of effluent into Commissioners Creek would create a path from the wastewater treatment plant almost directly into the Edwards

Aquifer, which provides drinking water for 1.7 million Texans via numerous local wells. This effluent would have high levels of nitrogen and phosphorous and contain Contaminants of Emerging Concern (CECs) including pharmaceuticals, surfactants, and other pollutants. Thus, although SOS urges TCEQ not to issue a discharge permit, if such a permit is issued, it should have effluent limits at least as stringent as those found in 30 TAC § 213.6(c).

b) Impacts on Hondo Creek. In evaluating wastewater permits, TCEQ must consider the baseline conditions in the receiving stream, the physical and hydrological characteristics of the stream, water body uses, and the associated water quality standards that protect those uses. Hondo Creek is a classified stream segment (Segment 2114 of the Nueces River Basin), designated for primary contact recreation, high aquatic life use, public water supply, and aquifer protection. There are currently no permits allowing direct discharge into Hondo Creek or any streams in the upper Nueces River basin. The discharge authorized by the draft permit would impair the designated uses and degrade its high water quality.

Hondo Creek runs approximately sixty-seven miles from its source west of Tarpley, Texas to its confluence with the Frio River. The point at which the effluent would enter Hondo Creek (the confluence with Commissioners Creek) is approximately ten miles downstream from the Hondo Creek headwaters, so most of Hondo Creek (fifty-seven miles) would be impacted by this permit. The four miles of Hondo Creek below the confluence with Commissioners Creek consists of shallow, clear water that is used for recreation (swimming, fishing, and tubing) and watering of cattle by local ranches that border Hondo Creek.

Because of the low-nutrient levels in this section of Hondo Creek, the introduction of high-nutrient effluent would have a dramatic impact on water quality, creating algae blooms and contaminating Hondo Creek. Beyond this four-mile segment, Hondo Creek enters the Edwards Aquifer recharge zone. The ten-mile section of Hondo Creek in the recharge zone consists of alternating reaches of dry limestone creek and often lengthy pools of clear water which are home to fish and other aquatic life. These pools are small local ecosystems, supporting surrounding wildlife and vegetation. The proposed permit would not only impact the Edwards Aquifer as described in section (a) above, but these pools would change from clear, pristine life-supporting water bodies to algae-covered, low-oxygen nutrient-heavy ponds. Fish and other aquatic life that reside in these pools would not be able to escape to more oxygenated water and would likely die off.

More specifically, the phosphorus allowed in the draft permit will degrade Commissioners Creek and Hondo Creek in violation of the Clean Water Act and harm aquatic life. The total phosphorus (TP) limit in the draft permit is 0.5 mg/L, which is much higher than likely background levels in both Commissioners Creek and Hondo Creek.

Dr. Ryan King has published a number of studies on the low levels of background TP limits in Hill Country streams, as well as the effects of adding TP to these naturally low-nutrient streams. These studies show that the addition of TP into such creeks in amounts even smaller than those allowed under the draft permit can cause irreversible environmental

and ecological effects on the receiving streams. One of the studies was commissioned by TCEQ for the purposes of developing nutrient criteria for waters in Texas.

The studies are attached to these comments and include:

King, R.S. Development of Biological Indicators of Nutrient Enrichment for Application in Texas Streams. Prepared for TCEQ, Section 106 Water Pollution Control Grant #98665304.

Taylor, J.M., King, R.S., Pease, A.A., & Winemiller, K.O. (2014). Nonlinear response of stream ecosystem structure to low-level phosphorus enrichment. *Freshwater Biology*, 59, 969-984.

Taylor, J.M., Back, J.A., Brooks, B.W., & King, R.S., (2018), Spatial, temporal and experimental: Three study design cornerstones for establishing defensible numeric criteria in freshwater ecosystems, *Journal of Applied Ecology*, 2018; 1-10.

2. The Minimum Dissolved Oxygen Levels Should be Increased to at least 6.0 mg/L.

The draft permit requires that the effluent have a minimum dissolved oxygen level of 4.0 mg/L. This level, in combination with the oxygen-reducing effects of algae caused by the increased nutrients, will have negative effects on the existing aquatic life in Commissioners Creek and Hondo Creek. Hondo Creek is designated as having high aquatic life use with a corresponding numeric criterion of 5.0 mg/L dissolved oxygen. However, existing dissolved oxygen levels are likely much higher, and it is these baseline conditions against which the effects of the discharge must be measured.

Allowing the discharge with nutrient levels far above background levels and dissolved oxygen levels far below background levels will cause a reduction in dissolved oxygen levels representing degradation beyond a de minimis amount.

3. The Mandate to Reuse Wastewater is a Positive Step, but the Applicant Should Not Be Limited in the Amount to be Reused, and Adequate Storage Should be Required.

The draft permit, Other Requirement 8, states that “the permittee shall reuse 75% of the treated effluent generated on a monthly basis.” We support the beneficial reuse of effluent both to reduce discharge into sensitive Hill Country streams and to minimize use of potable water for irrigation. However, we suggest amending the language to include the words “at a minimum” or “at least” in the above sentence. As currently written, the draft permit suggests that the permittee must reuse 75% of its effluent—no more, no less. But if there are circumstances under which there is a beneficial use for more than 75% of the effluent (for example, in dry years), the Applicant should be allowed to use more.

We further insist that the mandate to use reclaimed water also include a requirement that a reservoir of sufficient size be constructed into which reclaimed water can be stored until it can be used for beneficial reuse. This will maximize the beneficial reuse of the effluent and thus minimize the need to discharge water into the creeks. Additionally, the storage

reservoirs can provide a buffer in the event of a malfunction at the plant, reducing the risk that untreated water be discharged.

In the event the Applicant is unable to reuse all of the wastewater generated, SOS recommends that the remaining amounts be land applied, with RR 417 purchasing the necessary land for such and obtaining the requisite Texas Land Application Permit (TLAP) from TCEQ. Alternatively, any permit issued should include a required minimum streamflow in Commissioners and/or Hondo Creeks below which discharge is prohibited, to ensure that effluent does not overwhelm the creeks under no or low-flow conditions.

We urge the TCEQ to protect Commissioners Creek, Hondo Creek, the Frio and Nueces Rivers, and the Edwards Aquifer from degradation by denying RR 417's application and issuing a no-discharge permit. SOS files these comments specifically in response to the TCEQ's preliminary decision, and we reserve our right to request a contested case hearing.

Thank you for your consideration.

Sincerely,



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Final Report

For the Project

**DEVELOPMENT OF BIOLOGICAL INDICATORS OF NUTRIENT
ENRICHMENT FOR APPLICATION IN TEXAS STREAMS**

6 October 2009

§106 Water Pollution Control Grant # 98665304

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ACKNOWLEDGMENTS

Financial support of this study was provided by the Texas Commission on Environmental Quality to Ryan S. King at Baylor University and Kirk O. Winemiller at Texas AgriLife for “Development of biological indicators of nutrient enrichment for application in Texas streams,” Section 106 Water Pollution Control Grant # 98665304, and to Kirk O Winemiller and Ryan S. King for “Refinement and Validation of Habitat Quality Indices (HQI) and Aquatic Life Use (ALU) Indices for Application to Assessment and Monitoring of Texas Surface Waters” Contract 582-6-80304. We thank Mark Fisher, Clay Sebek, Lori Hamilton, Gregg Easley, and others from the TCEQ for assistance in managing both of these contracts. Numerous graduate and undergraduate students from Baylor and Texas A&M contributed to the collection of the data used in this report. We especially thank Dr. Barbara Winsborough, of Winsborough Consulting, Leander, TX, for her hard work and rapid delivery of algae species identifications from both the field and experimental studies. Dr. Winsborough conducted all algal species identifications reported in this document.

The TCEQ has committed to the development of nutrient criteria for waters in Texas as presented in the November 3, 2006 draft of the Nutrient Criteria Development Workplan. Under that plan the TCEQ is exploring several complementary strategies to develop nutrient criteria. Strategies now being investigated include the following: 1) basing criteria on concentrations of nutrients; 2) basing criteria on direct indicators of eutrophication, such as chlorophyll a; 3) developing “translator” procedures that relate concentrations of nitrogen and phosphorus to direct indicators of eutrophication; 4) basing criteria on historical “ambient” averages with a statistical allowance for variability; and 5) developing criteria based on the effect of nutrients or indicators of eutrophication on uses.

This study is only one component of the larger water quality standard criteria development process that will involve a diverse stakeholder workgroup and formal public participation process to establish regulatory criteria. For this study the choice of data analysis, presentation and interpretation of results, and the report conclusions and recommendations are those of the Principal Investigators and not those of the Texas Commission on Environmental Quality (TCEQ). No official endorsement of the TCEQ should be inferred.

GENERAL DESCRIPTION OF THE SPECIAL STUDY

Water quality criteria and standards are developed by States to improve water quality and to ensure that a water body is supportive of its designated aquatic life uses (ALU). Nutrient pollution caused by excessive loading of nitrogen (N) and/or phosphorus (P) can significantly limit the ability of streams to support their designated ALUs. In response to this problem, the U.S. EPA published its National Strategy for the Development of Regional Nutrient Criteria (1998), which detailed a comprehensive plan to be used by States for the development of scientifically defensible, numerical criteria for nutrients. The plan emphasized the need for the inclusion of endpoints that reflect the biological integrity of aquatic ecosystems and are supportive of ALUs. However, relatively few States have been sufficiently equipped with the necessary nutrient and biological data that could be used to develop defensible regional criteria, particularly for wadeable streams. Consequently, many States are either struggling to develop criteria or have had to implement numerical nutrient criteria based on regional EPA guidance without a clear understanding of the implications of these criteria to supporting biological integrity and ALUs in their respective States.

Texas has made significant progress in the development of nutrient criteria for reservoirs, but limited research has been done in wadeable streams. Because streams function differently than reservoirs, indicators of nutrient-related degradation that are used in reservoirs may have limited applicability in streams (e.g., surface-water chlorophyll a). In streams, attached vegetation (algae, bacteria, fungi, and macrophytes) and associated animals (macroinvertebrates, fish) are the biological indicators most likely to be affected by nutrient enrichment. Identifying linkages between surface-water nutrients (e.g., total phosphorus, total nitrogen) and biological indicators of aquatic life uses in streams is therefore imperative for development of defensible, numerical nutrient criteria.

We evaluated new indicators of nutrient-related alteration to wadeable stream ecosystems by bridging two complementary ongoing projects in the Subhumid Agricultural Plains (SAP) ecoregion of Texas. (1) a TCEQ-funded study on refinement and validation of habitat quality indices (HQI) and fish Index of Biotic Integrity in 64 streams in the SAP ecoregion, directed by

K. O. Winemiller of Texas A&M University (TAMU) with R. S. King of Baylor University (BU) (Winemiller et al. 2009), and (2) a US EPA Region 6 funded study on nutrient criteria development in the Cross Timbers subregion of the SAP of Texas, directed by R.S King at BU (Appendix B). At the initiation of the EPA study, BU intentionally selected 26 of their TCEQ HQI sites for their EPA study with the goal of maximizing physical, chemical and biological information for criteria development. However, sites sampled by TAMU located in the Blackland Prairies and East Central Texas Plains ecoregions of the SAP have not been sampled for nutrients or other nutrient indicators, as well as some additional HQI study sites sampled by BU in the Cross Timbers. Thus, we expanded sampling of surface-water nutrients and a few key biological indicators (periphyton metrics) to the 12 additional HQI sites not sampled for nutrients by BU and all 26 sites studied by TAMU as part of the TCEQ HQI study, thus adding nutrients and periphyton to the ongoing habitat and fish assessments at 64 stream sites in summer 2008. The linkage between the EPA and TCEQ studies effectively added a considerable amount of information for relatively little cost because the additional sample collection took place during site visits already planned for fish and habitat assessment. This overarching goal of the study was to help Texas in its ongoing effort to develop defensible, effects-based nutrient criteria in wadeable streams.

The specific objectives of this study were:

- 1) Compare surface-water total phosphorus (TP) and total nitrogen (TN) values measured by Baylor University with values measured by TCEQ. BU and TCEQ labs differ in minimum detectable limits (MDL) between methods for TP, and use different methods altogether for TN;
- 2) Evaluate the utility of soft-substrate periphyton (epilithon/epilithon) as an indicator of nutrient enrichment in soft-bottomed streams of the Texas Blackland Prairies and E. Central Texas Plains, and contrast responses of periphyton to nutrients in these ecoregions with those of the Cross Timbers (epilithon, or rock-substrate periphyton).
- 3) Estimate thresholds, if present, in periphyton (nutrient content, biomass, and species composition) and other biological variables measured in the habitat assessment (e.g., microalgae/biofilm cover, macrophyte cover) in response to surface-water TP, TN, and other indicators of stream condition such as sedimentation (e.g., mud-silt cover, substrate

embeddedness), as well as the potential drivers of these stressors (pasture, rowcrop, impervious cover, WWTP outfalls);

- 4) Evaluate responses of fish communities to nutrient enrichment, sedimentation, and drivers of those stressors;
- 5) Recommend responsive ecological indicators and identify nutrient concentrations (thresholds) that correspond to changes in ecological indicators for potential use in nutrient criteria development and stream assessments.

STUDY AREA AND STREAM SAMPLING

Study area

Data were collected from 64 wadeable streams in the Brazos and Trinity River basins within the Cross Timbers, Blackland Prairies, and East Central Texas Plains ecoregions (Figures 1-3). The Cross Timbers ecoregion (ECO 29) is a mosaic of forest, woodland, savanna, and prairie and is currently used mostly for rangeland and pastureland. The Texas Blackland Prairies ecoregion (ECO 32) is a disjunct ecological region distinguished from neighboring regions by fine-textured, clayey soils. This region was historically tallgrass prairie and now contains a higher percentage of cropland than adjacent ecoregions. In addition, large areas of the ecoregion are being converted to urban and industrial uses. The East Central Texas Plains ecoregion (ECO 33) was historically covered by post oak savanna and now is used primarily for pasture and rangeland (Griffin et al. 2004).

Watershed variables describing physical characteristics and topography, land use, and distribution of hydrologic disturbance points (outfalls and dams) were calculated for each site. Watershed boundaries for each sample site were automatically digitized in ArcGIS 9.2 with the ArcHYDRO 9 extension using a 1:24,000 scale digital elevation model (DEM) expressed as a 30 m raster, available from the U. S. Geological Survey. Mean slope and elevation were calculated for each watershed using the digital elevation model. Mean annual precipitation was calculated for each watershed from a polygon coverage of average monthly and annual precipitation. This dataset was obtained from USDA-NRCS. Number of wastewater outfalls and cumulative permitted outfall discharge (MGD) were calculated for each watershed based on the TCEQ municipal and industrial wastewater outfall shapefile available from <http://www.tceq.state.tx.us/gis/sites.html>. Landcover class percentages were calculated for each watershed using National Land Cover Database (NLCD 2001) available from http://www.mrlc.gov/nlcd_multizone_map.php. All watershed analyses were performed with ArcGIS 9.2 (ESRI, Redlands, CA.).

We sampled the 64 streams in the summer (June, July, and August) of 2008. At each stream site, a 160-500 m study reach was designated for periphyton and water chemistry sampling, fish

collection and local habitat measurements. Reach length was determined based upon the wetted width of the stream (approximately 40 times the average width). Study reach selection, fish collection, and habitat measurements were performed following the protocols of TCEQ Surface Water Quality Monitoring Procedures (TCEQ 2003, 2004).

Local-scale environmental variables

At each study site, we measured 57 habitat variables (Appendix A1) including substrate composition, instream cover, wetted width, depth, canopy cover, bank slope, riparian buffer width, dissolved oxygen (instantaneous), conductivity, and pH on the same dates as fish sampling. We made these measurements at 5 to 6 evenly spaced transects (depending on reach length). Some measurements, such as number of riffles, maximum pool depth, stream sinuosity, and composition of riparian vegetation, were summarized for the entire study reach. Discharge (in ft³/sec) was also measured along a representative transect within each reach using a portable electromagnetic flow meter (Marsh-McBirney Flo-Mate Model 2000). These variables and their relation to the HQI and fish communities were analyzed in Winemiller et al. (2009). A few of these variables were examined carefully in this study because they were shown to be related to fish communities in Winemiller et al. (2009), and of their potential as indicators of nutrient or nutrient-related stressors (e.g., microalgae cover, macrophyte cover, substrate embeddedness, mud-silt cover).

Water chemistry and periphyton sampling

Water chemistry sampling consisted of two sets of surface-water instantaneous grab samples and one reach-scale composite of epilithic (removal and compositing of periphyton from surface of at least 25 rocks, if present) or episammic (composite of several fixed-area samples of sand or finer sediments) periphyton.

The first set of surface-water grab samples for total phosphorus (TP) and total nitrogen (TN) analysis at BU were collected in triplicate in accordance with BU's EPA-approved project QAPP. The second set of surface-water grab samples for TCEQ Houston Laboratory analysis of total kjeldahl nitrogen (TKN), nitrate-nitrite-N (NO₃-NO₂-N), ammonia-nitrogen (NH₃-N), total phosphorus, orthophosphate-P (PO₄-P), seston chlorophyll-*a* (CHLA), total alkalinity, chloride,

total suspended solids, volatile suspended solids, sulfate, total dissolved solids, fluoride, and total phosphorus were sampled in accordance with TCEQ Surface Water Quality Monitoring Procedures Volume 1. All field sampling was accomplished by BU and TAMU investigators. In accordance with the applicable procedures and QAPPs, all samples were preserved where necessary, stored at 4°C upon collection, and shipped to BU and TCEQ, respectively, within 24 hours in coolers. The periphyton samples were collected in accordance with the BU nutrient study QAPP.

Periphyton composite samples were handled and analyzed in accordance with the project QAPP. All periphyton physical and chemical analysis was conducted by BU. Periphyton was shipped to BU on ice (4°C) within 24 hours of collection. Periphyton was homogenized and aliquots of known volume were analyzed for the following: total carbon (C), N, and P in the organic (OM) fraction of the periphyton (%); C, N, P per unit dry mass of bulk periphyton (no separation into OM or sediment fractions); ash-free dry mass (AFDM) (g/m²); chlorophyll a (mg/m²); and cell densities of the algae species in the periphyton (no/cm²). Periphyton OM fractions were separated from the bulk (unfractionated) periphyton by suspending aliquots in colloidal silica and centrifuging the mixture to separate sediment or other heavy particles from the lighter algae, bacteria, and other organic matter. Following centrifugation, the OM fraction was rinsed to remove colloidal silica, dried at 60°C for 24 h, pulverized to a fine powder, and analyzed for C, N, and P following Back et al (2008) and Scott et al (2008).

Algae species samples were homogenized, preserved, and identified in accordance with taxonomic methods for soft and diatom algae described in TCEQ (2005). One soft and one diatom taxonomic sample was identified per stream per year. At least 500 diatom and 300 soft algae cells per respective sample were identified (TCEQ 2005). Dr. Barbara Winsborough, an expert periphyton taxonomist from central Texas, performed all of the species identifications in accordance with the approved project plan.

Fish sampling

Within each study reach, all available habitats were sampled using a backpack electrofisher (Smith-Root Model LR-24) and seine net (15' x 6' or 6' x 6'). Crews of 3-4 people electrofished

each study reach in a single upstream pass with a minimum effort of 900 seconds. The reach was then sampled with a seine net with a minimum of six 10-m hauls. Sampling continued beyond the minimum effort until all habitats were sampled and no new species were captured within the study reach. Collected fishes were identified, separated into juvenile and adult age classes, counted, and either released into the habitat or preserved in 10% buffered formalin for later identification. A detailed description of fish community composition and important environmental correlates among the 64 sites is included in Winemiller et al. (2009).

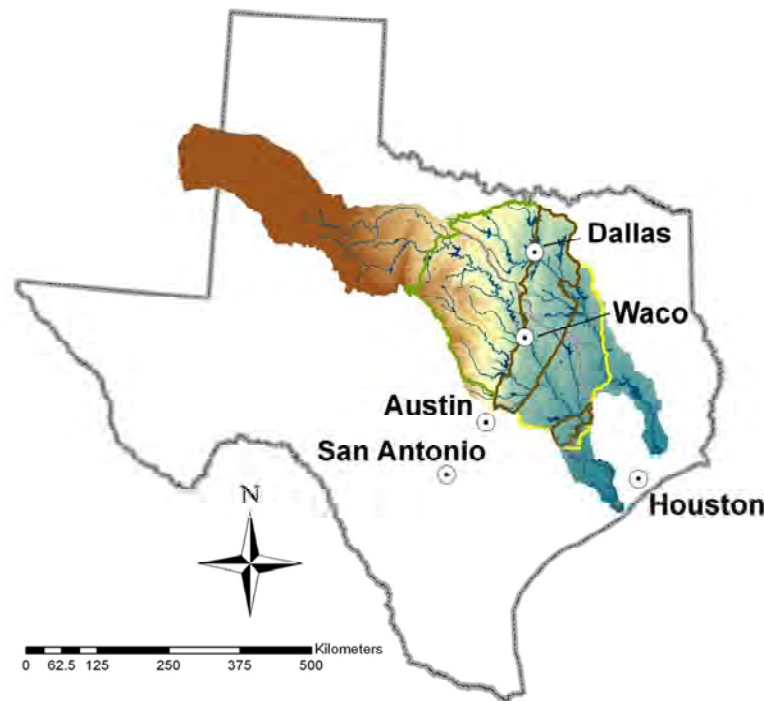


Figure 1. Map showing the study region in the Brazos and Trinity watersheds. Colored lines delineate ecoregion boundaries (green = Cross Timbers, brown = Texas Blackland Prairies, yellow = East Central Texas Plains).

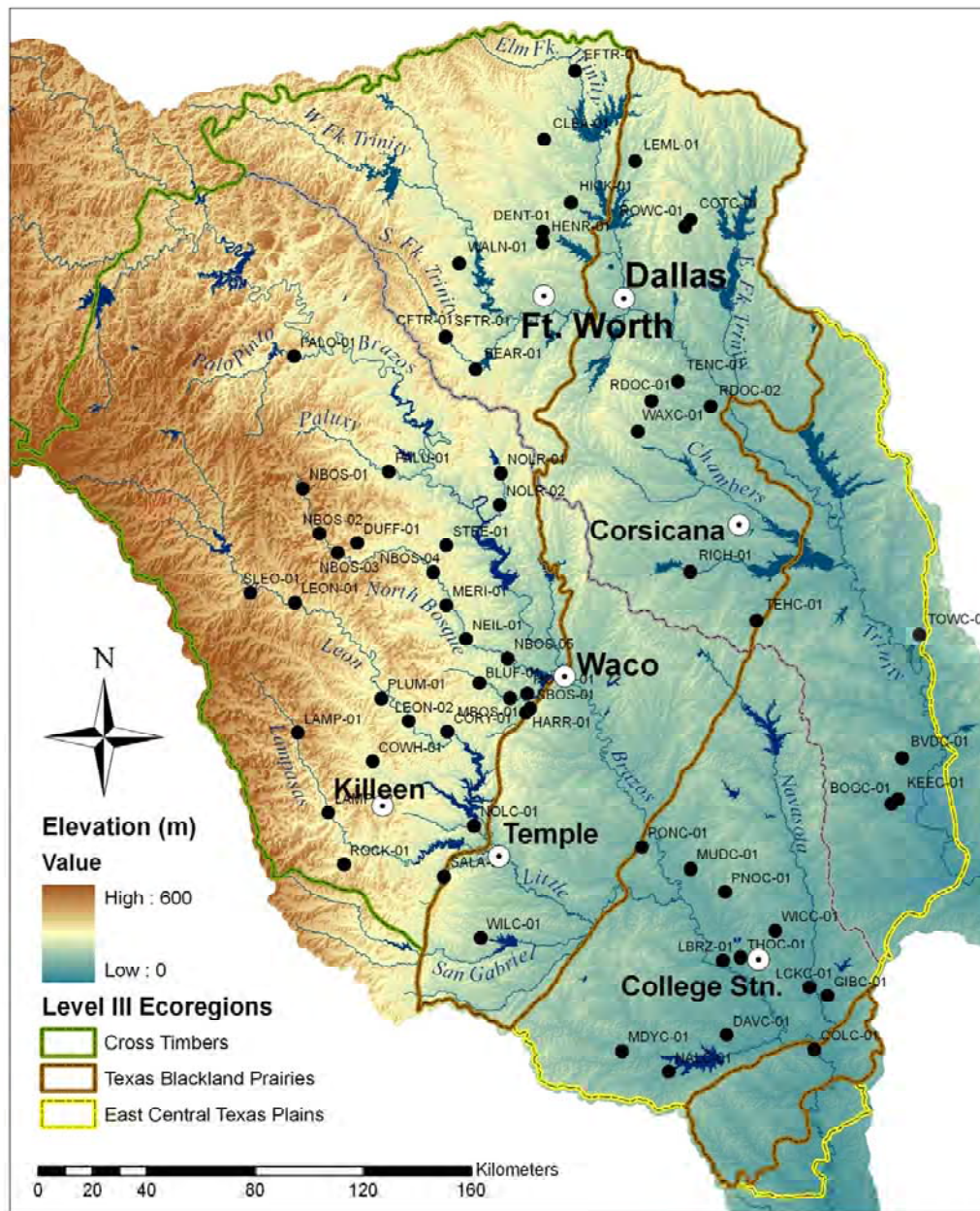


Figure 2. Map showing the 64 study sites and elevation gradients across the Trinity and Brazos basins and the three ecoregions within the SAP.

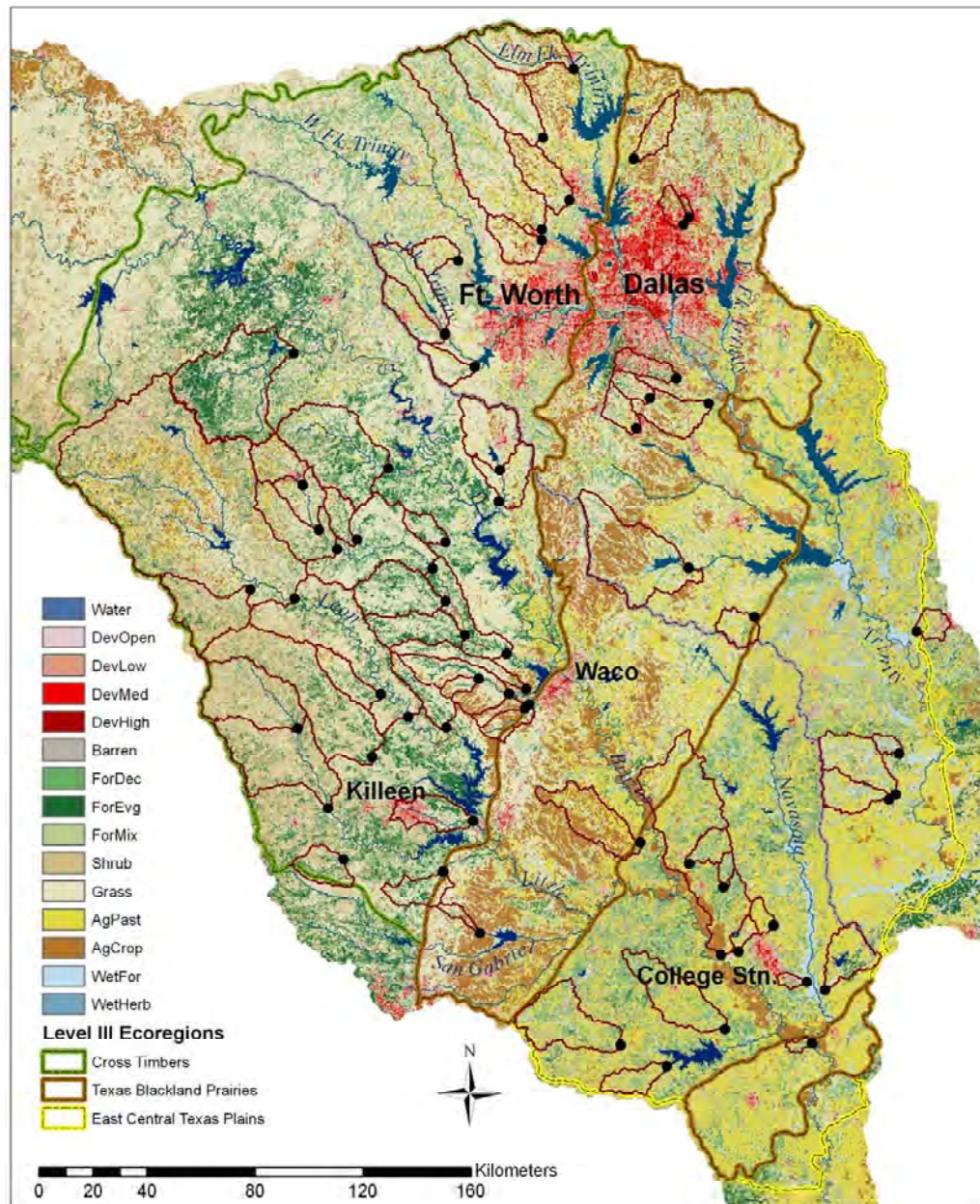


Figure 3. Spatial distribution of dominant land-cover classes among the 26 study watersheds (NLCD 2001).

DATA ANALYSES

We followed the analysis framework outlined by King and Richardson (2003) and the aforementioned EPA Region 6 study (King et al. 2009; Appendix B) to identify variables that were candidate indicators of nutrient-related reductions in biological integrity. Nutrient and response variable data were graphically evaluated to initially screen variables and data sets for relationships that could be reasonably analyzed using threshold statistical techniques, as biological responses to nutrients were likely to be nonlinear and heteroscedastic. Conditional dot plots were used to examine distributions of variables among ecoregions, whereas lattice scatterplots were used to visualize and contrast stressor-response relationships.

We estimated potential threshold responses of univariate biological variables (e.g., periphyton nutrient ratios, chlorophyll a, macrophyte cover, etc) to numerical levels of nutrients or nutrient-related stressors using nonparametric changepoint analysis (nCPA), a technique explicitly designed for detecting threshold responses using ecological data (King and Richardson 2003, Qian et al. 2003). This analysis is based on the fact that structural change in an ecosystem may result in a change in both the mean and the variance of an ecological response variable used to indicate a threshold. **When observations are ordered along an environmental variable (gradient), a changepoint is simply the value that separates the data into the two groups that have the greatest difference in means and variances.** This can also be thought of as the degree of within-group variance relative to the between group variance, or *deviance* (*D*). Analytically, the nCPA examines every point along the stressor gradient and seeks the point that maximizes the reduction in deviance.

There is one particular value of the predictor *y* (e.g, TP) that maximizes the reduction in deviance in the response data (in this case, the selected biological responses); however, there is uncertainty associated with that value. It is unlikely that any one value of the predictor (e.g., TP) is the only value that could represent a changepoint. In reality, depending on the acuteness of the biological change in response to TP, several observations of TP could represent the changepoint, each with varying probabilities. Thus, to assess the risk associated with particular levels of TP, nCPA incorporates estimates of uncertainty in the changepoint (King and Richardson 2003). These estimates are calculated using a bootstrap simulation. This simulation resamples (with

replacement) the original dataset and recalculates the changepoint with each simulation.

Bootstrap simulations are repeated 1,000 times. The result is a distribution of changepoints that summarizes the uncertainty among multiple possible changepoints. This uncertainty is expressed as a cumulative threshold frequency based on the relative frequency of each changepoint value in the distribution.

Multivariate algal and fish species abundance data were handled differently than the univariate biological data. First, important differences (gradients) in species composition and environmental correlates of those gradients were identified using non-metric multidimensional scaling (nMDS). NMDS is a distance based procedure that ordinales study units based on rank dissimilarities (Minchin 1987, Clarke 1993, Legendre and Legendre 1998). We used Bray-Curtis dissimilarity (BCD) as the distance measure, a coefficient that has been repeatedly demonstrated to be robust for ecological community data (Faith and Norris 1989). A two-dimensional solution was used for all analyses as stress values (a measure of agreement between BCDs and the configuration of the ordination) were relatively low and did not substantially decrease when additional axes were included in ordinations. Before running ordinations on the data sets, algae or fish species occurring at only two sites (algae) and one site (fish) within a data set were excluded, and abundances were log transformed. Algae and fish data matrices were analyzed separately. Variables from the watersheds and environmental measurements with high skewness (> 1) were also log transformed to improve linear relationships with the ordinations. Ordinations were performed in PC-Ord version 5.20 (MjM Software, Gleneden Beach, OR, U.S.A.).

We used rotational vector fitting to relate environmental and watershed variables to gradients in algal and fish community composition quantified by the NMS ordinations (Faith and Norris 1989). Vector fitting was used to find the direction of the maximum correlation for each environmental variable. Significance ($P \leq 0.05$) of each environmental vector was estimated using 1,000 random permutations of the data. Vector fitting was performed using the ECODIST package in R version 2.5.1 (© 2007, The R Foundation for Statistical Computing).

To estimate species thresholds to nutrients or other stressors identified in the ordinations, we employed a new analytical approach, Threshold Indicator Taxa ANalysis (TITAN; King and

Baker), with the goals of (1) exploring and identifying abrupt changes in both the occurrence frequency and relative abundance of individual taxa along nutrient gradients, (2) quantifying uncertainty associated with both observed distributions of each taxon and the broader sample, and (3) estimating the relative synchrony of those changes as a non-parametric assessment of a community threshold. Current statistical methods used for grouping samples and detecting community ecological thresholds are not developed for distinguishing responses of individual taxa with low occurrence frequencies or highly variable abundances (Dufrêne and Legendre 1997, Brenden et al. 2008, Andersen et al. 2008). Some methods assume a linear, univariate response along all or part of an environmental gradient (e.g., Toms and Lesperance 2003), whereas others focus solely on aggregate, community-level dissimilarity (e.g., De'Ath 2002, King et al. 2005) or species turnover between samples (i.e., beta-diversity). Noisy, non-linear, and poorly distributed occurrences are typical properties of the vast majority of taxa in multivariate community data matrices (McCune and Grace 2002). Multivariate or multi-metric analysis can obscure distinct responses of taxa subsets in a community data set, especially if both predominant and rare species do not respond in a similar fashion or focal species do not respond as expected. TITAN circumvents these problems.

TITAN represents a combination and extension of change-point and indicator species analysis. In TITAN, we use normalized indicator species taxa scores (z) to identify the value of a continuous variable, x , resulting in the optimal partitioning of sample units, such that the indicator score is maximized either for individual taxa or the additive response of all normalized indicator z -scores at the community level. Negatively responding taxa (z^-) are distinguished from those responding positively (z^+) to yield taxa-specific change-point distributions as well as cumulative responses of declining [$\text{sum}(z^-)$] and increasing [$\text{sum}(z^+)$] subsets of the community. Resampling procedures are used to measure both indicator reliability and purity, and to assess estimate uncertainty surrounding the existence of community change-points.

TITAN analysis was performed on the same species data sets as in the ordinations using log-transformed abundance data. Predictors included important nutrient variables identified from the environmental vector fitting analysis. TITAN was conducted in R version 2.5.1 (© 2007, The R

Foundation for Statistical Computing) using the custom package TITAN written by M. E. Baker and R. S. King (Baker and King, in revision; King and Baker, in revision).

RESULTS AND INTERPRETATION

Comparison of BU and TCEQ TP and TN laboratory methods

Baylor and TCEQ total phosphorus (TP, ug/L) data corresponded closely above the TCEQ lab method detection limit (LOD) of 50 ug/L (Figure 4). Importantly, 34 of the 64 sites had TP concentrations below the TCEQ LOD, whereas all sites fell above the BU lab MDL of 3.6 ug/L (Appendix C, BU TP method). This result is particularly significant given the results of King et al. (2009; Appendix B) and new results reported in this document that provide compelling evidence of numerous biological changes in response to TP concentrations above 20 ug/L, a level well below the TCEQ LOD.

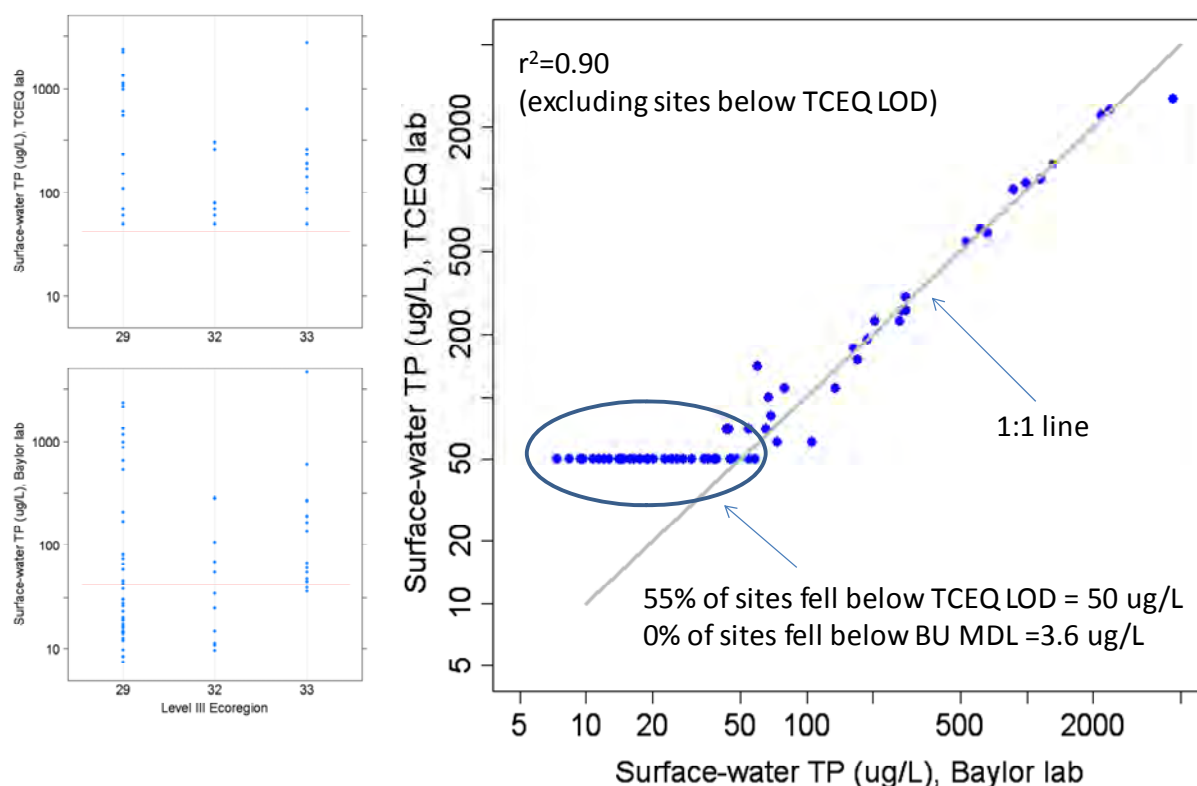


Figure 4. Distribution of surface water TP (ug/L) values among ecoregions (29=Cross Timbers, 32=TX Blackland Prairies, 33=E.Central.TX Plains) and analytical methods (BU=Baylor, TCEQ). The main panel (right) shows that above the TCEQ LOD of 50 ug/L, the two methods match quite well ($r^2=0.90$, close to 1:1 correspondence), but over half of the streams in the study area fell below the TCEQ LOD. The two small panels (left) show that most of samples that fell below the TCEQ LOD (red line) were in Ecoregions 29 and 32.

The BU and TCEQ results for total nitrogen (TN) corresponded quite well for most of the distribution of values (Figure 5; $r^2=0.91$). Two discrepancies between methods were evident: 1) variance in the TCEQ TN data began to increase at the low end of the distribution, and 2) TCEQ values were consistently above the 1:1 line between methods. Both of these were likely due to the way the TCEQ TN value was computed in this report. TCEQ measures TN as total Kjeldahl nitrogen + nitrite-nitrate-N + ammonia-N, each with its own method and LOD. Baylor (BU) converts all forms of nitrogen by digestion (Appendix D) to nitrate-N and measures it with one method. Because some of the nitrogen components in the TCEQ methods fell below the method LOD, we assumed that the LOD was the measured value (we could not assume that it was zero), thus the sum of the nitrogen parameters typically included a LOD value that artificially elevated the TN estimate. It appears that, except for low levels of TN, the TCEQ and BU TN methods yield similar results and the TCEQ LODs may not be an important source of error in TN estimation.

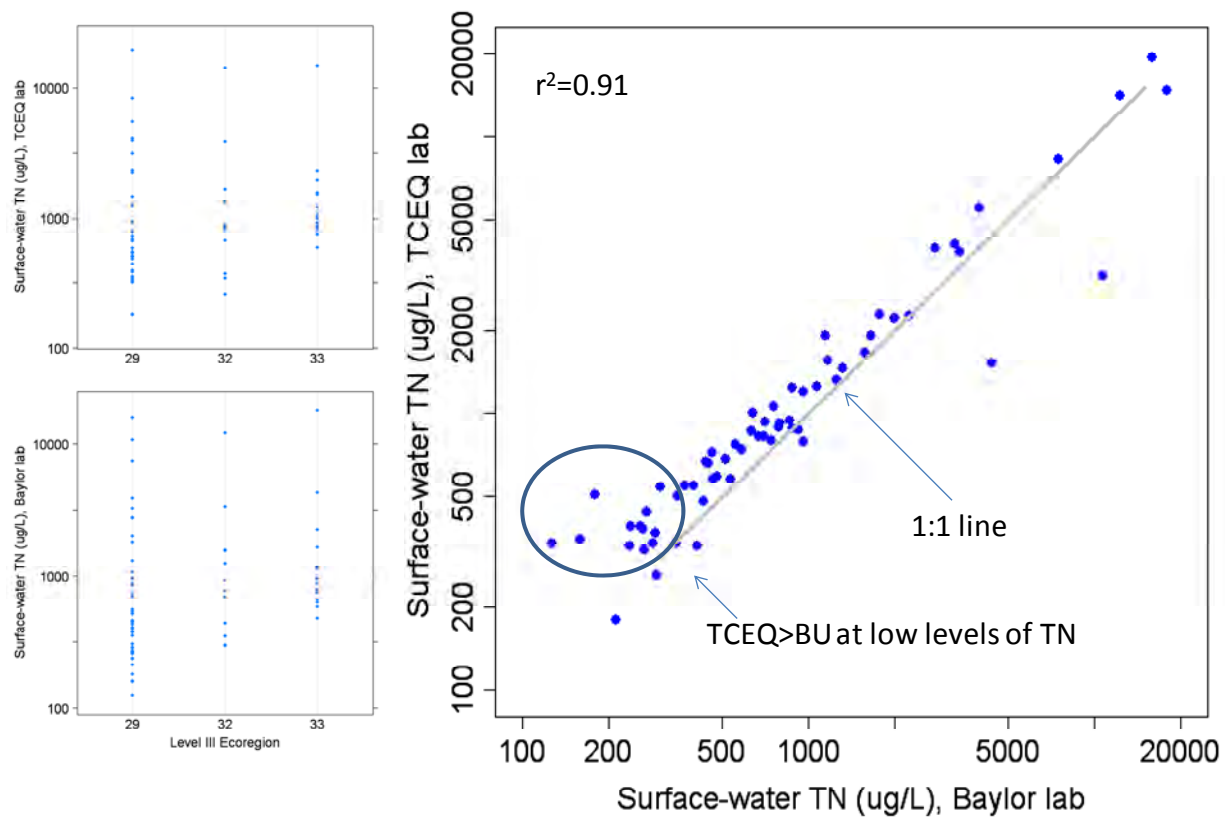


Figure 5. Distribution of surface water TN (ug/L) values among ecoregions (29=Cross Timbers, 32=TX Blackland Prairies, 33=E.Central.TX Plains) and analytical methods (BU=Baylor, TCEQ).

Surface-water TN and TP (hereafter, BU lab data for these two analytes) were positively correlated among the 38 sites in the Cross Timbers (Ecoregion 29) and the 15 sites in the East Central Texas Plains (Ecoregion 33), but no relationship was evident among the 11 sites from the Blackland Prairies (Ecoregion 32; Figure 6).

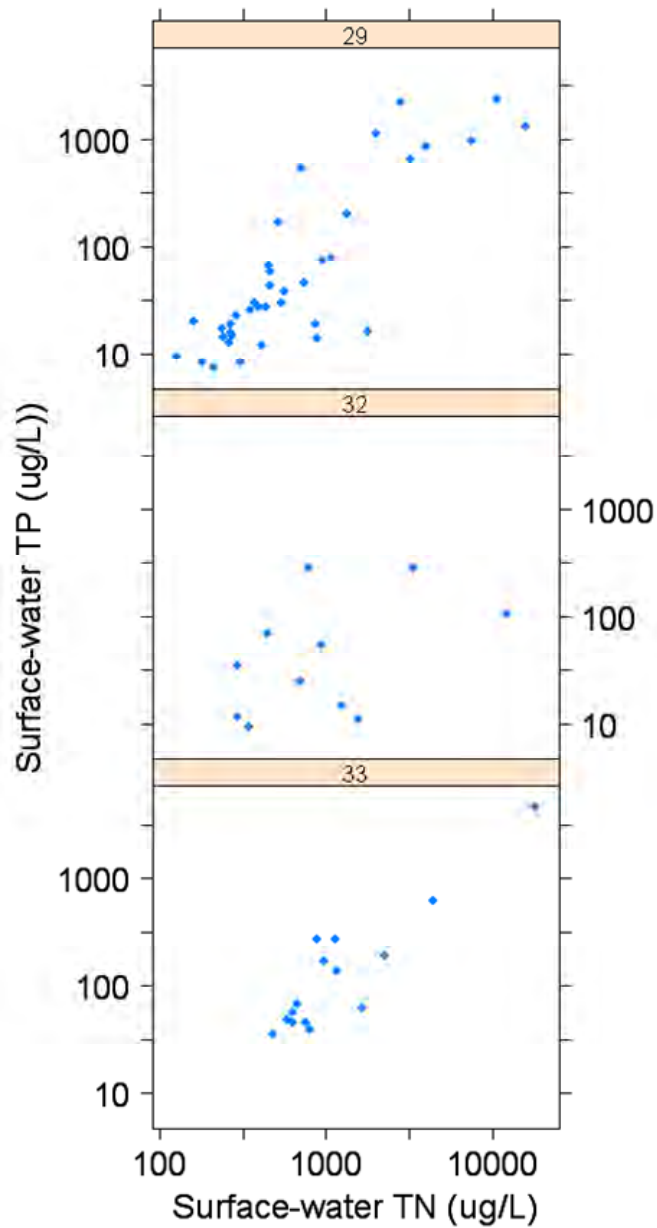


Figure 6. Scatterplots of TN vs. TP among the 3 ecoregions.

Ecoregion 29 had a wide range of TN and TP values, spanning a gradient from <10 and <100 ug/L to >2,000 and >15,000 ug/L TP and TN, respectively (Figure 6, top panel). There also were sites, particularly at low levels of TP, that tended to have relatively high concentrations of TN, which would potentially be important for evaluating whether P or N were more responsible for biological changes, if any were evident.

Ecoregion 32 had a much narrower range of TP than Ecoregion 29, with a few values near 20 ug/L and none above 500 ug/L TP (Figure 6, middle panel). With only 11 sites, and only 2 of those in the Brazos basin, coupled with the narrow range of TP values, statistical analysis of biological responses to nutrient gradients would yield results that would be uncertain and more likely to be confounded by other variables or outliers.

Ecoregion 33 had an even narrower of TN and TP values than Ecoregion 32, which is undesirable for characterization of biological responses to nutrients (Figure 6, bottom panel). All of the TP values in Ecoregion 33 were > 40 ug/L, much higher than the biological thresholds observed by King et al. (2009; Appendix B). Low sample size (n=15) likely resulted in an insufficient characterization of the distribution of nutrient levels in Ecoregion 33.

RESULTS AND INTERPRETATION, CONTINUED

Periphyton nutrient content across ecoregions

Periphyton from rocks (Ecoregion 29) and sand/mud (Ecoregions 32, 33) had considerably different distributions of nutrient ratios (C:P, C:N, N:P, Figure 8). Not surprisingly, there was a much larger difference in ratios between the bulk and OM fractions in the sand/mud samples than rock samples, likely because of the much higher proportion of sediment to OM in these samples.



Figure 7. Photograph of a subsample of homogenized periphyton suspended in water (middle tube) following laboratory processing (bulk, or unfractionated periphyton), and four tubes containing aliquots of periphyton that were suspended in colloidal silica and centrifuged to separate the organic matter (algae, bacteria, detritus, fungi) from heavier, mostly inorganic particles (silt, clay, sand). The lighter organic material is pulled to the top of the suspension, whereas the sediment is pulled to the bottom during the centrifugation process. Following centrifugation, the organic fraction is removed using a pipettor, dried, pulverized, and analyzed for total carbon, nitrogen and phosphorus. The unfractionated bulk periphyton sample is dried, pulverized, and analyzed in the same manner but without separation from inorganic particles. See Appendix B for details.

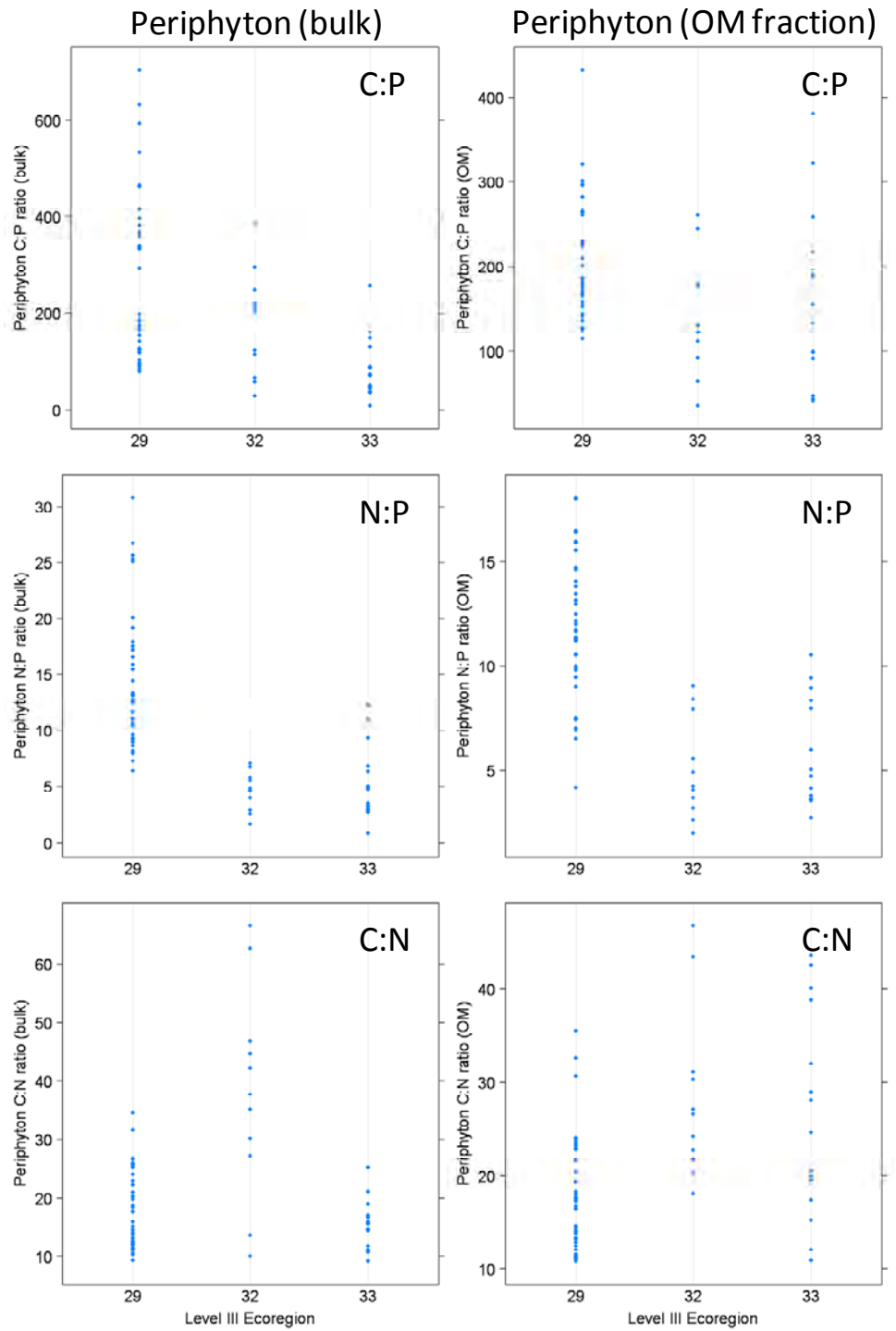


Figure 8. Distribution of C:P, N:P, and C:N ratios among streams in Ecoregions 29, 32, and 33 and between bulk and OM fractions of periphyton.

Rock periphyton (Ecoregion 29) nutrient ratios were strongly, and nonlinearly, related to surface-water nutrient concentrations (Figure 9, 10). Periphyton C:P and N:P ratios declined sharply with small increases in TP. The difference between OM C:P ratios and bulk C:P ratios was very high for periphyton in streams with low levels of TP, but rapidly diminished with TP enrichment. This implied that the bulk periphyton, which contained both sediment and the exopolysaccharide bacterial matrix, was storing as much phosphorus as the cellular organic matter (algae, fungi, bacteria). This was consistent with results of King et al (2009; Appendix B), reinforcing the strong connection between surface-water enrichment and rapid uptake, storage, and recycling of nutrients, particularly phosphorus, in the periphyton.

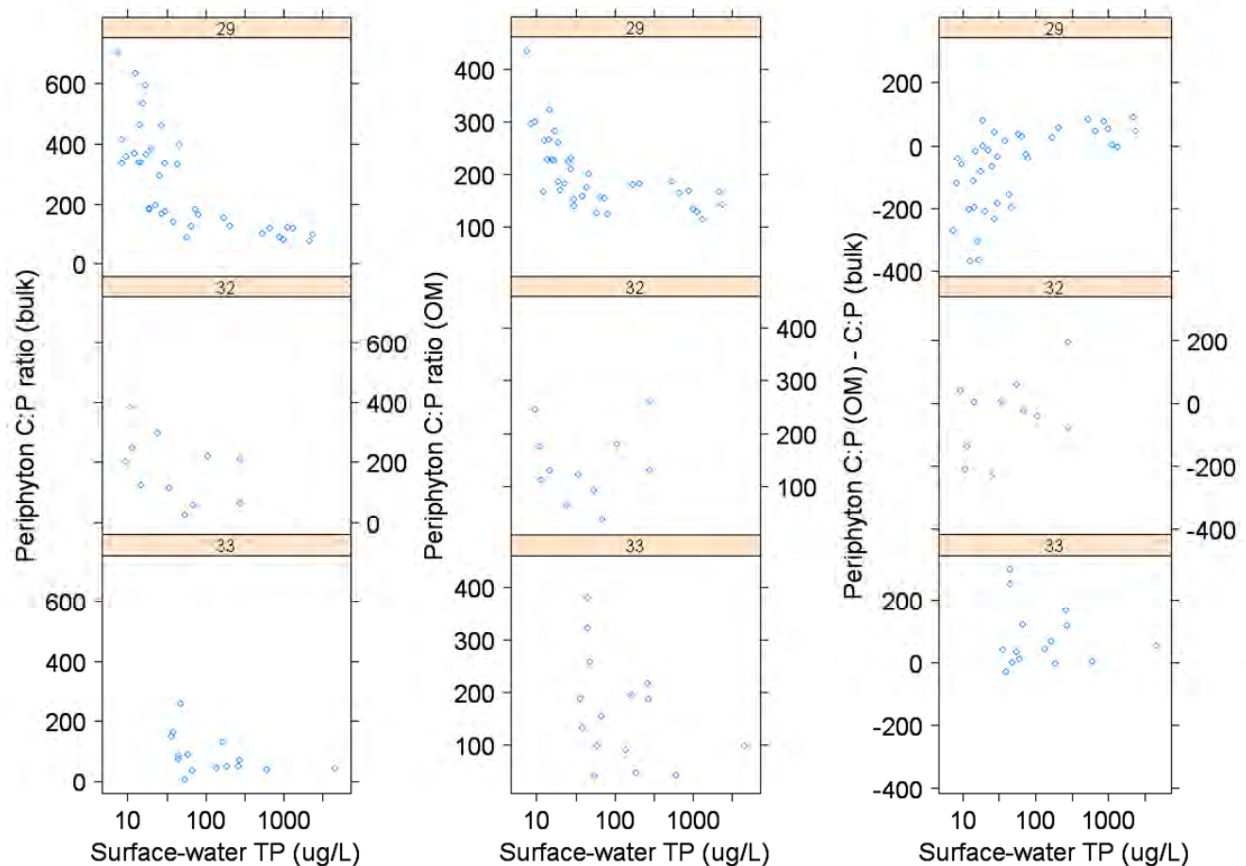


Figure 9. Scatterplots of periphyton C:P ratios (bulk, OM, and OM minus bulk) in response to surface-water TP across the three ecoregions.

Unfortunately, little of the variance in sand/mud periphyton ratios corresponded to surface-water nutrient concentrations (Figure 9, 10). Sand/mud C:P, C:N, and N:P ratios were unrelated to surface-water TP or TN in Ecoregion 32. There was a subtle relationship between C:P ratios and TP in Ecoregion 32, but the pattern was noisy and interpretation was difficult with so few samples.

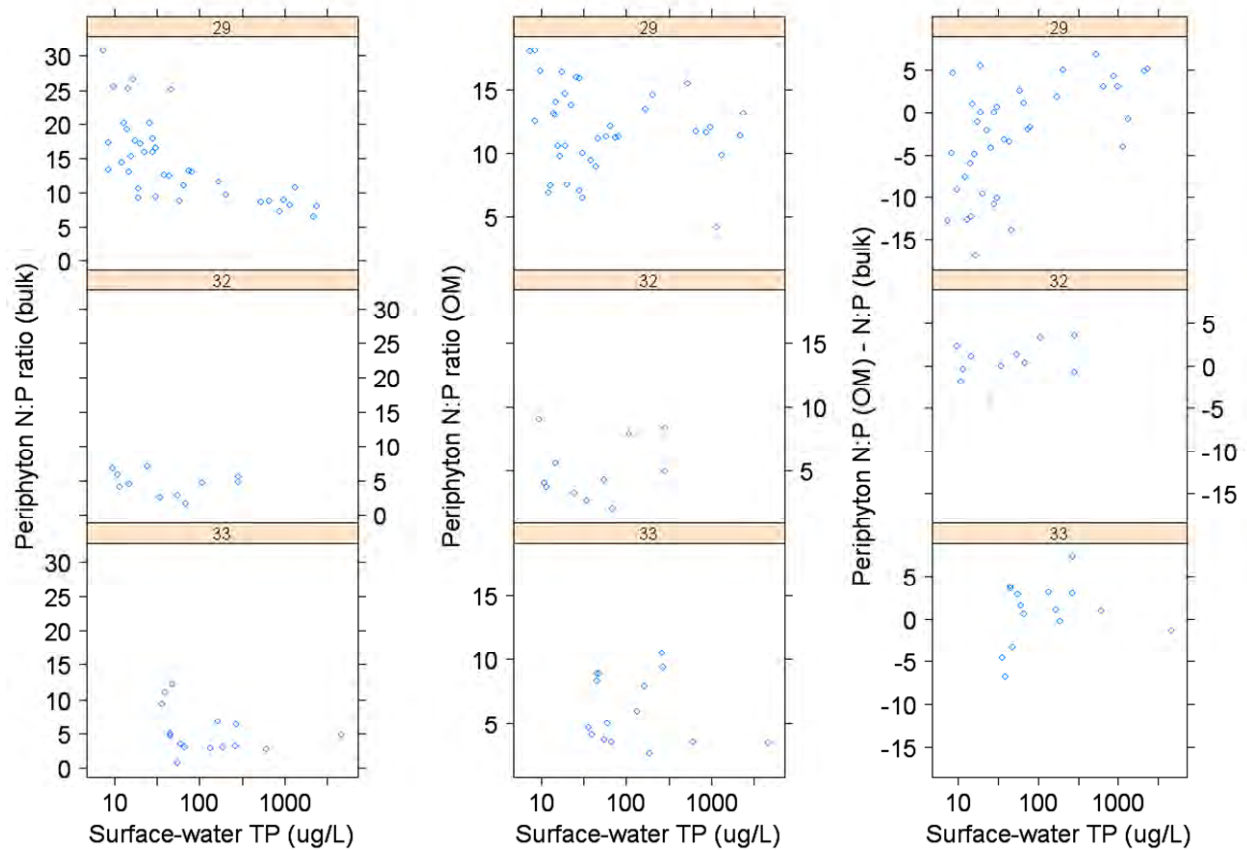


Figure 10. Scatterplots of periphyton N:P ratios (bulk, OM, and OM minus bulk) in response to surface-water TP across the three ecoregions.

RESULTS AND INTERPRETATION, CONTINUED

Surface-water and periphyton chlorophyll across ecoregions

Surface-water chlorophyll-a increased sharply in response to TP and TN in Ecoregion 29 (Figure 11, TP results only). Most of the sites with values below detection limits for chlorophyll were at low levels of TP. This pattern was not clear in Ecoregion 32 and 33, but was confounded by sample size and gradient length.

Periphyton chlorophyll-a per unit area trended toward a slight increase in Ecoregion 29, but was very noisy. However, the ratio of chlorophyll a to AFDM (ash-free dry mass) increased with TP, reflecting a shift from more calcareous periphyton to a community comprised of more filamentous and colonial green algae (King et al. 2009; Appendix B). Periphyton chlorophyll appeared to decline in response to TP in Ecoregion 32, whereas no relationship was evident in Ecoregion 33.

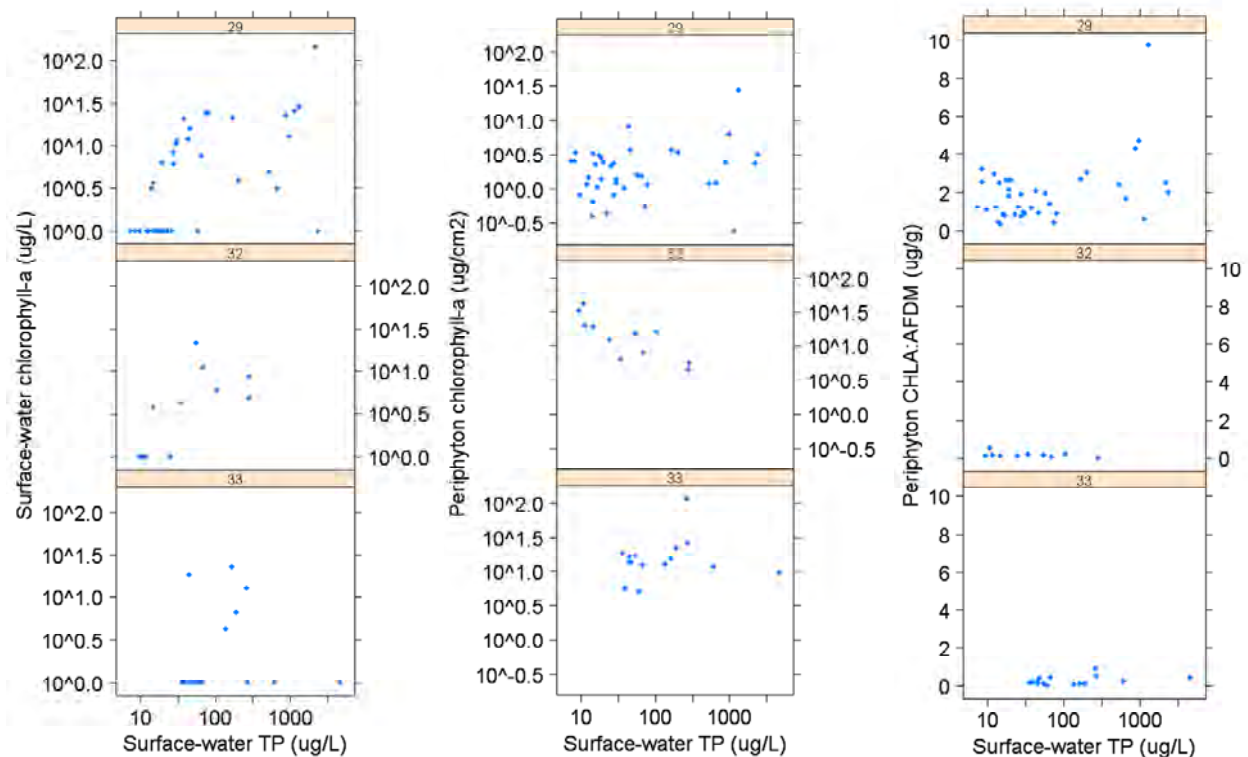


Figure 11. Scatterplots of chlorophyll-a (ug/L, water), chlorophyll-a (mg/m², periphyton), and the ratio of periphyton chlorophyll-a to AFDM (mg/g) across the three ecoregions.

RESULTS AND INTERPRETATION, CONTINUED

Estimation of thresholds for univariate biological indicators, Ecoregion 29

Qualitative results revealed that insufficient sample sizes and noisy data rendered threshold analysis in Ecoregions 32 and 33 to be impractical. However, the large number of sites, graphically obvious nonlinear changes in several variables, and wide range of nutrient concentrations in Ecoregion 29 was suitable for statistical analysis of thresholds.

Surface-water chlorophyll-a and nonfiltrable residue showed very similar responses to TP in Ecoregion 29 (Figure 12; note that outlier for both variables did not influence the changepoint estimate). Both were near or below detection limits at TP < 25 ug/L, showed a sharp, significant increase above 25 ug/L TP (Table 1). Both variables also increased significantly above a TN threshold of ~350 ug/L (Table 1).

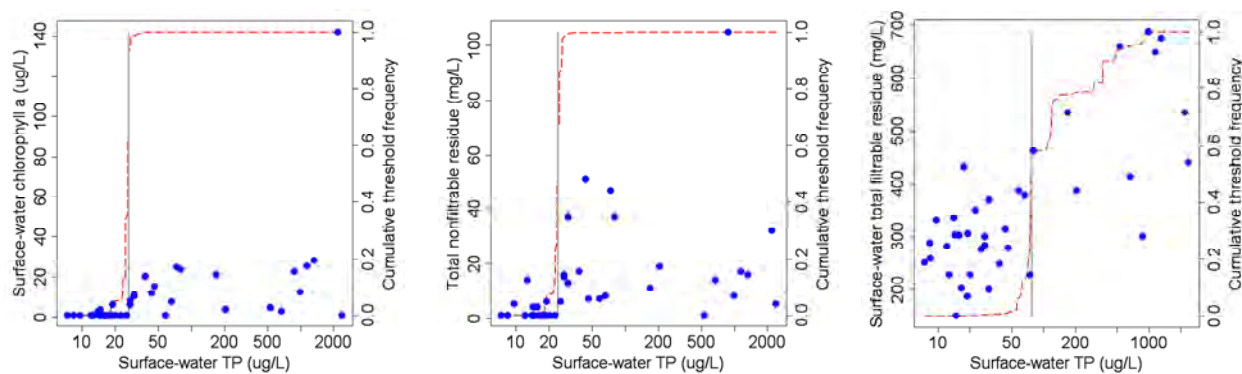


Figure 12. Results from nonparametric changepoint analysis using **surface-water TP** as a predictor of threshold changes in **surface-water chlorophyll-a, nonfiltrable residue, and filterable residue** in **Ecoregion 29**. Each blue dot represents one of the 38 sites sampled in summer 2008. The gray vertical line is the observed TP threshold (the level of TP resulting in the greatest difference in the response variable to the left and right of that value). The dotted red line is the cumulative threshold frequency, an estimate of uncertainty based on 1,000 bootstrap samples of the data (see King and Richardson 2003). The cumulative threshold frequency illustrates the range of possible threshold values; different quantiles of this distribution can be interpreted as confidence intervals around the observed threshold. See Table 1 for summary of the corresponding statistical results.

Total filtrable residue also increased significantly with increasing TP and TN (Figure 12; TP results only). However, its threshold level of TP and TN were less certain (Table 1).

Periphyton C:P, N:P, and C:N ratios sharply declined in response to TP (Table 1, Figure 13). Periphyton C:P (bulk) and C:P (OM) both declined significantly at <20 $\mu\text{g/L}$ (Table 1, Figure 13), reinforcing periphyton C:P ratios as a very sensitive, robust indicator of nutrient enrichment in Cross Timber streams (King et al. 2009; Appendix B). The bulk samples also appeared to be nearly as sensitive to TP as the OM samples.

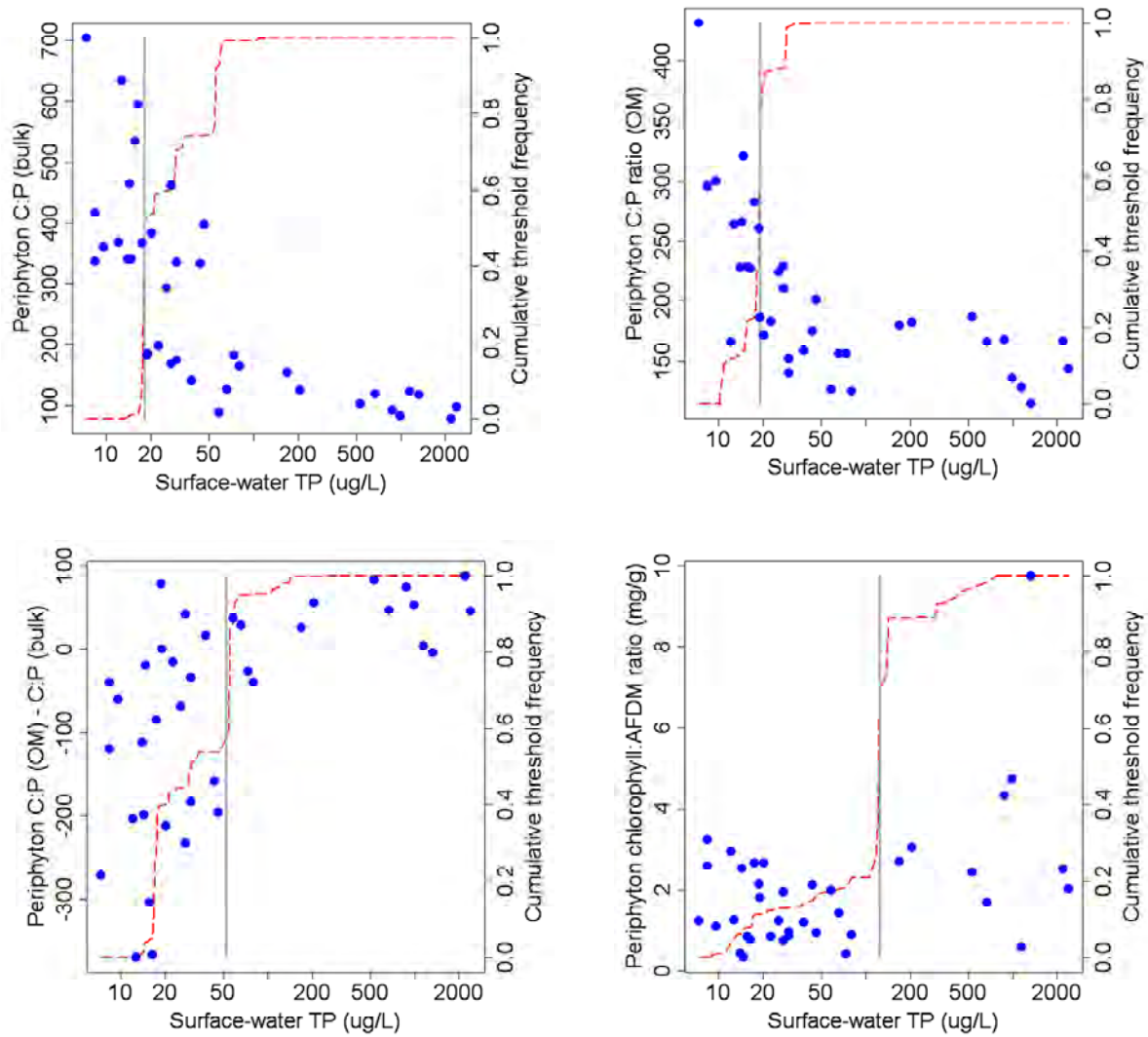


Figure 13. Results from nonparametric changepoint analysis using **surface-water TP** as a predictor of threshold changes in **periphyton variables** in **Ecoregion 29**. Each blue dot represents one of the 38 sites sampled in summer 2008. The gray vertical line is the observed TP threshold (the level of TP resulting in the greatest difference in the response variable to the left and right of that value). The dotted red line is the cumulative threshold frequency, an estimate of uncertainty based on 1,000 bootstrap samples of the data (see King and Richardson 2003). The cumulative threshold frequency illustrates the range of possible threshold values; different quantiles of this distribution can be interpreted as confidence intervals around the observed threshold. See Table 1 for summary of the corresponding statistical results.

Table 1.. Results of nonparametric changepoint analysis using nutrients and nutrient-related predictors of threshold responses in fish community indicators of biological integrity in Ecoregion 29. See figures 12 through 13 for graphical display of some of these results.

Predictor	Response	Response > threshold	Threshold (obs)	P value	Bootstrap threshold quantiles				
					10%	50%	90%	Mean<obs	Mean>obs
Total N (ug/L)	AFDM_M2	ns	280.17	0.5335	277.17	462.50	1661.17	1.36	1.08
Total N (ug/L)	CHLA_UGL	Increase	362.00	0.0010	338.83	374.00	487.83	1.10	8.21
Total N (ug/L)	CHLA:AFDM	Increase	3246.67	0.0043	685.95	1660.00	2786.67	1.61	5.21
Total N (ug/L)	CHLA_M2	ns	3246.67	0.0665	271.00	1140.50	2786.67	1.53	5.98
Total N (ug/L)	C:N (OM)	Decline	440.83	0.0206	280.17	440.83	1143.17	21.23	15.24
Total N (ug/L)	C:N (BULK)	Decline	440.83	0.0061	277.17	440.83	511.00	21.71	14.41
Total N (ug/L)	C:N OM-BULK	ns	1891.67	0.2213	261.83	468.25	1891.67	-0.38	3.01
Total N (ug/L)	C:P ALG	Decline	362.00	0.0004	277.17	328.17	377.58	270.93	167.78
Total N (ug/L)	C:P BULK	Decline	266.00	0.0029	263.83	384.67	918.17	472.70	219.39
Total N (ug/L)	C:P OM-BULK	Increase	266.00	0.0186	263.83	284.50	1891.67	-192.74	-36.83
Total N (ug/L)	EMBEDDED	ns	420.17	0.0506	280.17	420.17	918.17	25.78	45.41
Total N (ug/L)	TFILRESI	Increase	1891.67	0.0039	440.83	1016.00	1891.67	314.00	528.57
Total N (ug/L)	MACRPHYT	ns	362.00	0.1196	280.17	362.00	800.17	4.24	0.68
Total N (ug/L)	MCRPH_AB	ns	362.00	0.0607	295.67	374.00	918.17	0.54	0.15
Total N (ug/L)	MICRALG	ns	800.17	0.0630	295.67	792.67	807.67	9.86	0.41
Total N (ug/L)	MUDSILT	Increase	328.17	0.0318	318.83	337.08	918.17	0.27	12.19
Total N (ug/L)	NFILRESI	Increase	328.17	0.0051	318.83	338.83	918.17	0.25	1.02
Total N (ug/L)	N:P (OM)	ns	362.00	0.0615	238.83	362.00	414.33	13.72	10.91
Total N (ug/L)	N:P (BULK)	Decline	261.83	0.0104	251.83	423.17	1891.67	21.39	13.08
Total N (ug/L)	N:P OM-BULK	ns	2393.33	0.0560	261.83	445.83	2285.00	-3.87	3.31
Total P (ug/L)	AFDM_M2	ns	368.33	0.3764	12.44	69.78	368.33	1.23	0.90
Total P (ug/L)	CHLA_UGL	Increase	26.78	0.0003	24.00	26.78	26.78	1.28	10.65
Total P (ug/L)	CHLA:AFDM	Increase	125.08	0.0291	17.03	125.08	303.10	0.10	0.43
Total P (ug/L)	CHLA_M2	ns	770.33	0.3042	14.27	40.73	665.00	1.60	2.99
Total P (ug/L)	C:N (OM)	Decline	30.18	0.0124	16.60	29.00	55.85	20.94	14.56

Total P (ug/L)	C:N (BULK)	Decline	21.43	0.0014	19.05	24.00	54.50	22.88	14.29
Total P (ug/L)	C:N OM-BULK	ns	932.17	0.1869	13.42	26.78	703.67	-0.30	3.81
Total P (ug/L)	C:P ALG	Decline	19.05	0.0002	10.89	19.05	28.95	274.22	166.07
Total P (ug/L)	C:P BULK	Decline	18.23	0.0004	17.77	18.32	55.85	454.67	188.74
Total P (ug/L)	C:P OM-BULK	Increase	52.08	0.0030	17.03	30.18	59.97	-129.82	33.50
Total P (ug/L)	EMBEDDED	Increase	21.43	0.0106	16.65	21.43	30.18	22.00	47.03
Total P (ug/L)	TFILRESI	Increase	77.03	0.0002	63.10	77.03	471.39	284.89	522.00
Total P (ug/L)	MACRPHYT	ns	26.78	0.2288	16.18	26.78	69.78	3.45	0.64
Total P (ug/L)	MCRPH_AB	ns	26.78	0.0909	16.18	26.78	69.78	0.47	0.13
Total P (ug/L)	MICRALG	ns	15.30	0.1400	15.12	25.20	598.33	13.33	4.54
Total P (ug/L)	MUDSILT	Increase	24.22	0.0088	23.02	24.22	25.35	0.67	14.07
Total P (ug/L)	NFILRESI	Increase	24.22	0.0003	22.38	24.22	26.78	0.24	1.17
Total P (ug/L)	N:P (OM)	Decline	10.89	0.0309	10.89	14.10	48.70	16.26	11.36
Total P (ug/L)	N:P (BULK)	Decline	52.08	0.0020	15.12	31.97	63.10	17.55	9.58
Total P (ug/L)	N:P OM-BULK	Decline	52.08	0.0048	17.03	55.85	125.08	-5.55	2.10

Several other variables showed significant changes that corresponded to TP and TN (Table 1). Sedimentation variables (substrate embeddedness, mud-silt cover) both increased sharply at levels of TP and TN that also corresponded to significant water quality and biological changes (chlorophyll-a, periphyton C:P, filtrable residue). These sedimentation indicators were shown by Winemiller et al (2009) to correspond with increasing cover of pasture in the study watersheds, suggesting that pasture may be an important driver of both elevated nutrients and sediment problems in Ecoregion 29.

Variables that were indicators of submersed macrophyte cover (MCRPH_AB, MACRPHYT) and microalgae/biofilm cover (MICRALG) were expected to decline in response to TN and TP. However, they were too variable in their responses to be statistically significant. We expected these to decline because of their consistent response to TP in the study by King et al. (2009; Appendix B), which included an assessment of these variables in June 2008 at 26 of these 38 streams. In that event, macrophytes and biofilm thickness both significantly declined in response to TP levels > 20 ug/L. King et al. (2009) study used the 100-point transect method for estimating reach-scale cover of macrophytes, filamentous macroalgae, biofilm thickness, substrate, and sediment film thickness and found this approach to yield an excellent characterization of these variables. This current study used the TCEQ physical habitat assessment method, which was constrained to just 5 or 6 cross-sectional transects. Because of the high degree of spatial heterogeneity in the length of these reaches, we suggest that these transects are more likely to under or over estimate cover of these variables, and this may explain why these variables were not as effective as the field survey indicators of nutrient enrichment in the King et al. (2009) study.

RESULTS AND INTERPRETATION, CONTINUED

Multivariate analysis of algal species composition among ecoregions

Ordination of sites based on the density of different algal species showed that periphyton communities growing on rocks (Cross Timbers) was clearly different than communities growing on sand/mud (Blackland Prairies, East Central Texas Plains; Figure 14-16). This was not unexpected. However, the ordination also revealed that algae growing on mud/silt in the Blackland Prairies was significantly different than East Central Texas Plains, with very little overlap (Figure 14; MRPP, $p < 0.01$). This implies that ecoregional differences observed for fish (Winemiller et al. 2009) were also true for algae, thus analyses based on taxonomic composition will need to be stratified by ecoregion for both of these indicator groups.

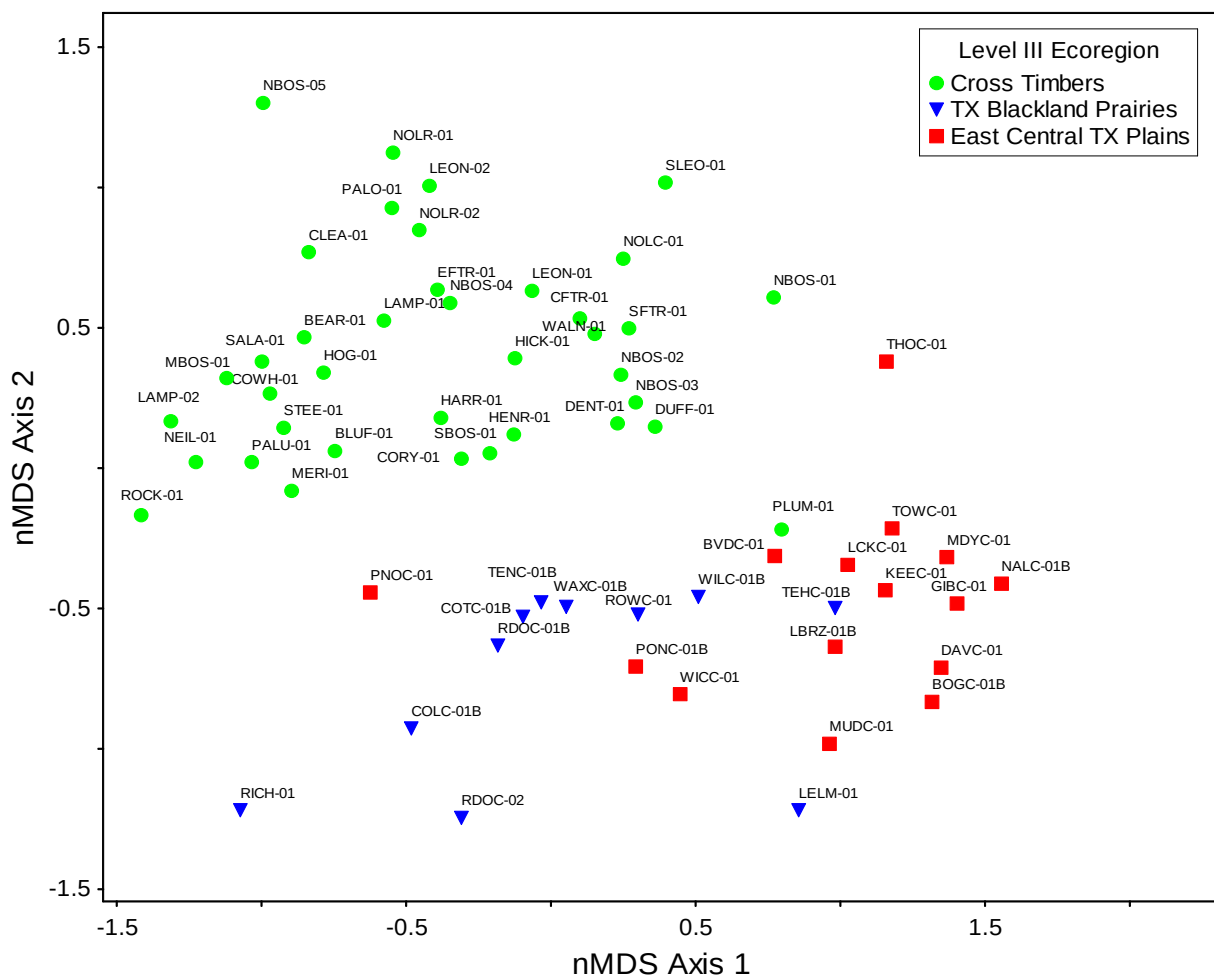


Figure 14. Nonmetric multidimensional scaling (nMDS) ordination of algal species composition among the three ecoregions.

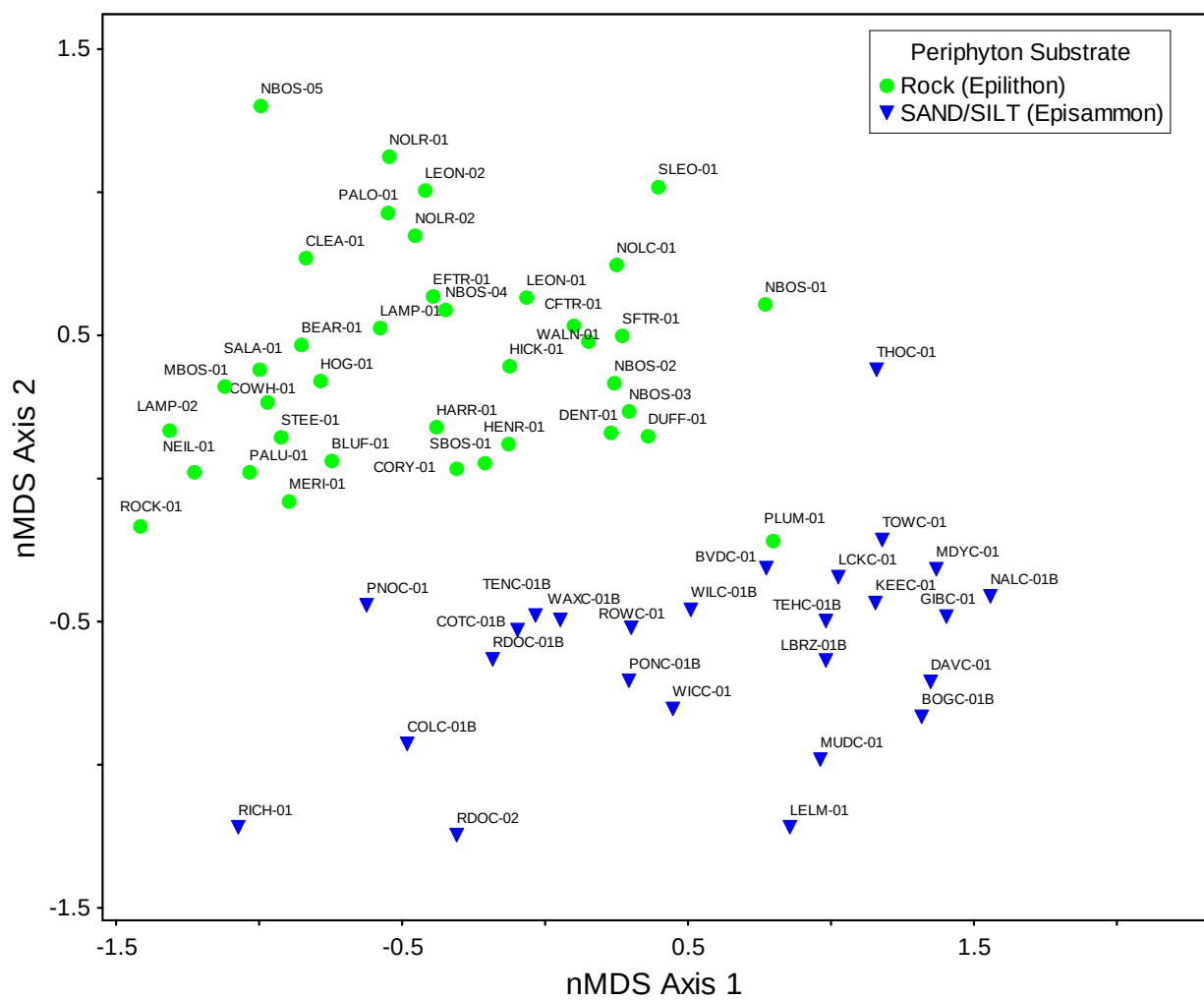


Figure 15. Nonmetric multidimensional scaling (nMDS) ordination of algal species composition between the two substrate types.

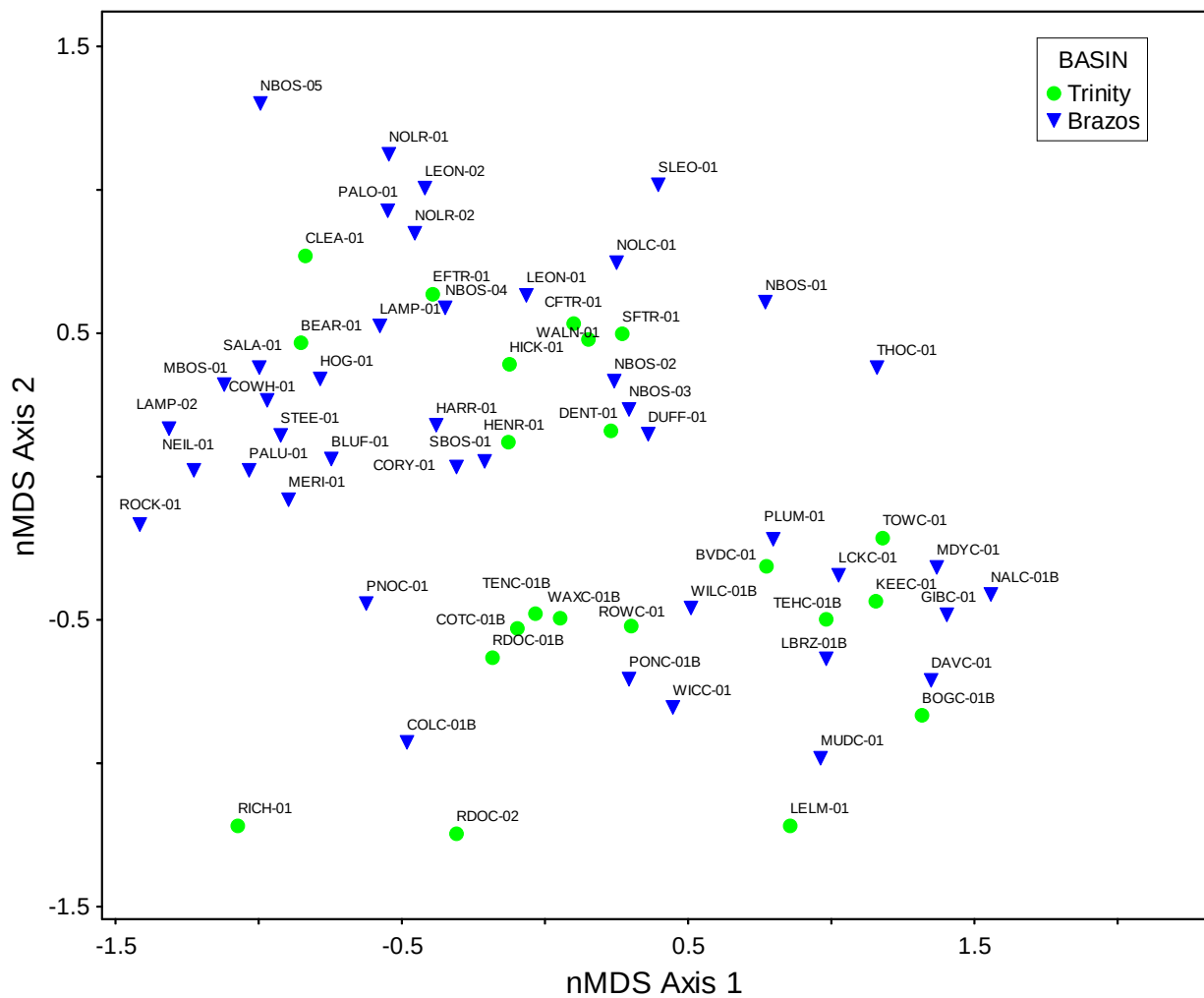


Figure 16. Nonmetric multidimensional scaling (nMDS) ordination of algal species composition among the two major river basins.

Algal species composition did not differ by the major river basins (Brazos and Trinity), however. Trinity sites were mostly enclosed within the cluster of Brazos sites in the ordination, or vice versa, regardless of substrate or ecoregion. This is important because it suggests that taxonomic composition metrics likely do not need to be stratified by basin for analyses or index development.

Ordination of algal species composition within Ecoregion 29 revealed a strong gradient along axis 1 that was highly correlated with numerous nutrient and nutrient-related environmental variables (Figures 17-19). Sites with low TP, TN, pasture, outfalls, sediment, and chloride and high periphyton C:N, C:P, and N:P ratios were grouped on the left side of the ordination, whereas sites with high values for these stressors were consistently grouped on the right side. This result is very similar to 2006 and 2007 data reported in King et al. (2009; Appendix B).

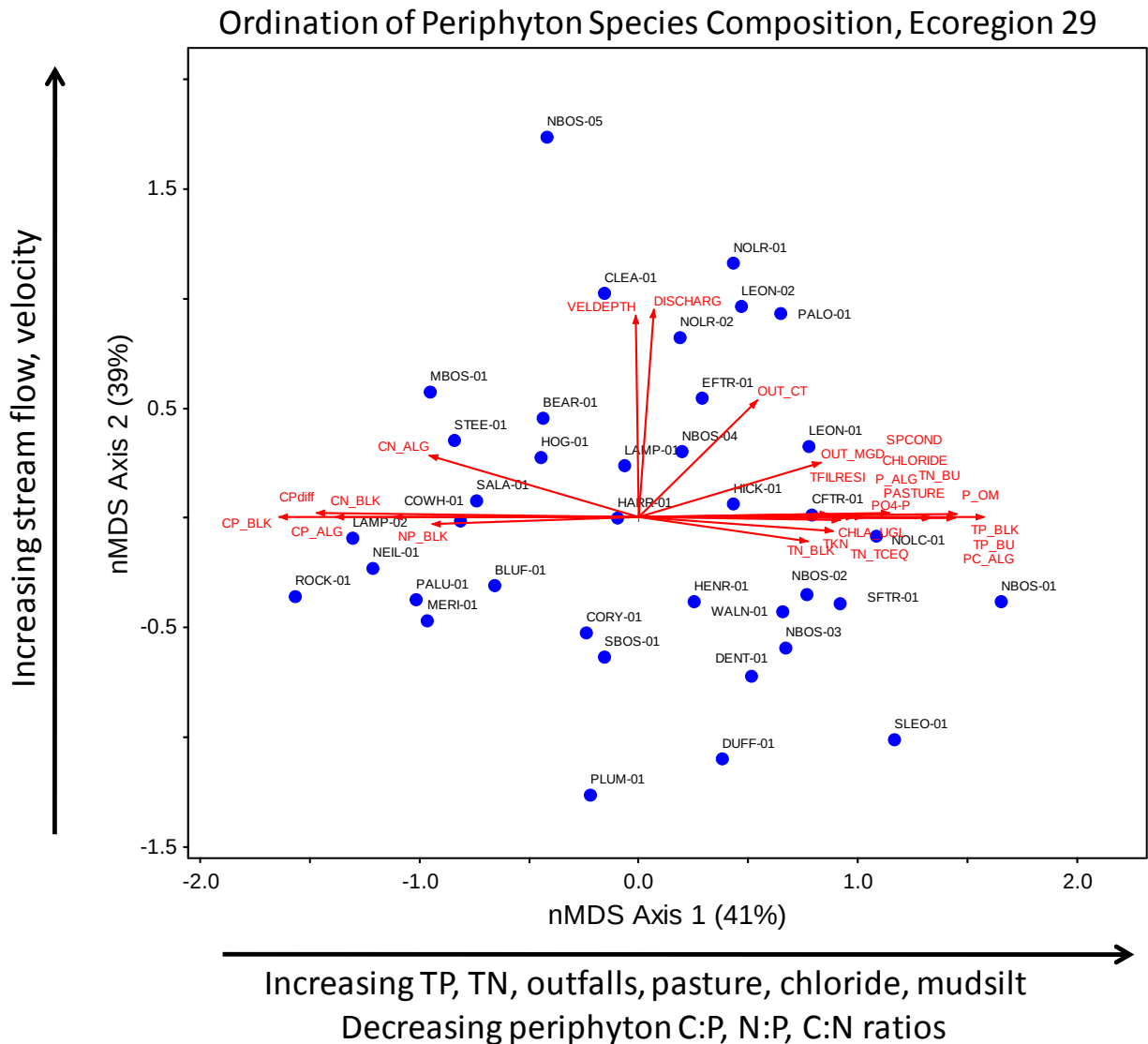


Figure 17. Nonmetric multidimensional scaling ordination of algal species composition among the 38 sites in Ecoregion 29 in summer 2008. Abundance data (no. of cells/cm²) was log₁₀(x+1) transformed prior to analysis. Bray-Curtis distance was used as the dissimilarity metric. Distances between sites in the ordination space are proportional to taxonomic dissimilarity (near=similar, far=dissimilar). In each figure, the red arrows (vectors) represent the direction and magnitude of significant ($p < 0.05$) correlations between environmental variables and algal species composition. See Appendices A1-3 for full variable names.

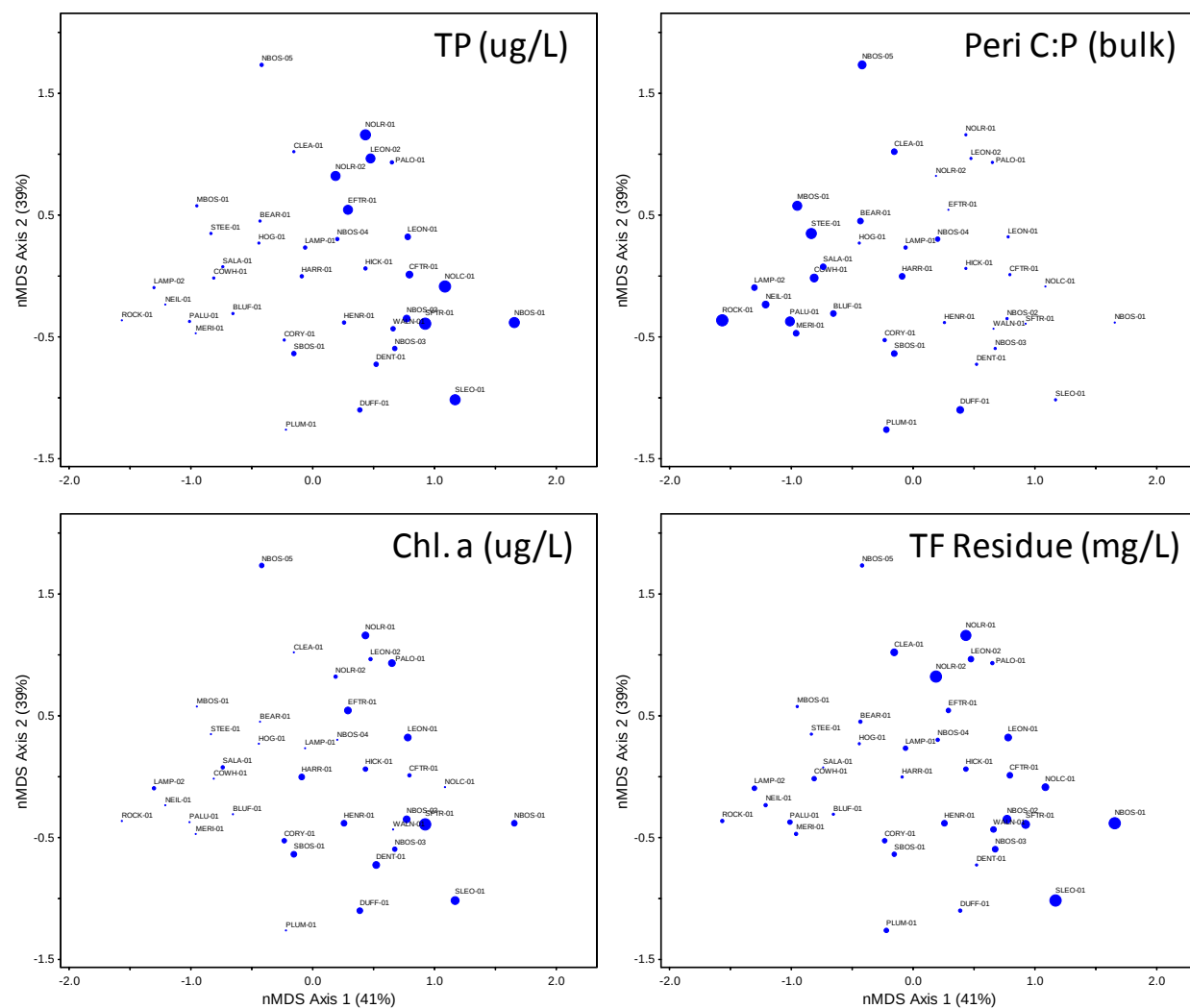


Figure 18. Nonmetric multidimensional scaling ordination of algal species composition among the 38 sites in Ecoregion 29 in summer 2008. The ordination diagram is identical to Figure 17, except that site symbols are scaled in proportion to measured values of surface-water TP, periphyton C:P (bulk), chlorophyll-a (water), and total filtrable residue (water).

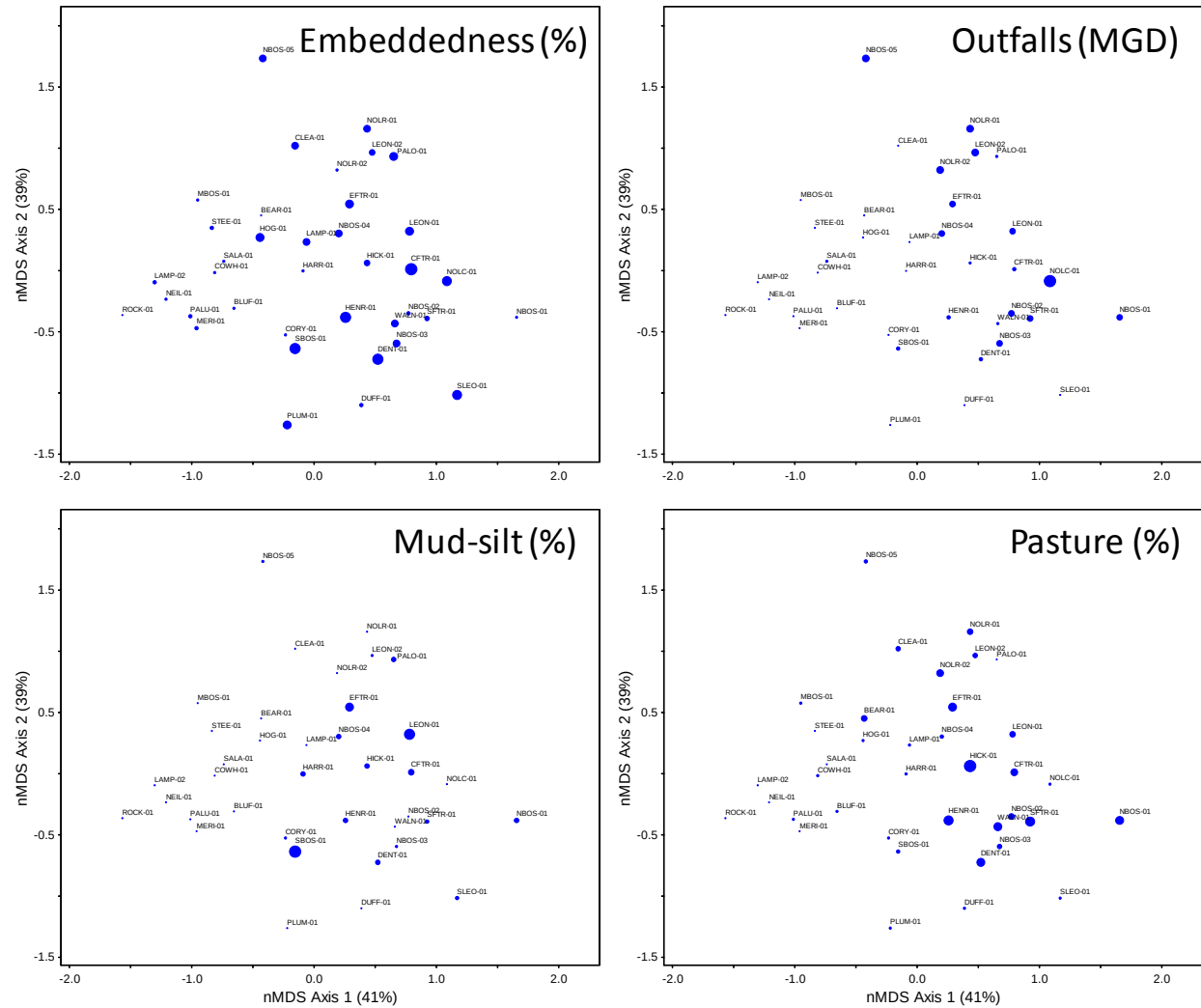


Figure 19. Nonmetric multidimensional scaling ordination of algal species composition among the 38 sites in Ecoregion 29 in summer 2008. The ordination diagram is identical to Figure 17, except that site symbols are scaled in proportion to measured values to substrate embeddedness, outfalls (permitted mgd in watershed), mud-silt cover (%), and pasture cover (% of watershed).

Algal species composition was not related to any nutrient or nutrient-related variable in Ecoregions 32 or 33 (Figure 20, 21). Even with the small sample sizes, algal taxonomic composition should have corresponded more closely to surface-water and periphyton chemistry than it did in these data sets. This implied that sand/mud periphyton samples were too variable to use reliably as nutrient indicators, and that alternative substrates (wood, artificial) should be considered for biological assessment in these soft-bottomed stream ecosystems.

Ordination of Algal Species Composition, Ecoregion 32 No significant relationships with nutrients

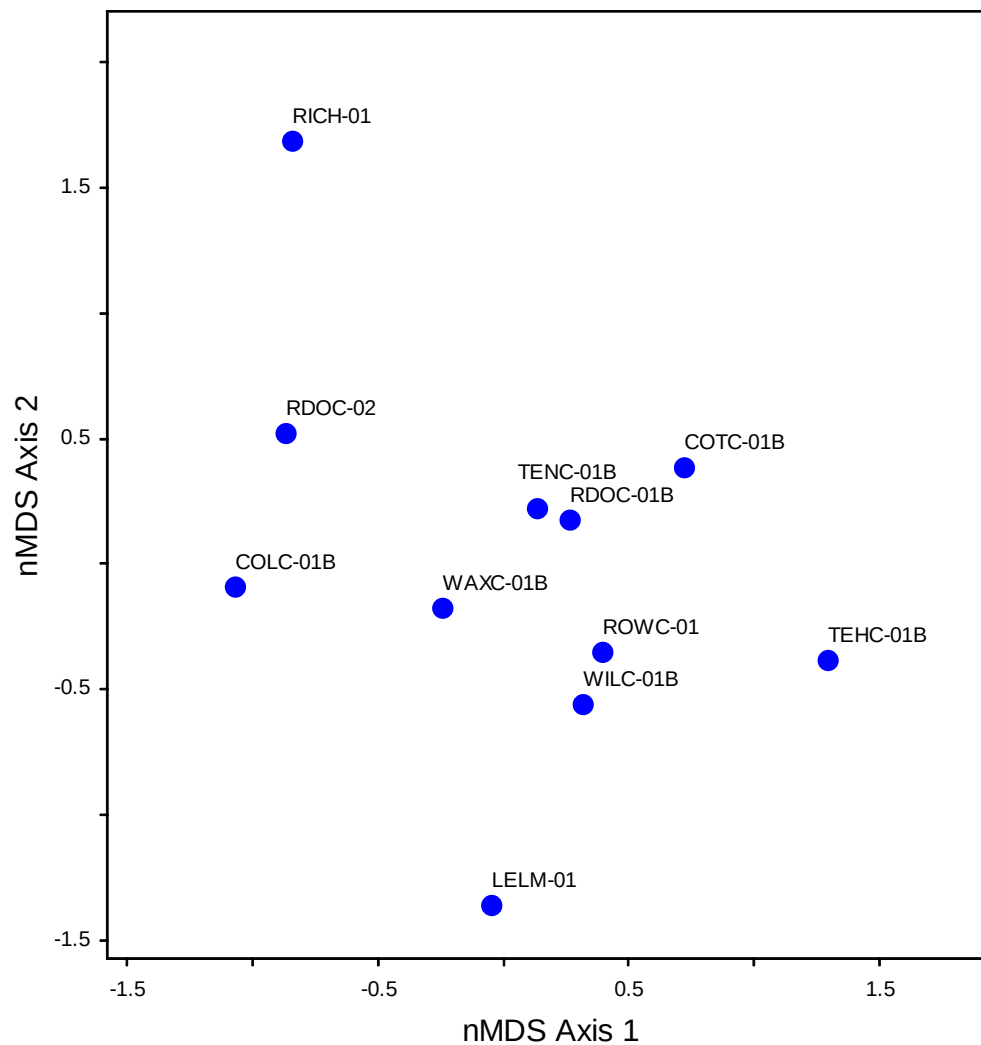


Figure 20. Nonmetric multidimensional scaling ordination of algal species composition among the 11 sites in Ecoregion 32 in summer 2008.

Ordination of Algal Species Composition, Ecoregion 33
No significant relationships with nutrients

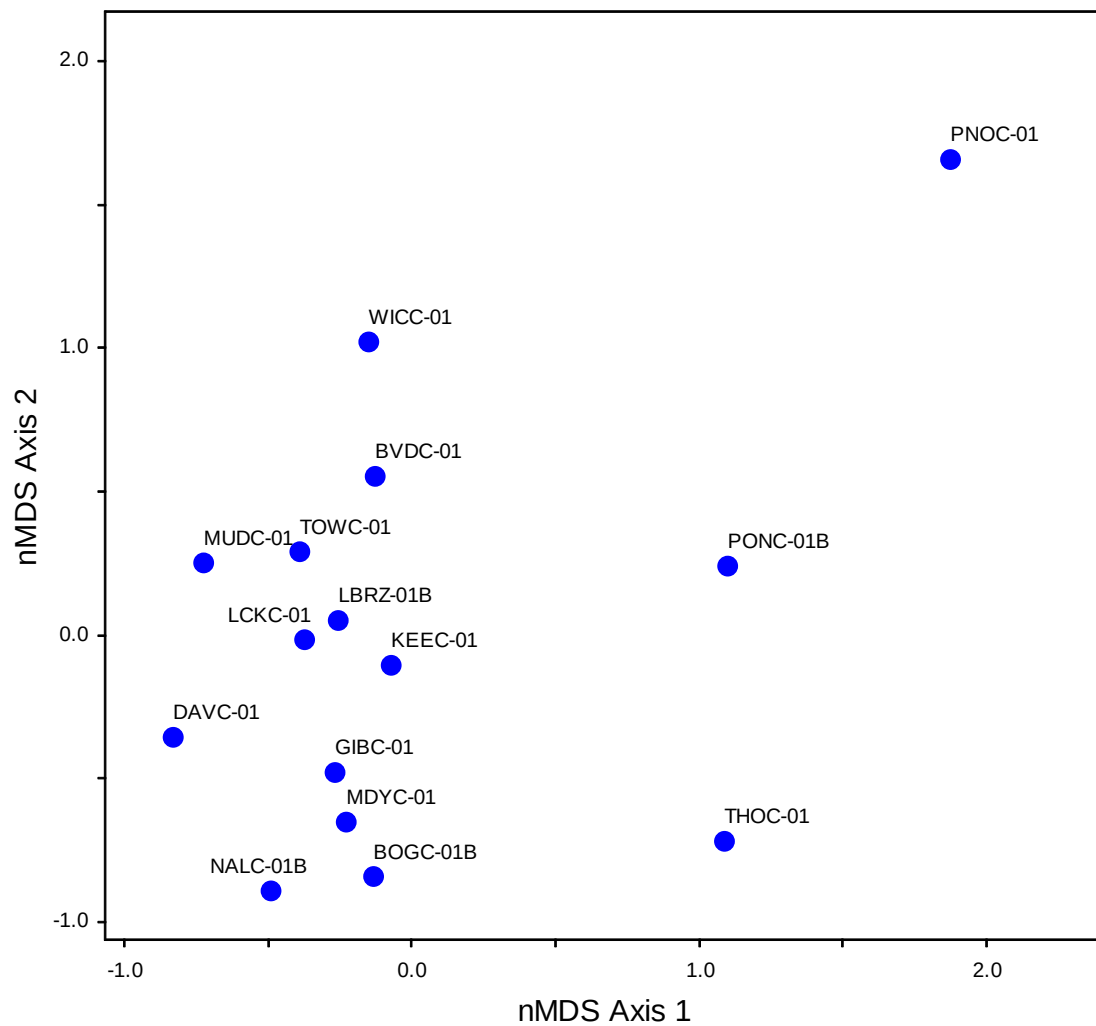


Figure 21. Nonmetric multidimensional scaling ordination of algal species composition among the 15 sites in Ecoregion 33 in summer 2008.

RESULTS AND INTERPRETATION, CONTINUED

Threshold responses of algal species to nutrient gradients in Ecoregion 29

Thirty-one algal species declined significantly in response to surface-water TP (Figure 22; Appendix A6). Most of these taxa declined between 15 and 25 ug/L TP. The TP level most likely to result in a community level decline (sum z-; Table 2) was 21 ug/L. Bootstrap confidence limit estimates suggested that this threshold may have been as low as 12 ug/L and highly likely to occur if TP exceeded 28 ug/L (Table 2).

TITAN also detected 36 algal species that proliferated rapidly with increasing TP in the wake of declines of other species (Figure 22; Appendix A6). Most of these species increased between 20 and 50 ug/L TP, but a few did not begin to appear until TP exceeded 500 ug/L (Figure 22). The community level threshold for increasing (positive responding) taxa was 40 ug/L TP (Table 2).

Fifteen and 28 taxa declined in response to TN and chloride, respectively (Figure 24; Appendix A6, Table 2). Some of these taxa differed from those that declined in response to TP, but the magnitude of the aggregate community response was lower than that of TP. Community-level threshold declines in algal species composition were most likely at 320 ug/L TN and 20 ug/L chloride (Table 2).

Most of the same taxa that declined in response to increasing TP declined in response to decreasing C:P ratios in the periphyton (Figure 25, Appendix A6). The consistency of this response is important because it demonstrates that changes in the amount of phosphorus in the periphyton itself results in sharp community changes that mirror the changes in response to surface-water TP. The level of C:P in the periphyton that led to the greatest overall decline in algal species was below 225 for OM samples and 335 for bulk periphyton.

The percentage cover of pasture in watersheds and the permitted volume of outfalls in watersheds (millions of gallons per day) both resulted in similar threshold declines in algal species as nutrients and nutrient related stressors (Figures 26, 27; Appendix A6). These variables also corresponded to sharp increases in taxa not found at sites with low levels of nutrients, sediment, and chloride (Figures 26, 27; Appendix A6). Watersheds exceeding 3.3% pasture cover and 0.31 MGD of permitted outfalls had the greatest overall declines in algal species, whereas pollution-indicator species proliferated in watersheds with > 7% pasture and >0.31 MGD of outfalls (Table 2).

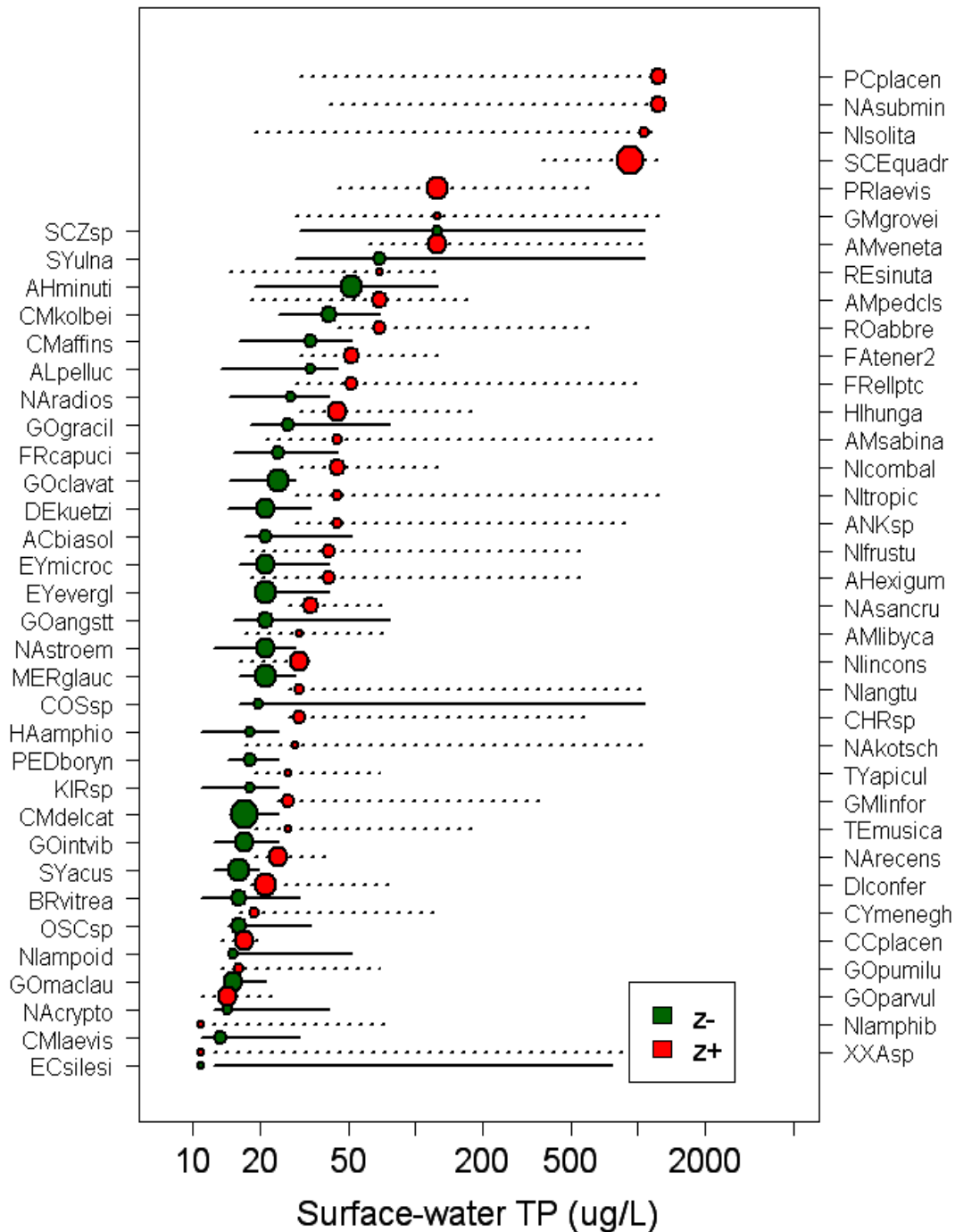


Figure 22. Results of Threshold Indicator Taxa Analysis (TITAN) using surface-water TP as a predictor of threshold changes in individual algal species in Ecoregion 29 in summer 2008. Taxa are classified as either negative (z-) or positive (z+) threshold indicators based on the direction of response to TP. The observed TP threshold value (colored symbols) correspond to each taxon deemed to change significantly. Taxon IDs (see Appendix A5) are shown on the left (negative indicators) and right (positive indicators) y-axes, in rank order of their TP thresholds. Line segments around each symbol are 90% confidence intervals around the TP threshold. Symbol sizes correspond to the indicator score.

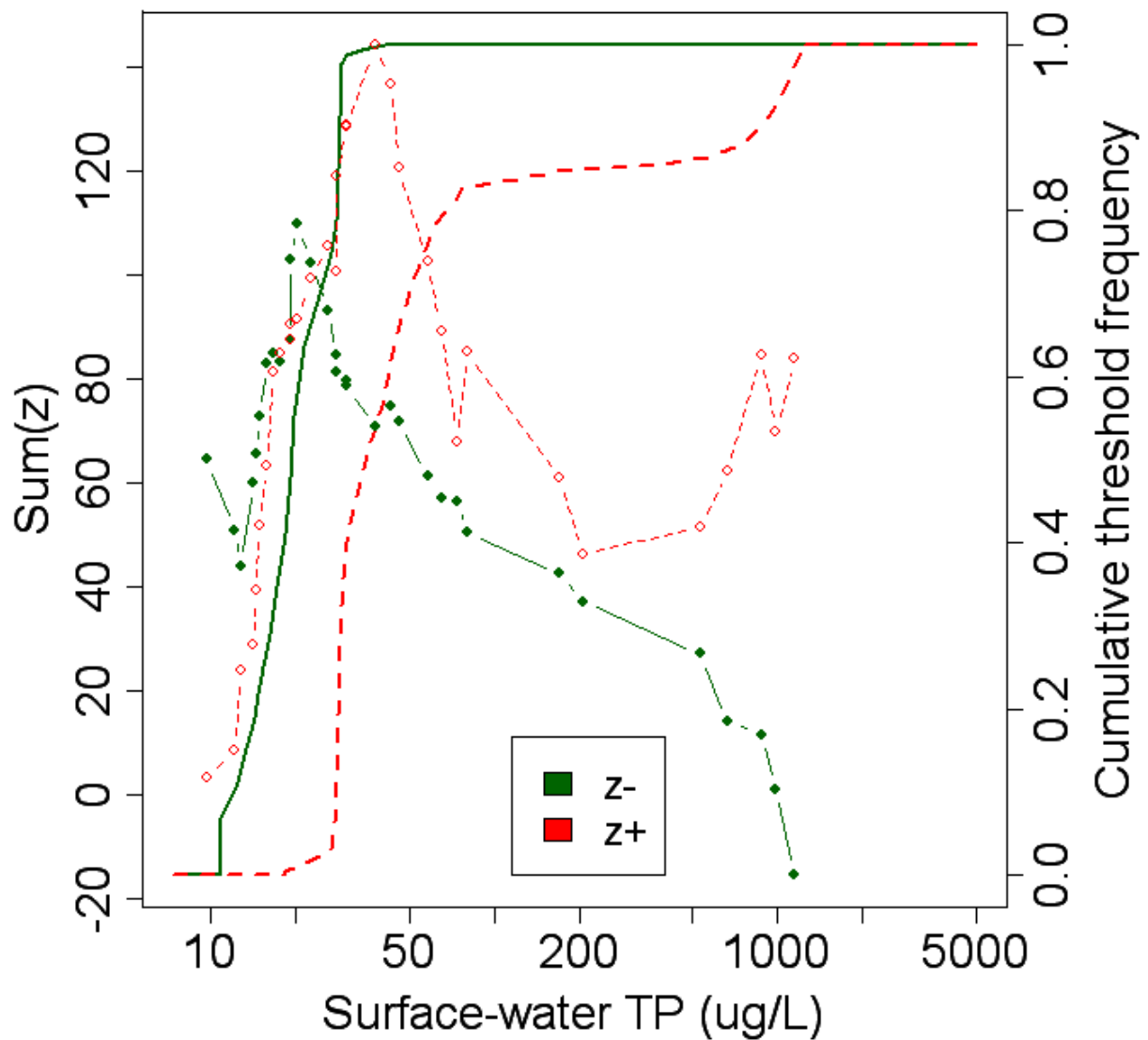


Figure 23. Results of Threshold Indicator Taxa Analysis (TITAN) using surface-water TP as a predictor of threshold changes in community-level algae abundance data in Ecoregion 29 in summer 2008. Community responses are separated between the aggregate response of negative ($\text{sum}(z^-)$) and positive ($\text{sum}(z^+)$) threshold indicator taxa. The TP value resulting in the highest $\text{sum}(z)$ value is the point in which the greatest cumulative negative (z^-) or positive (z^+) occurs. Bootstrapping is used to estimate the cumulative threshold frequency for negative (green) and positive (red) responses, respectively. See Table 2 for community level ($\text{sum}(z)$) thresholds.

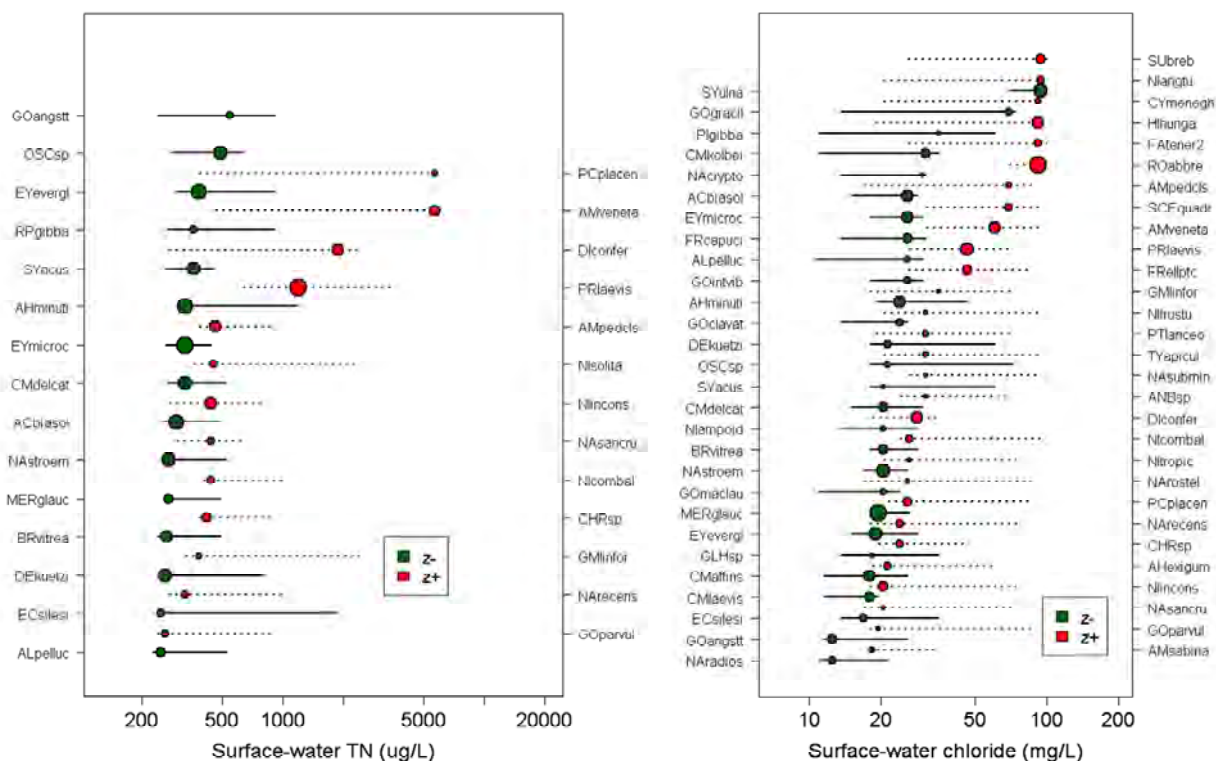


Figure 24. Results of Threshold Indicator Taxa Analysis (TITAN) using surface-water TN (left panel) and chloride (right panel) as predictors of threshold changes in individual algal species in Ecoregion 29 in summer 2008. Taxa are classified as either negative (z-) or positive (z+) threshold indicators based on the direction of response to these predictors. The observed TN or chloride threshold value (colored symbols) correspond to each taxon deemed to change significantly, and the size of the symbol corresponds to the magnitude of the response. Taxon IDs (see Appendix A5) are shown on the left (negative indicators) and right (positive indicators) y-axes, in rank order of their thresholds. Line segments around each symbol are 90% confidence intervals around each observed threshold. Symbol sizes correspond to the indicator score.

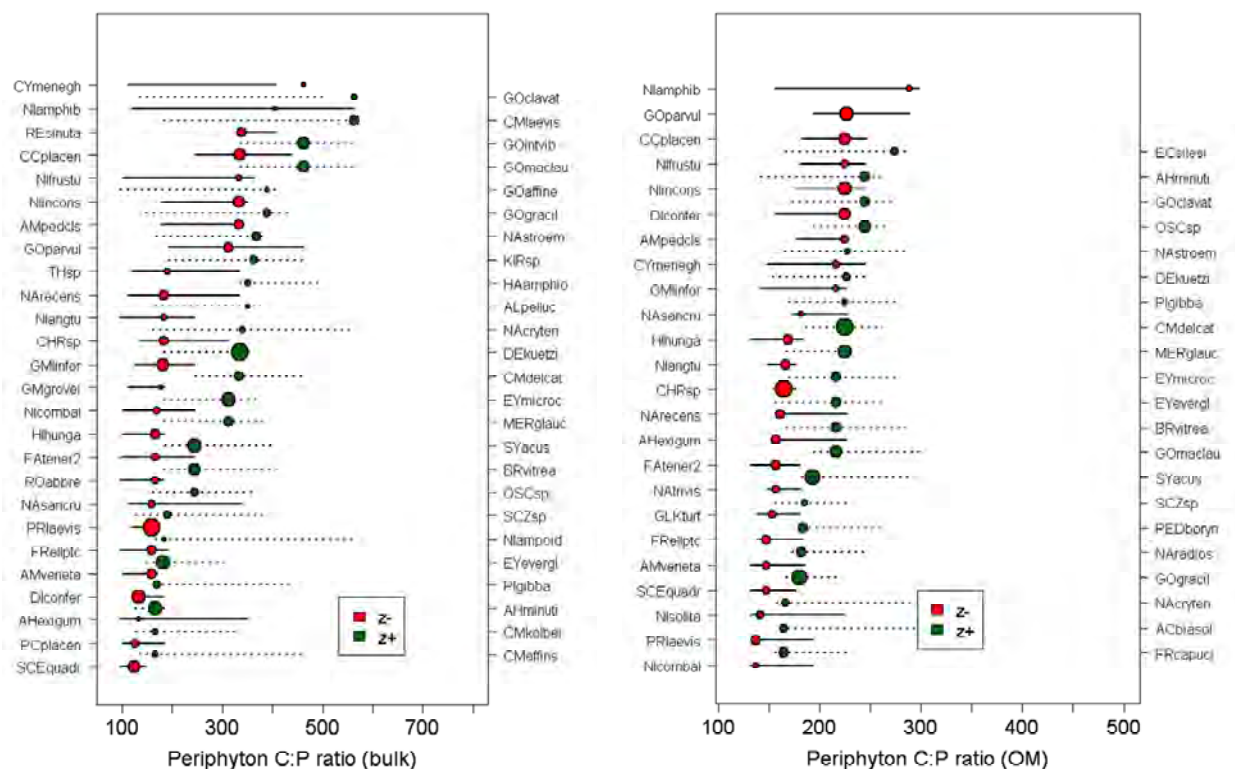


Figure 25. Results of Threshold Indicator Taxa Analysis (TITAN) using periphyton C:P bulk (left panel) and periphyton C:P OM (right panel) as predictors of threshold changes in individual algal species in Ecoregion 29 in summer 2008. Taxa are classified as either negative (z-) or positive (z+) threshold indicators based on the direction of response to the C:P ratios in the periphyton. The observed C:P threshold value (colored symbols) correspond to each taxon deemed to change significantly, and the size of the symbol corresponds to the magnitude of the response. Taxon IDs (see Appendix A5) are shown on the left (negative indicators) and right (positive indicators) y-axes, in rank order of their C:P thresholds. Line segments around each symbol are 90% confidence intervals around the C:P threshold. Symbol sizes correspond to the indicator score.

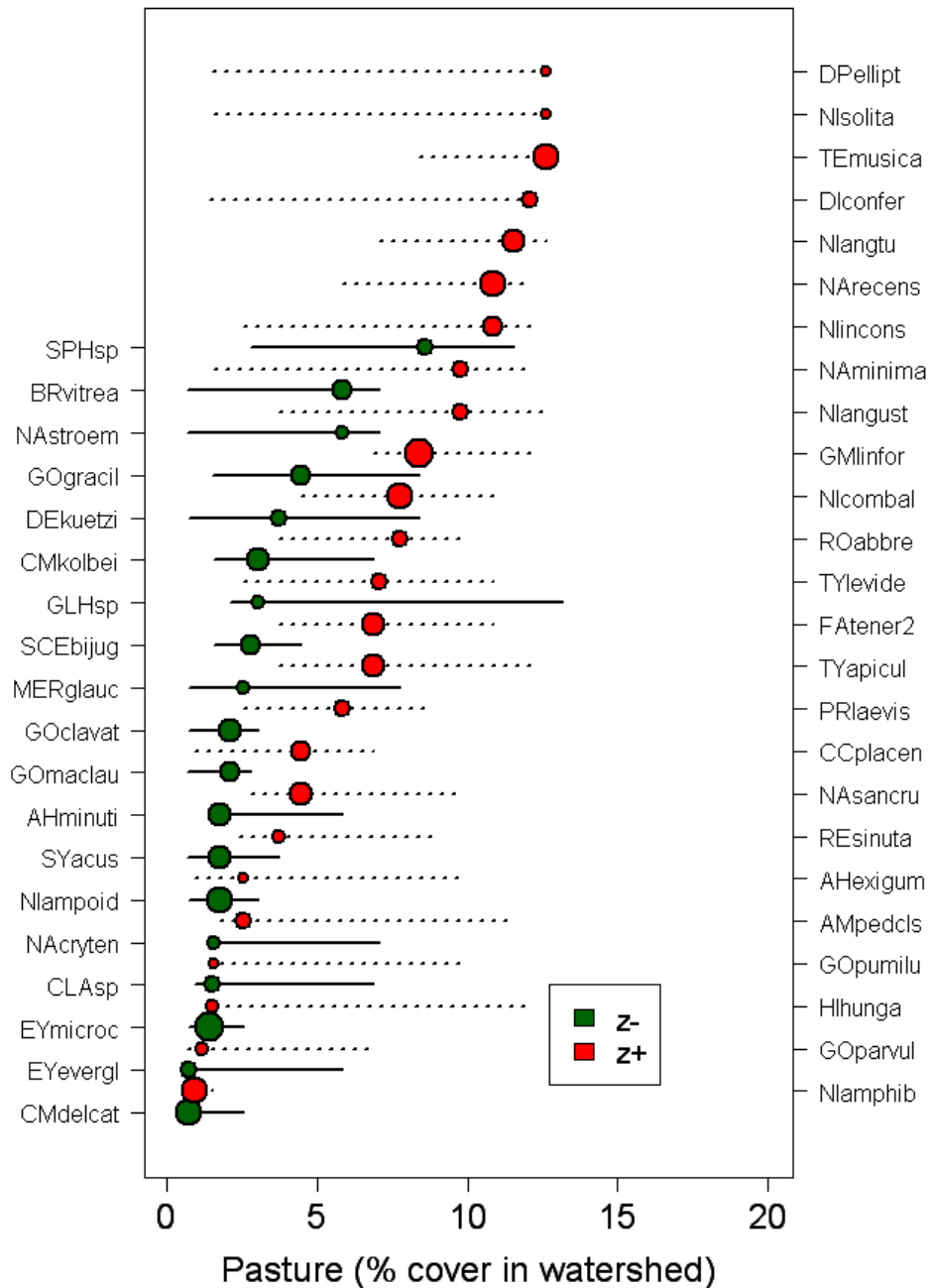


Figure 26. Results of Threshold Indicator Taxa Analysis (TITAN) using % pasture cover in watersheds as a predictor of threshold changes in individual algal species in Ecoregion 29 in summer 2008. Taxa are classified as either negative (z-) or positive (z+) threshold indicators based on the direction of response to % pasture. The observed % pasture threshold value (colored symbols) correspond to each taxon deemed to change significantly. Taxon IDs (see Appendix A5) are shown on the left (negative indicators) and right (positive indicators) y-axes, in rank order of their % pasture thresholds. Line segments around each symbol are 90% confidence intervals around the % pasture threshold. Symbol sizes correspond to the indicator score.

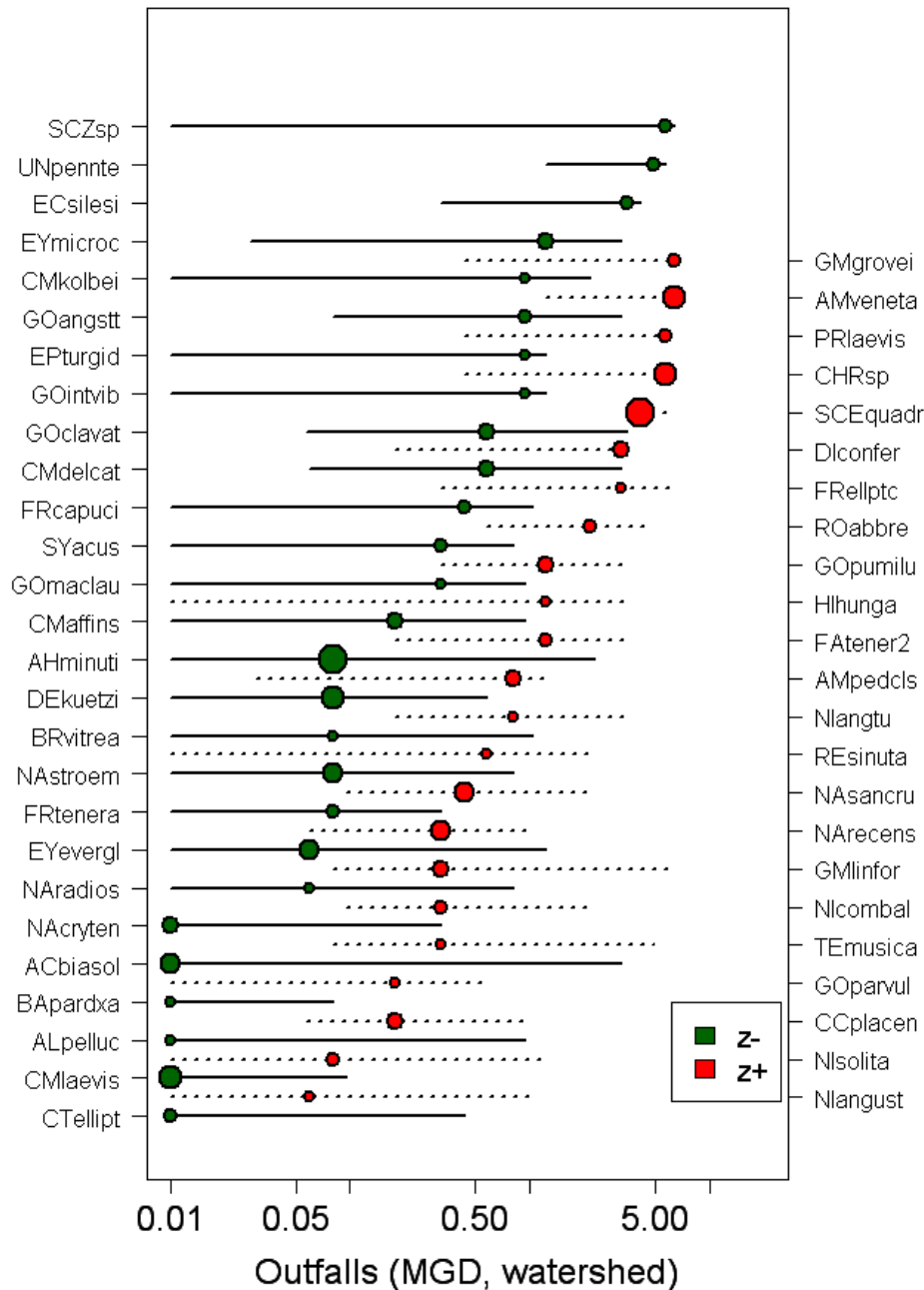


Figure 27. Results of Threshold Indicator Taxa Analysis (TITAN) using outfalls (mgd) in watersheds as a predictor of threshold changes in individual algal species in Ecoregion 29 in summer 2008. Taxa are classified as either negative (z-) or positive (z+) threshold indicators based on the direction of response to outfalls. The observed outfall (mgd) threshold value (colored symbols) correspond to each taxon deemed to change significantly. Taxon IDs (see Appendix A5) are shown on the left (negative indicators) and right (positive indicators) y-axes, in rank order of their outfall thresholds. Line segments around each symbol are 90% confidence intervals around the outfall threshold. Symbol sizes correspond to the indicator score.

Table 2. Community-level results from Threshold Indicator Taxa Analysis (TITAN) on **algal species** composition from **Ecoregion 29** in response to water and periphyton nutrient concentrations, sedimentation, outfalls, pasture, and chloride. Thresholds (*Obs.*) are based on the value of the predictor resulting in the greatest aggregate decrease (sum(z-)) or increase (sum(z+)) in the frequency and abundance of taxa in the community. Taxa responses associated with lower nutrient or stressor conditions are shown in **bold**. The lower (10%), middle (50%), and upper (90%) quantiles of 1,000 bootstraps represent measures of uncertainty around the observed threshold. **Note that lower C:P values = higher P enrichment relative to organic carbon in the periphyton, thus taxa that “decrease” sharply in response to increasing C:P are associated with higher levels of P-enrichment,, whereas “increaser” taxa are associated with lower levels of P enrichment..* See previous figures for details.

	Threshold Indicator	Taxa response > threshold	Obs. threshold	Bootstrap Threshold Quantiles		
				10%	50%	90%
TP (ug/L)	sumz-	Decline	21.43	12.44	19.68	28.95
	sumz+	Increase	40.73	28.95	40.73	932.17
TN (ug/L)	sumz-	Decline	384.67	225.33	271.00	462.50
	sumz+	Increase	440.83	402.50	462.50	5723.33
Periphyton C:P (OM)	sumz-	Decline	216.60	141.57	182.03	216.60
	sumz+	Increase	225.08	182.03	227.57	295.89
Periphyton C:P (bulk)	sumz-	Decline	159.00	95.50	165.95	245.12
	sumz+	Increase	335.81	178.01	191.55	438.57
Pasture (%)	sumz-	Decline	3.26	0.77	2.42	3.26
	sumz+	Increase	7.05	3.26	8.61	12.36
Outfalls (MGD)	sumz-	Decline	0.32	0.01	0.18	0.58
	sumz+	Increase	0.32	0.18	0.58	5.69
Mud-silt (%)	sumz-	Decline	0.00	0.00	1.21	3.75
	sumz+	Increase	15.42	2.20	13.75	21.17
Chloride (mg/L)	sumz-	Decline	20.50	18.00	20.50	26.00
	sumz+	Increase	31.00	24.00	30.00	92.50

RESULTS AND INTERPRETATION, CONTINUED

Multivariate analysis of fish species composition among ecoregions

Winemiller et al (2009) thoroughly describe the relationships between fish communities, habitat variables, and watershed physiographic variables among ecoregions using the summer 2008 data set. Therefore, results presented here are limited to nutrient and nutrient-related variables not included in that report.

Fish community structure in Ecoregion 29 was strongly related to many nutrient and nutrient-related variables (Figure 28). Sites with low TP, chloride, substrate embeddedness, mud-silt, chlorophyll-a, filtrable and nonfiltrable residue and high periphyton C:P, C:N, and N:P ratios were grouped on the left end of axis 1, the most important axis of community structure (Figures 28-30). Watershed outfalls and pasture also were significantly related to fish communities along this axis and suggested that both were potential drivers of these biological changes. These local and watershed variables were therefore selected as predictors of potential threshold changes in fish species and subsequent metrics based on combinations of fish species (see *Threshold responses of fish species to nutrient gradients in Ecoregion 29*, next section).

Fish communities in Ecoregion 32 and 33 were weakly related to a few nutrient or nutrient-related variables (Figures 31, 32). Because of small sample sizes, insufficient sites with low levels of nutrients, and outliers, none of these relationships was sufficiently strong to be reliable or interpretable. However, these weak trends imply that watershed stressors such as outfalls, rowcrop, pasture, and impervious cover are likely influencing fish communities in these ecoregions, and that nutrient enrichment and sedimentation probably play a role in reduction of biological integrity in these stream ecosystems. Additional sites that fill gaps in the spatial distribution of sites in these ecoregions are needed to adequately evaluate biological responses to habitat, water quality, and watershed sources of abiotic stressors.

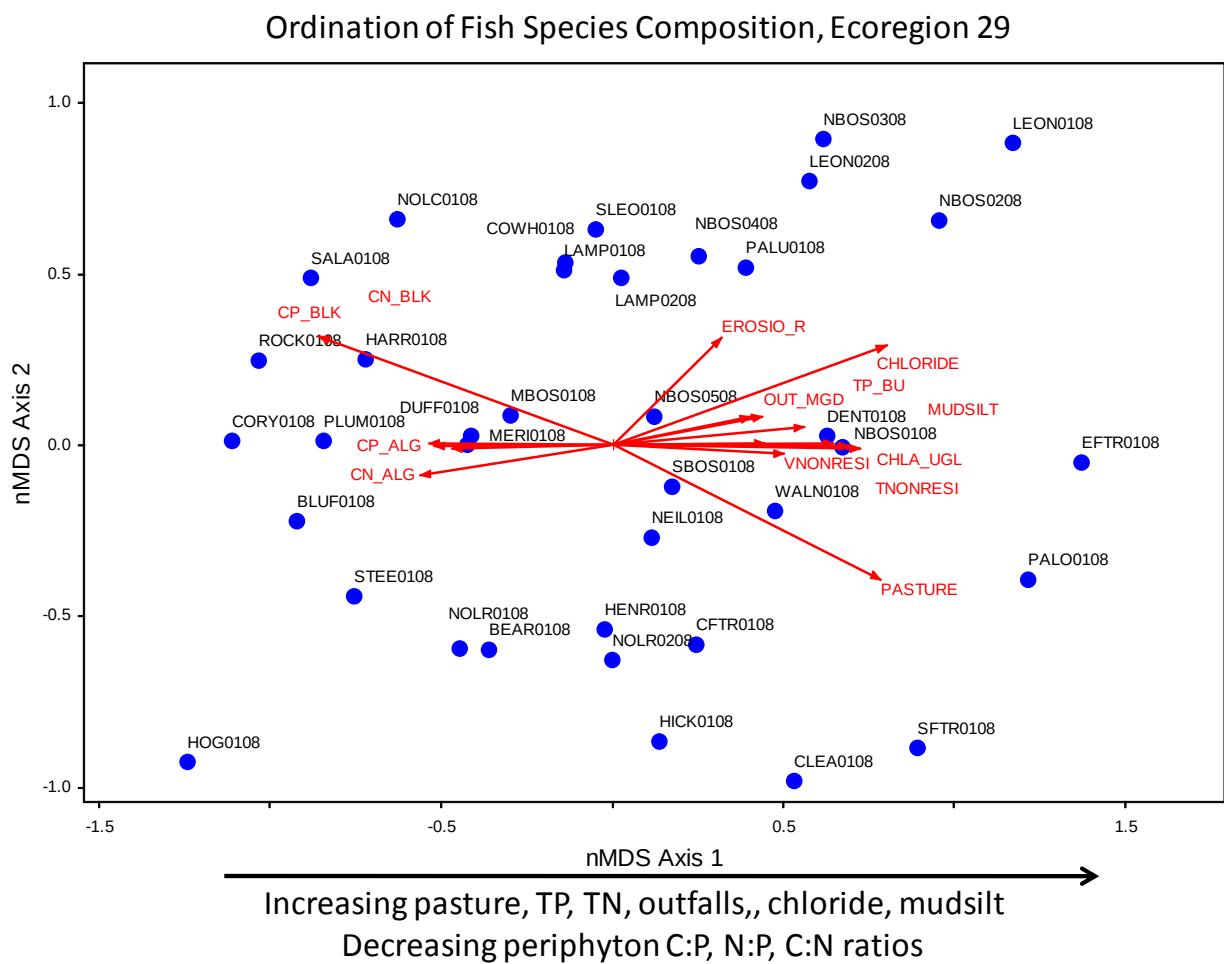


Figure 28. Nonmetric multidimensional scaling ordination of fish species composition among the 38 sites in Ecoregion 29 in summer 2008. Abundance data was $\log_{10}(x+1)$ transformed prior to analysis. Bray-Curtis distance was used as the dissimilarity metric. Distances between sites in the ordination space are proportional to taxonomic dissimilarity (near=similar, far=dissimilar). In each figure, the red arrows (vectors) represent the direction and magnitude of significant ($p < 0.05$) correlations between environmental variables and fish species composition. See Appendices for full variable names.

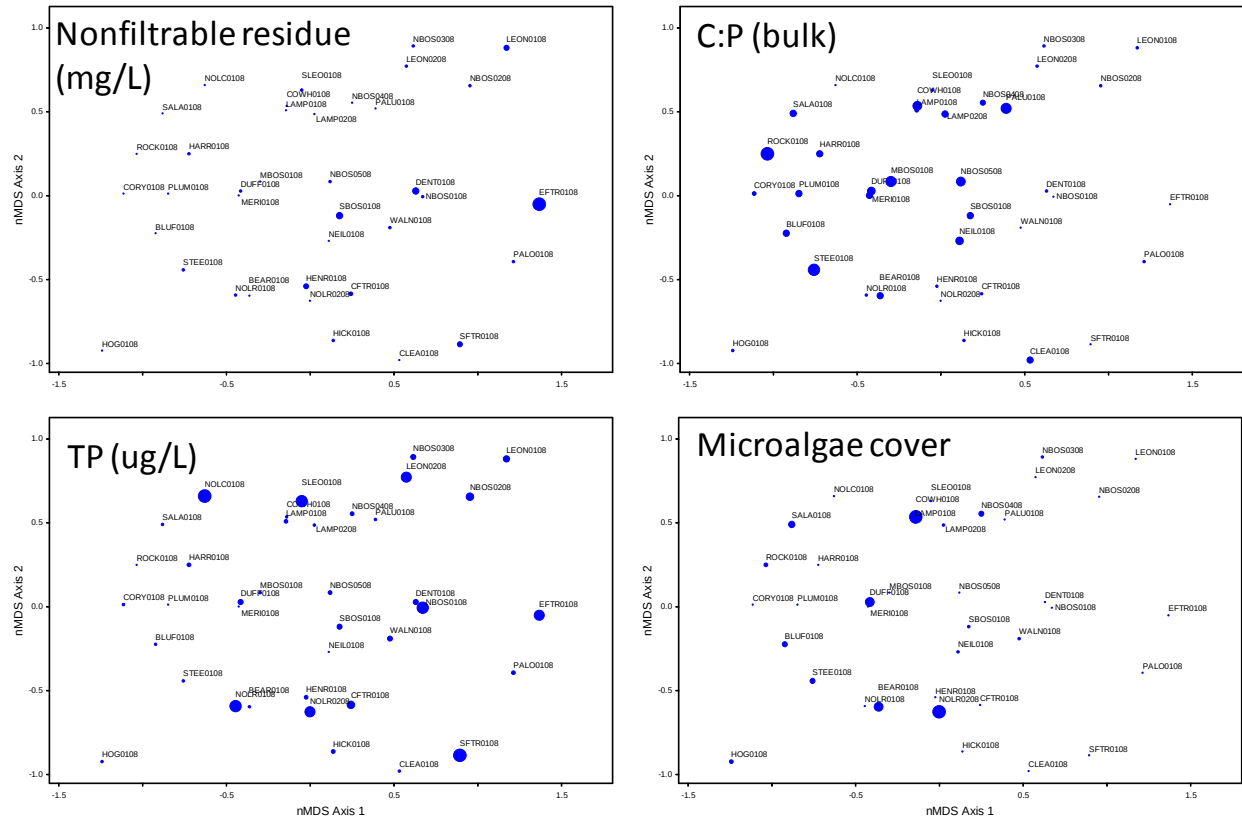


Figure 29. Nonmetric multidimensional scaling ordination of fish species composition among the 38 sites in Ecoregion 29 in summer 2008. The ordination diagram is identical to Figure 28, except that site symbols are scaled in proportion to measured values of surface-water TP, periphyton C:P (bulk), microalgae cover, and total nonfiltrable residue (water).

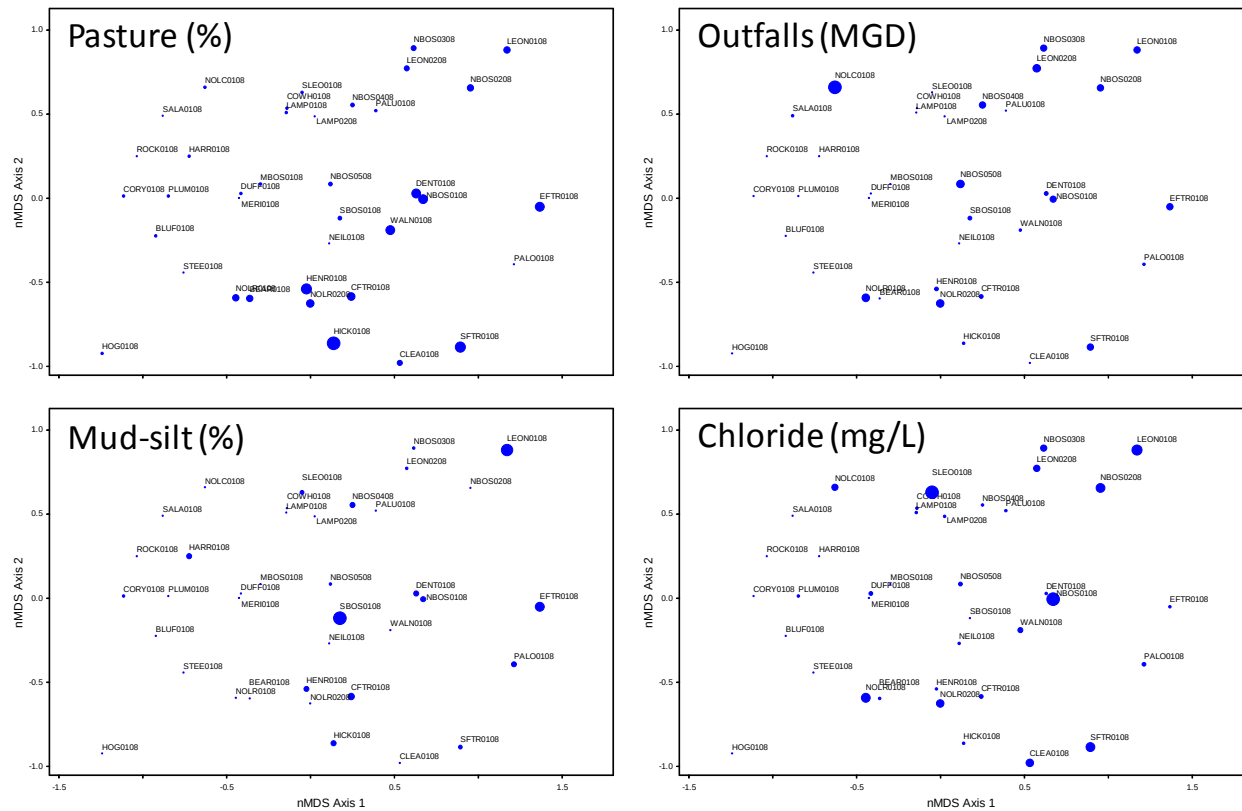


Figure 30. Nonmetric multidimensional scaling ordination of fish species composition among the 38 sites in Ecoregion 29 in summer 2008. The ordination diagram is identical to Figure 28, except that site symbols are scaled in proportion to measured values of pasture (%), outfalls (mgd), mud-silt (%), and chloride (mg/L).

Ordination of Fish Species Composition, Ecoregion 32

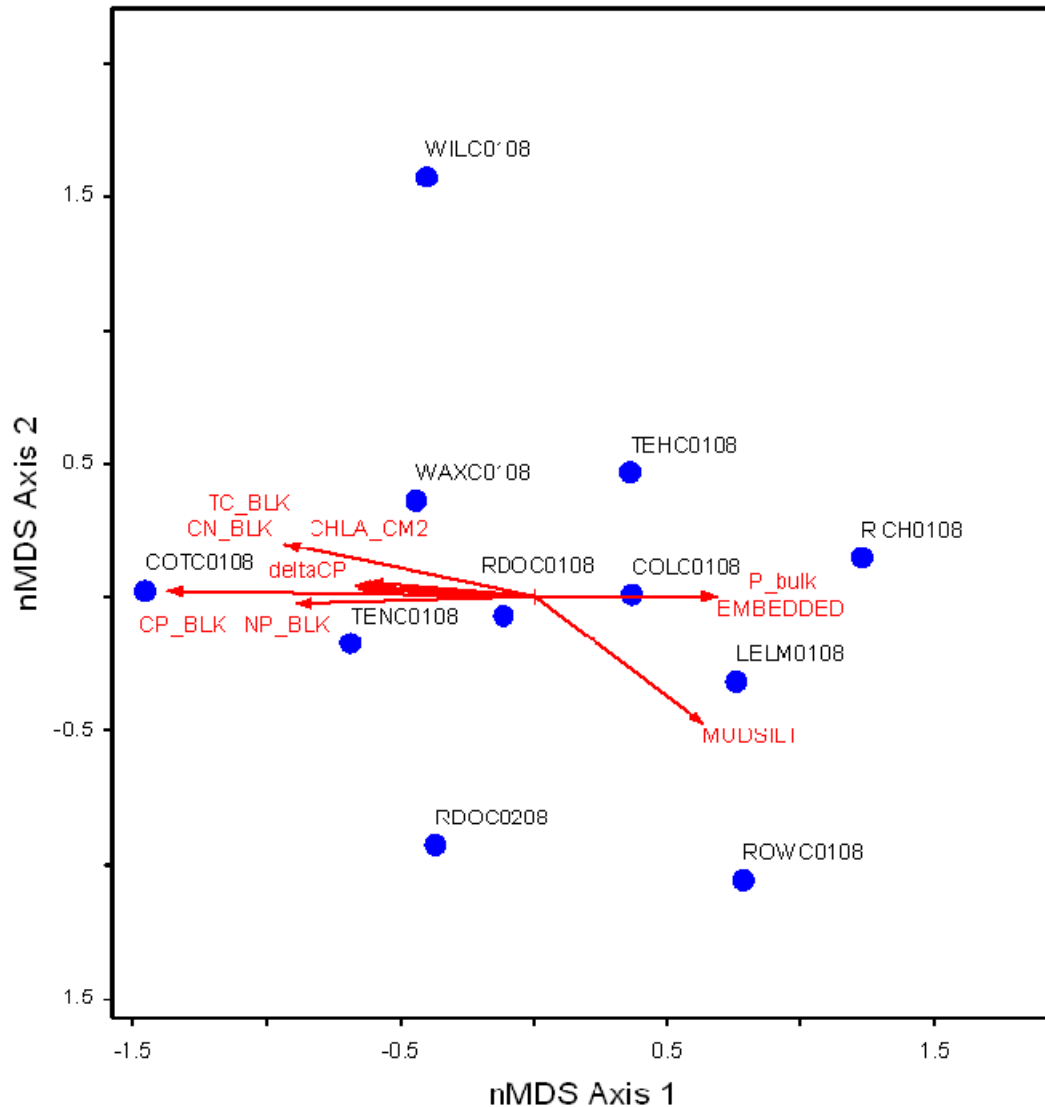


Figure 31. Nonmetric multidimensional scaling ordination of fish species composition among the 11 sites in Ecoregion 32 in summer 2008. Abundance data was $\log_{10}(x+1)$ transformed prior to analysis. Bray-Curtis distance was used as the dissimilarity metric. Distances between sites in the ordination space are proportional to taxonomic dissimilarity (near=similar, far=dissimilar). In each figure, the red arrows (vectors) represent the direction and magnitude of significant ($p < 0.05$) correlations between environmental variables and fish species composition. See Appendices for full variable names.

Ordination of Fish Species Composition, Ecoregion 33

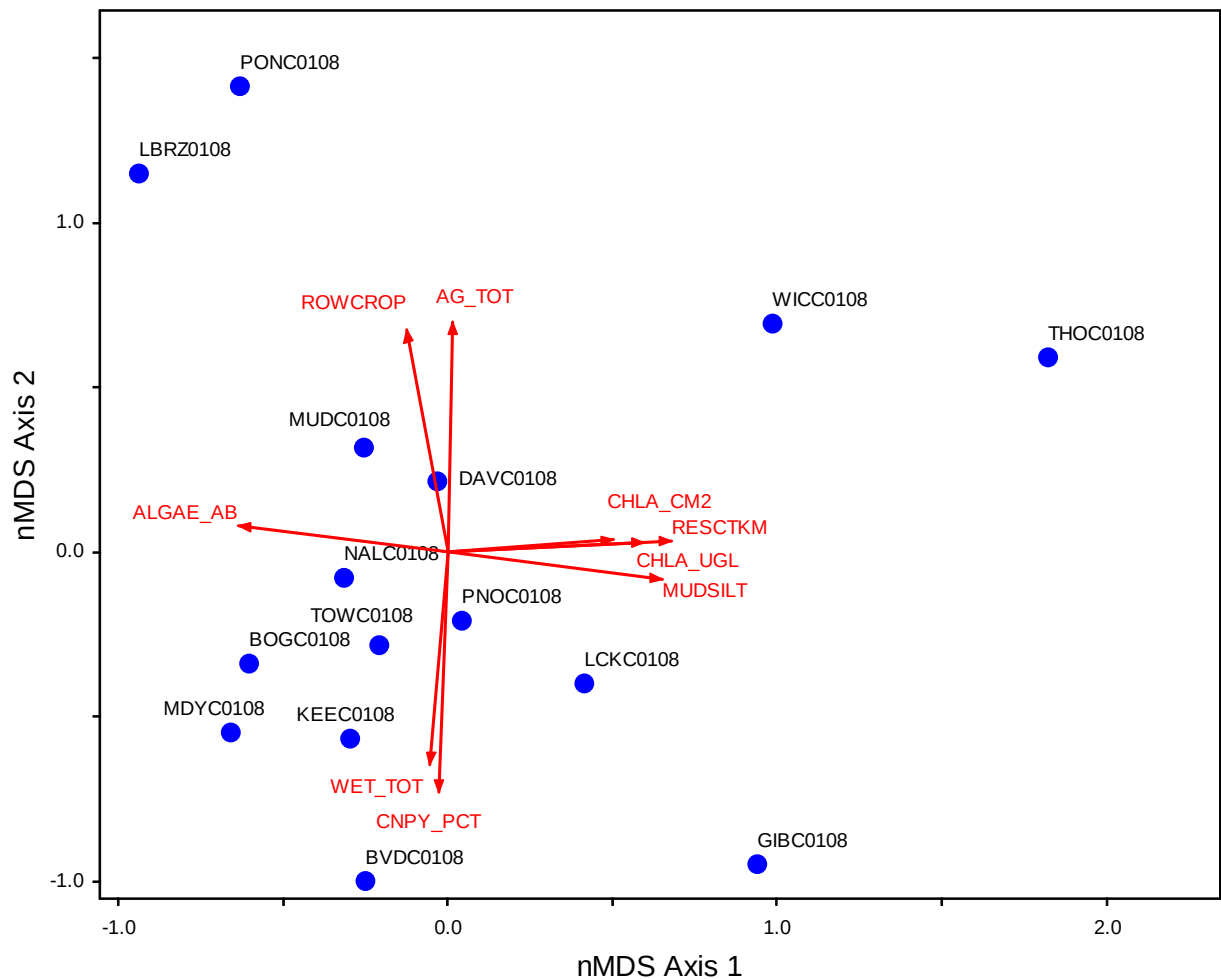


Figure 32. Nonmetric multidimensional scaling ordination of fish species composition among the 15 sites in Ecoregion 33 in summer 2008. Abundance data was $\log_{10}(x+1)$ transformed prior to analysis. Bray-Curtis distance was used as the dissimilarity metric. Distances between sites in the ordination space are proportional to taxonomic dissimilarity (near=similar, far=dissimilar). In each figure, the red arrows (vectors) represent the direction and magnitude of significant ($p<0.05$) correlations between environmental variables and fish species composition. See Appendices for full variable names.

RESULTS AND INTERPRETATION, CONTINUED

Threshold responses of fish species to nutrient gradients in Ecoregion 29

TITAN revealed that four fish species significantly declined in response to surface-water TP (Figure 33; Appendix A7). Three of these species (CYPRVENU=*Cyprinella venusta*, blacktail shiner; ETHESPEC=*Etheostoma spectabile*, orangethroat darter; CAMPANOM=*Camptostoma anomalum*, central stoneroller) had observed thresholds between 15 and 25 ug/L TP. The TP level most likely to result in a community level decline (sum z-) was 28 ug/L (Table 3).

Four fish species sharply increased in abundance and frequency of occurrence in sites with elevated TP: CYPRLUTR (*Cyprinella lutrensis*, red shiner), PIMEVIGI (*Pimephales vigilax*, bullhead minnow), LEPIOSSE (*Lepisosteus osseus*, longnose gar), and CARPCARP (*Carpionodes carpio*, river carpsucker) (Figure 33; Appendix A7). The community-level threshold for taxa that proliferated with TP enrichment was 30 ug/L (Table 3).

Most of these same species either declined or increased in response to periphyton C:P ratios, chloride, mud-silt cover, substrate embeddness, outfalls, and pasture (Table 3; Figure 34). Additional fish species that significantly declined in response to one or more of these stressors included *Fundulus notatus* (FUNDNOTA), *Lepomis gulosus* (LEPOGULO), *Notropis volucellus* (NOTRVOLU), *Moxostoma congestum* (MOXOCONG), and *Lepomis cyanellus* (LEPOCYAN) (Figure 34, Appendix A7).

Additional species that proliferated with increasing levels of nutrients or nutrient-related stressors included *Cyprinus carpio* (CYPRCARP), *Lythurus umbratilis* (LYTHUMBR), *Dorosoma cepedianum* (DOROCEPE), *Pylodictis olivaris* (PYLOOLIV), and *Pomoxis annularis* (POMOANNU) (Figure 34; Appendix A7). Most of these species are typically associated with turbid low gradient streams or reservoirs.

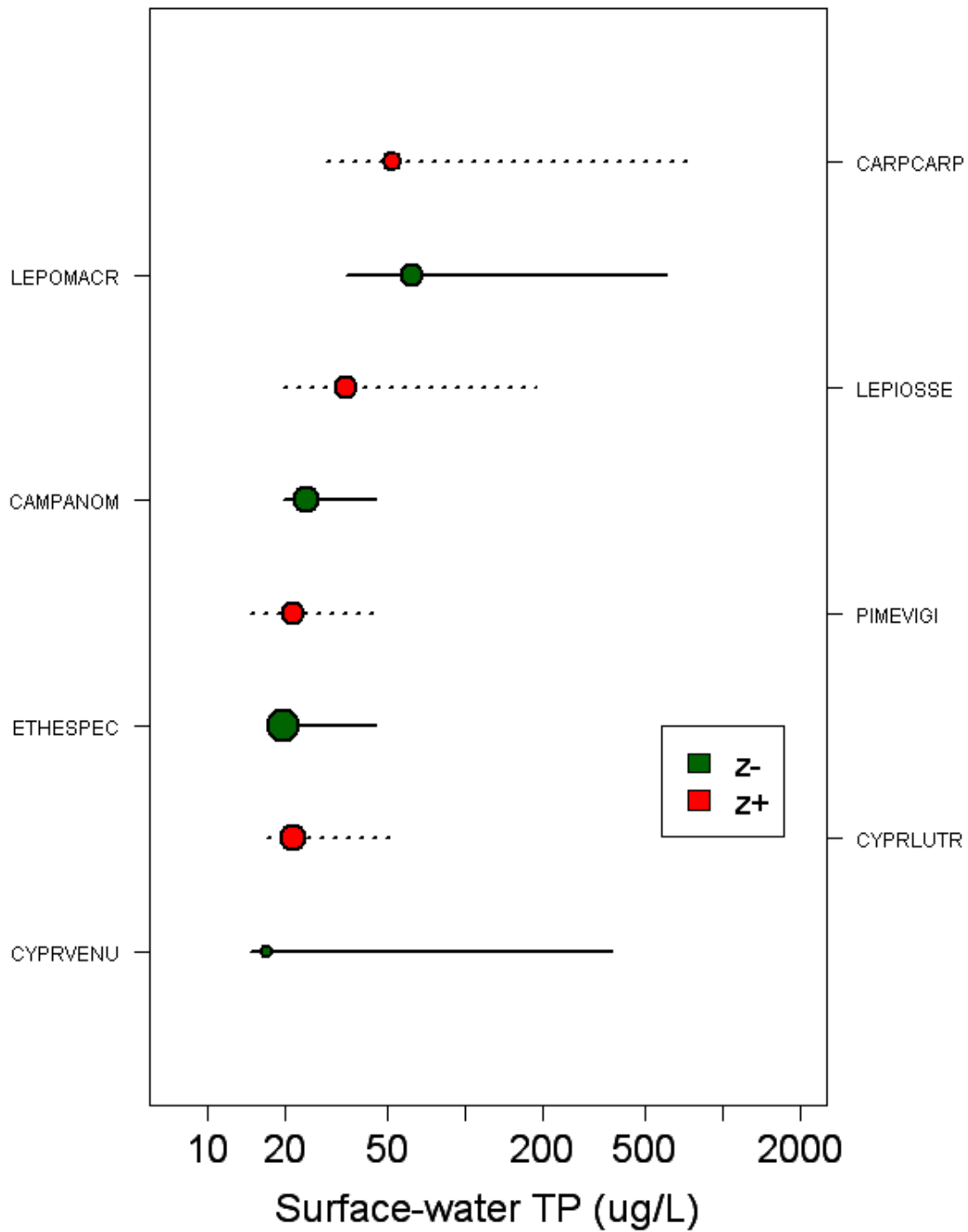


Figure 33. Results of Threshold Indicator Taxa ANalysis (TITAN) using surface-water TP as a predictor of threshold changes in individual fish species distributions in Ecoregion 29 in summer 2008.

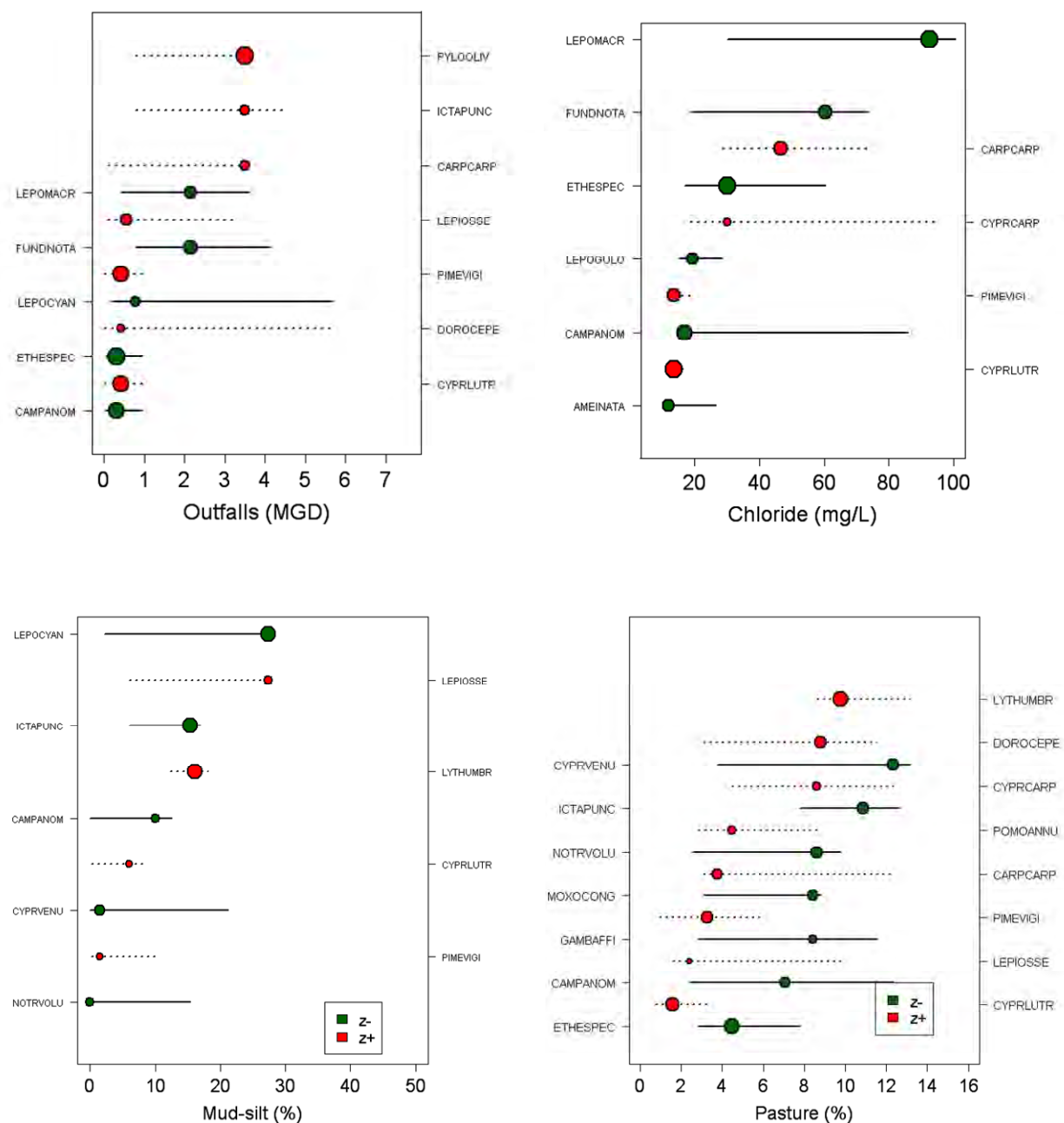


Figure 34. Results of Threshold Indicator Taxa Analysis (TITAN) using outfalls, chloride, mud-silt cover, and pasture as predictors of threshold changes in individual fish species distributions in Ecoregion 29 in summer 2008.

Table 3 Community-level results from Threshold Indicator Taxa Analysis (TITAN) on **fish** species composition from **Ecoregion 29** in response to TP, sedimentation, outfalls, pasture, and chloride. Thresholds (*Obs.*) are based on the value of the predictor resulting in the greatest aggregate decrease (sum(z-)) or increase (sum(z+)) in the frequency and abundance of taxa in the community. Taxa responses associated with lower nutrient or stressor conditions are shown in **bold**. The lower (10%), middle (50%), and upper (90%) quantiles of 1,000 bootstraps represent measures of uncertainty around the observed threshold.

Predictor	Threshold Indicator	Taxa response > threshold	Obs. threshold	Bootstrap Threshold Quantiles		
				10%	50%	90%
TP (ug/L)	sumz-	Decline	27.77	17.03	30.18	81.84
	sumz+	Increase	30.18	24.22	34.18	69.78
Mud-silt (%)	sumz-	Decline	0.92	0.00	7.25	16.04
	sumz+	Increase	15.00	6.00	16.04	21.17
Outfalls (MGD)	sumz-	Decline	2.16	0.02	0.80	3.25
	sumz+	Increase	0.31	0.09	0.80	3.27
Pasture (%)	sumz-	Decline	8.61	3.26	7.05	9.79
	sumz+	Increase	3.26	2.81	7.05	12.07
Chloride (mg/L)	sumz-	Decline	18.50	12.50	21.50	60.50
	sumz+	Increase	35.00	13.50	24.00	72.50

Based on these and results described in Winemiller et al. (2009), we evaluated five univariate variables as potential new fish metrics of nutrient or nutrient-related problems in streams of Ecoregion 29:

- Fish community index (nMDS Axis 1). Site values are scores along the primary axis of variation in fish community structure from non-metric multidimensional scaling ordination of the 38 sites in Ecoregion 29 during summer 2008. Low (negative) scores represent sites that are most dissimilar from sites with high levels of outfalls, pasture, nutrients, chloride, and sediment (high, positive scores on axis 1).
- Percent abundance of the key grazing herbivore (*Campostoma anomalum*, central stoneroller). *Campostoma* was found to decline significantly in response to pasture, outfalls, embeddedness, and mud-silt in Winemiller et al. (2009), and additionally to chloride, TP, and C:P periphyton in this study. *Campostoma* plays a fundamental role in stream ecosystem processes in these streams by grazing on periphyton, recycling nutrients, exporting sediment, and as a primary food resource for native predator fishes such as spotted bass (*Micropterus punctulatus*).
- Percent abundance of darters (*Etheostoma*). *Etheostoma spectabile* was the dominant benthic invertivore in clear-water, low nutrient streams in Ecoregion 29, but rapidly declined with increasing nutrient enrichment, sedimentation, chloride, and drivers of these stressors (outfalls, pasture). Other related species (*Percina* spp.) were too infrequently collected to determine statistical significance but likely were negatively affected by these stressors as well. Primarily riffle, crevice-dwelling fish, these fish are mechanistically linked to benthic processes in streams and are another key indicator of biological integrity in these ecosystems.
- Percent abundance of nutrient-intolerant cyprinids (*Cyprinella venusta*, *Notropis volucellus*). Blacktail shiners are common in most streams in Ecoregion 29, but their percent contribution to community structure clearly declined as nutrient enrichment and sedimentation increased. Mimic shiner was also sensitive to these stressors. Note that classification of “intolerant” here is independent of TCEQ or other tolerant/intolerant classifications.

- Percent abundance of nutrient-tolerant cyprinids (*Cyprinella lutrensis*, *Pimephales vigilax*). Both of these species showed sharp increases in abundance with nutrient enrichment, as indicated by TITAN. Although these are native species and contribute positively to “number of native cyprinids”, a metric used in the TCEQ IBI, these species are in fact very tolerant of pollution and benefit from human alterations to streams. Red shiners have been shown through historical analysis of Brazos River seine data (T. Bonner, unpublished data) to have markedly increased in abundance in the past 30-50 years while other native cyprinids have declined, a phenomenon coincident with dam construction and water quality declines in the mainstem Brazos. *Pimephales vigilax*, or bullhead minnow, is a close relative to the toxicological test organism *Pimephales promelas*, or fathead minnow, used because of its ease in reproduction and hardiness. Note that classification of “tolerant” here is independent of TCEQ or other tolerant/intolerant classifications.

Some of the other species found to be negative or positive threshold indicators by TITAN may also serve as stressor-specific metrics of biological integrity, but these responses need further evaluation.

Figures 35-39 and Table 4 illustrate that indeed these univariate metrics all significantly showed threshold responses to all of the stressors identified in the ordination and TITAN analyses.

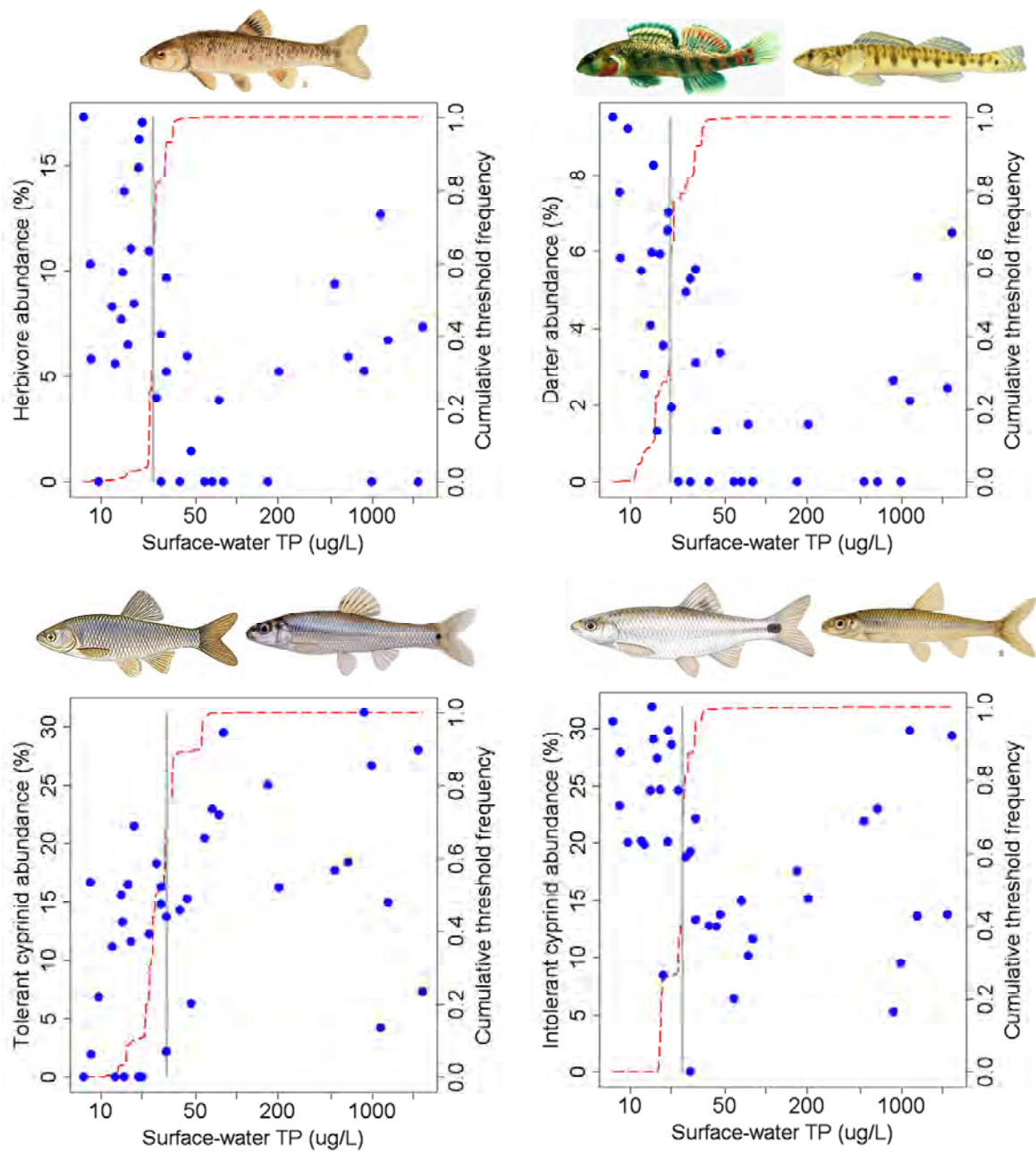


Figure 35. Results on nonparametric changepoint analysis using surface water TP as a predictor of threshold responses in the four proposed new fish metrics of nutrient-related reduction in biological integrity in Ecoregion 29.

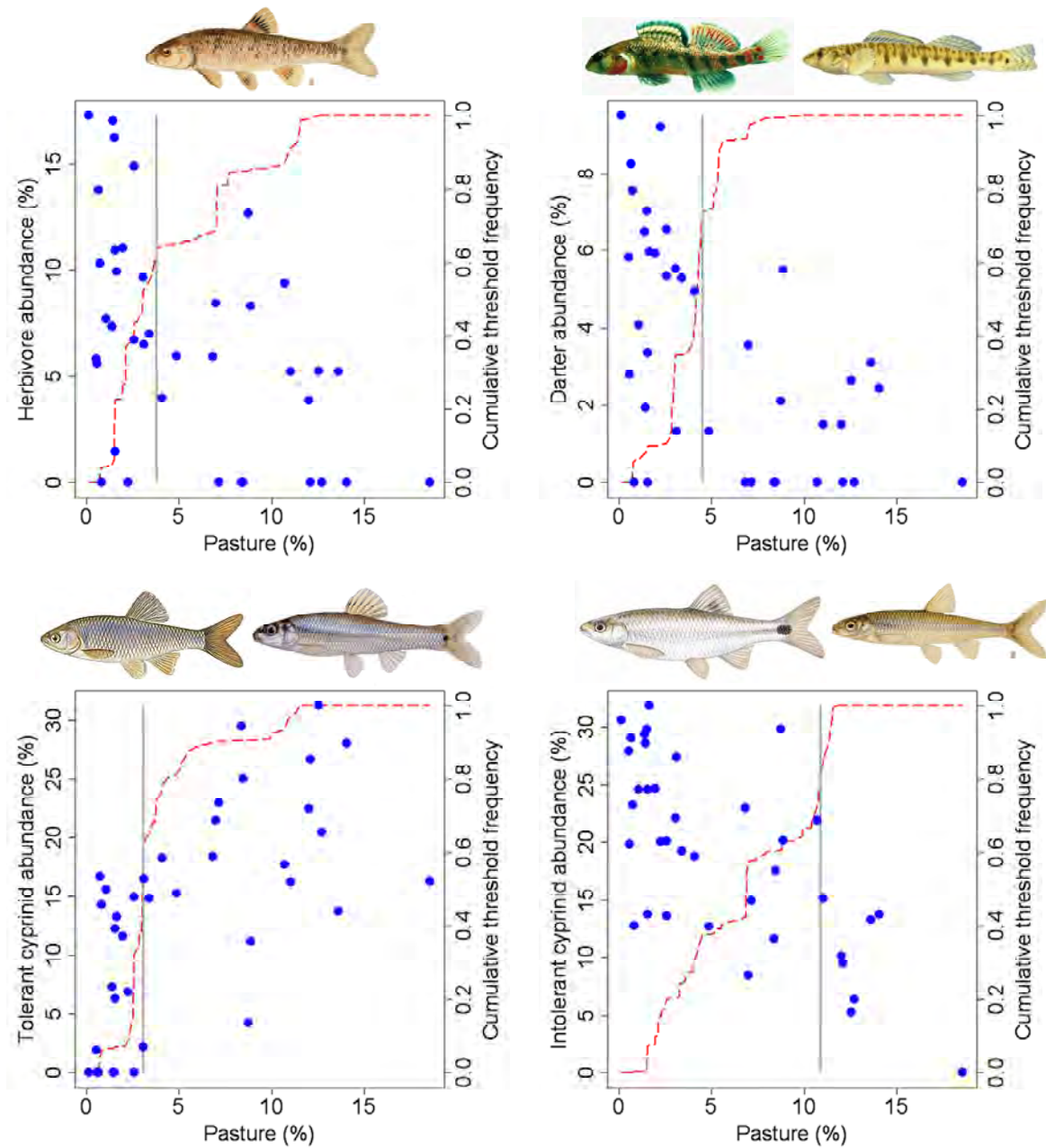


Figure 36. Results on nonparametric changepoint analysis using % pasture in the watershed as a predictor of threshold responses in the four proposed new fish metrics of nutrient-related reduction in biological integrity in Ecoregion 29.

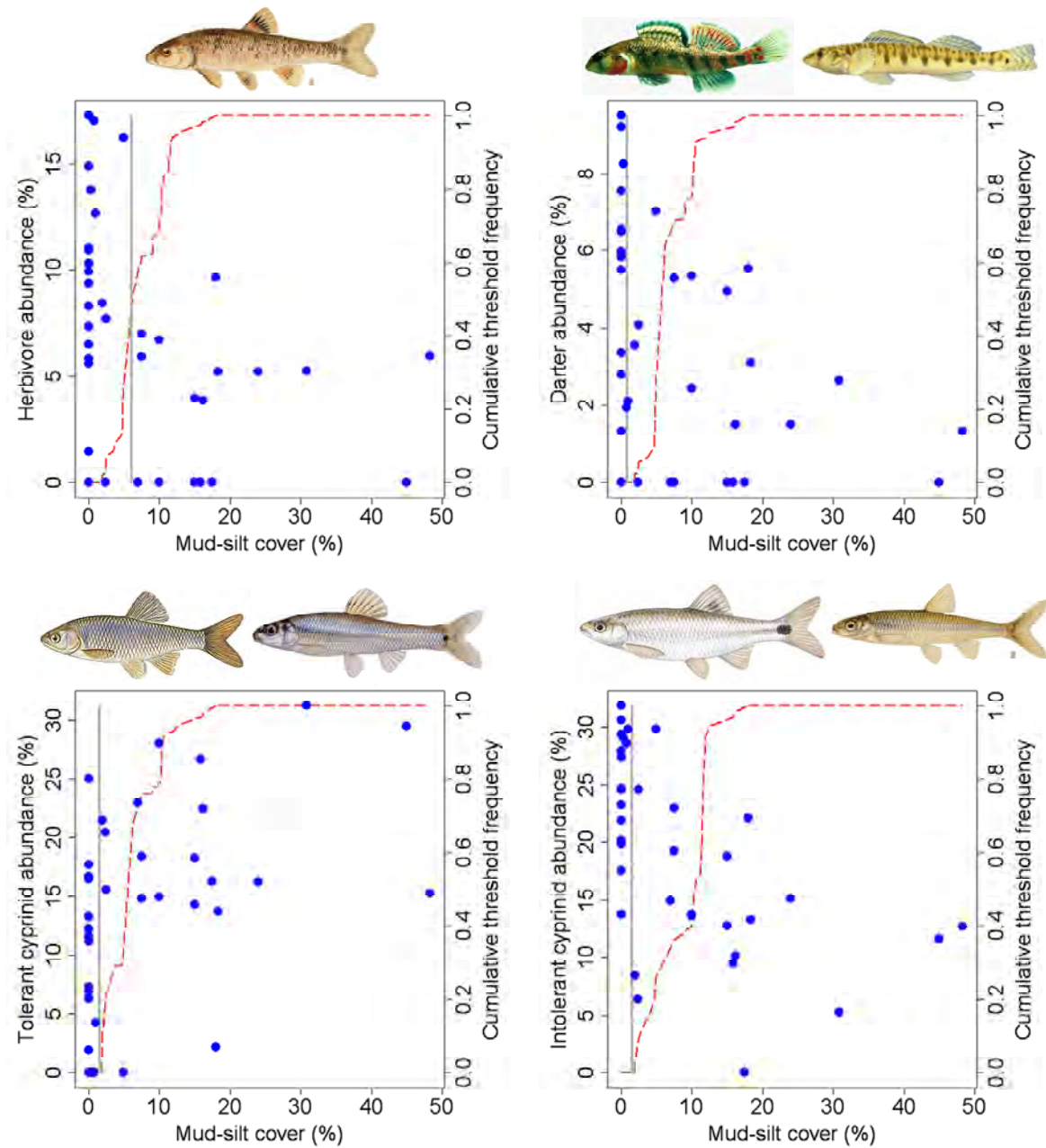


Figure 37. Results on nonparametric changepoint analysis using mud-silt cover (%) as a predictor of threshold responses in the four proposed new fish metrics of nutrient-related reduction in biological integrity in Ecoregion 29.

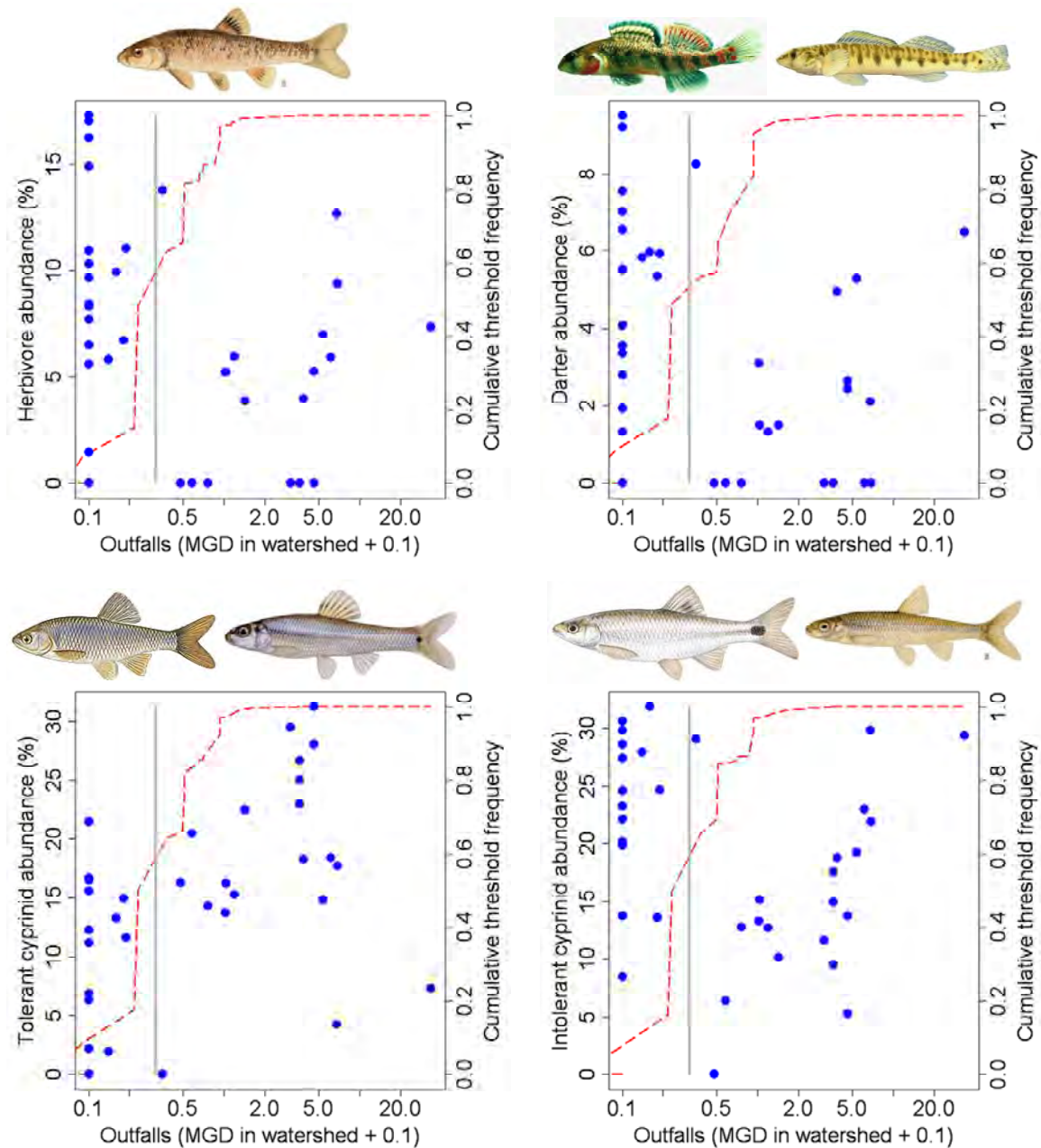


Figure 38. Results on nonparametric changepoint analysis using outfalls (permitted discharge in MGD; not necessarily the actual discharge) as a predictor of threshold responses in the four proposed new fish metrics of nutrient-related reduction in biological integrity in Ecoregion 29.

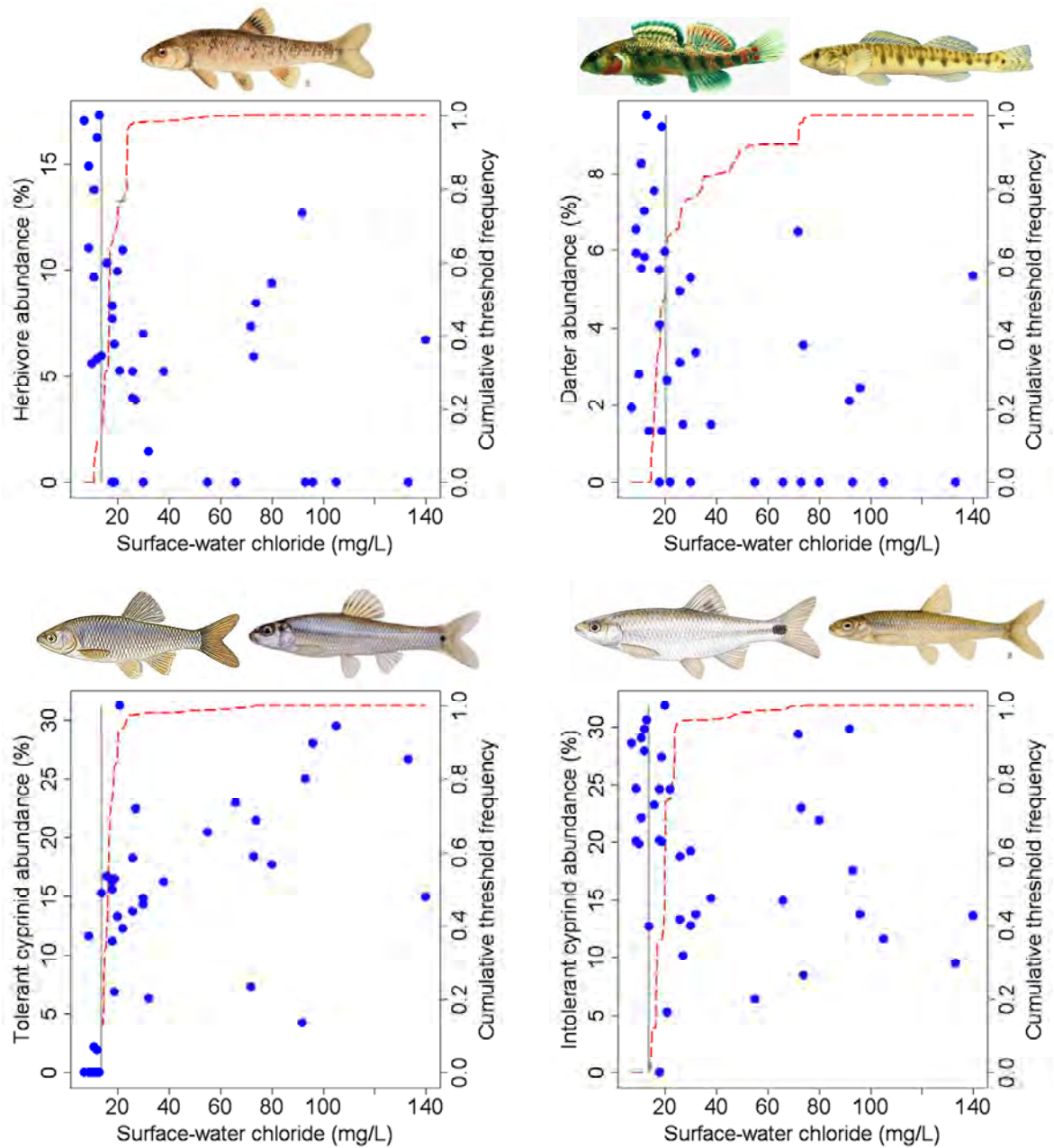


Figure 39. Results on nonparametric changepoint analysis using surface water chloride as a predictor of threshold responses in the four proposed new fish metrics of nutrient-related reduction in biological integrity in Ecoregion 29.

Table 4. Results of nonparametric changepoint analysis using nutrients and nutrient-related predictors of threshold responses in fish community indicators of biological integrity in Ecoregion 29. See figures xx through xx for graphical display of most of these results.

Predictor	Fish metric	Response > obs. threshold	Threshold (obs.)	P value	Bootstrap Threshold Quantiles			Mean < obs.	Mean > obs.
					10%	50%	90%		
Total phosphorus (ug/L)	Darters (%)	Decline	19.68	0.0015	15.12	19.68	30.18	5.93	1.98
Total phosphorus (ug/L)	Grazing herbivore (%)	Decline	24.22	0.0044	22.62	24.22	30.18	10.23	4.07
Total phosphorus (ug/L)	Nutrient-intolerant cyprinids (%)	Decline	24.22	0.0061	17.03	24.22	30.18	24.43	15.20
Total phosphorus (ug/L)	Community index (nMDS 1)	Increase	30.18	0.0041	17.03	25.20	55.15	-0.43	0.48
Total phosphorus (ug/L)	Nutrient-tolerant cyprinids (%)	Increase	52.08	0.0052	21.43	55.85	59.97	9.51	20.35
Periphyton C:P (bulk)	Darters (%)	Increase	178.01	0.0057	170.90	183.72	334.89	1.47	4.86
Periphyton C:P (bulk)	Grazing herbivore (%)	Increase	178.01	0.0159	142.56	178.01	334.10	3.60	8.89
Periphyton C:P (bulk)	Nutrient-intolerant cyprinids (%)	Increase	183.72	0.0180	95.50	183.72	368.01	14.89	22.87
Periphyton C:P (bulk)	Community index (nMDS 1)	Decline	178.01	0.0039	105.97	178.01	189.99	0.53	-0.39
Periphyton C:P (bulk)	Nutrient-tolerant cyprinids (%)	Decline	170.90	0.0096	95.50	173.06	313.72	19.53	9.57
Pasture (%)	Darters (%)	Decline	4.47	0.0023	2.11	4.47	5.45	5.09	1.39
Pasture (%)	Grazing herbivore (%)	Decline	3.75	0.0273	1.52	3.08	11.03	8.95	4.12
Pasture (%)	Nutrient-intolerant cyprinids (%)	Decline	10.87	0.0020	2.10	6.89	11.37	21.73	9.17

Pasture (%)	Community index (nMDS 1)	Increase	3.08	0.0024	2.42	3.08	6.93	-0.50	0.45
Pasture (%)	Nutrient-tolerant cyprinids (%)	Increase	3.08	0.0007	2.42	3.08	3.71	6.84	19.50
Embeddedness (%)	Darters (%)	Decline	12.50	0.0265	14.91	20.83	30.00	6.32	2.78
Embeddedness (%)	Grazing herbivore (%)	Decline	18.33	0.0074	12.50	18.33	30.83	11.17	4.83
Embeddedness (%)	Nutrient-intolerant cyprinids (%)	Decline	28.33	0.0137	17.50	30.00	47.92	23.68	15.37
Embeddedness (%)	Community index (nMDS 1)	Increase	20.83	0.0130	16.67	20.83	30.00	-0.52	0.30
Embeddedness (%)	Nutrient-tolerant cyprinids (%)	Increase	20.83	0.0190	15.83	20.83	32.50	7.67	16.91
Mud-silt (%)	Darters (%)	Decline	0.83	0.0484	5.00	6.25	10.63	4.72	2.40
Mud-silt (%)	Grazing herbivore (%)	Decline	6.00	0.0187	3.75	6.25	11.67	8.84	3.67
Mud-silt (%)	Nutrient-intolerant cyprinids (%)	Decline	1.50	0.0020	3.50	10.42	11.88	24.47	14.25
Mud-silt (%)	Community index (nMDS 1)	Increase	1.50	0.0025	2.00	6.25	10.63	-0.50	0.45
Mud-silt (%)	Nutrient-tolerant cyprinids (%)	Increase	1.50	0.0096	2.00	6.25	12.75	8.37	18.12
Outfalls (MGD)	Darters (%)	Decline	0.31	0.0030	0.22	0.38	0.93	5.22	1.65
Outfalls (MGD)	Grazing herbivore (%)	Decline	0.31	0.0071	0.22	0.38	0.93	9.55	3.77
Outfalls (MGD)	Nutrient-intolerant cyprinids (%)	Decline	0.31	0.0149	0.22	0.31	0.93	23.18	15.00
Outfalls (MGD)	Community index (nMDS 1)	Increase	0.31	0.0021	0.22	0.23	0.93	-0.49	0.49
Outfalls (MGD)	Nutrient-tolerant cyprinids (%)	Increase	0.31	0.0028	0.09	0.93	1.13	7.93	19.08
Chloride (mg/L)	Darters (%)	Decline	20.50	0.0091	15.50	20.00	49.00	5.19	2.01
Chloride (mg/L)	Grazing herbivore (%)	Decline	13.50	0.0029	12.00	17.00	24.00	12.36	4.89
Chloride (mg/L)	Nutrient-intolerant cyprinids (%)	Decline	13.50	0.0263	15.00	20.50	24.00	25.86	16.99
Chloride (mg/L)	Community index (nMDS 1)	Increase	13.50	0.0013	14.00	17.00	20.50	-0.91	0.28
Chloride (mg/L)	Nutrient-tolerant cyprinids (%)	Increase	13.50	0.0005	14.00	15.00	19.00	1.75	17.15

CONCLUSIONS AND RECOMMENDATIONS

The weight of evidence provided in this report, particularly when coupled with the 2-year EPA Region 6 study on nutrient criteria development (Appendix B), implies that nutrient enrichment is a very probable cause of numerous biological changes in streams in Ecoregion 29. Fine-sediment runoff from pastures and overgrazed riparian zones also appears to be stressor that covaries strongly with moderate levels of P enrichment, suggesting that sediment-bound P from pasture runoff is a potential source of enrichment. The highest levels of P enrichment are clearly associated with waste-water treatment plant outfalls. Streams with high volumes of effluent discharge host markedly different biota than relatively unenriched streams.

Specific findings of this study that have important implications for nutrient criteria development and biological assessment methods include the following:

- Because of the overwhelming evidence in this report and in King et al. (2009; Appendix B) of consistent biological changes in streams with > 20 ug/L TP, the current laboratory method used by TCEQ for determining total phosphorus (TP) should be modified to measure lower levels of TP than the current LOD of 50 ug/L. The BU method (Appendix C), which utilizes a Lachat Quik-chem 8500 flow-injection autoanalyzer with a 360 place autosampler, has a lab MDL of around 3.6 ug/L (recomputed based on each run) and has been used in numerous other labs across the country for detecting low levels of TP.
- The TCEQ method for computing total nitrogen based on the addition of nitrate-nitrite-N, ammonia-N and Kjeldahl N analytes yields results that are quite similar to the BU method of measuring total N in one analysis. However, at low levels of TN, the TCEQ method may overestimate TN if the LOD value for one or more of the component analytes is computed as part of the total. Most of the significant biological changepoints in response to TN were detected ranged from 261 to 440 ug/L, thus laboratory methods should ensure that levels at or below this range are within LODs.
- Periphyton nutrient content is a very robust and sensitive indicator of nutrient status in streams in Ecoregion 29, where limestone gravel, cobble, and bedrock are the dominant

substrates. It was also very strongly related to nonlinear changes in algal species composition and fish community structure. We recommend that periphyton C, N and P content be considered as an integrative measure of stream nutrient status, and a strong predictor of biological changes in hard-bottomed streams.

- However, in soft-bottom streams of Ecoregions 32 and 33, periphyton nutrient content measured from sand/mud substrate was highly variable and did not correspond well to surface-water nutrients or changes in algal species composition. We do not recommend continued evaluation of sand/mud periphyton in these systems as an indicator of nutrient-related degradation, and suggest that alternative substrates such as wood or artificial substrates be considered in future studies.
- The existing TCEQ habitat assessment method that relies on 5 to 6 cross-sectional transects to assess stream habitat variables may not be sufficient for adequately characterizing cover of some important structural and functional elements of central Texas streams. Biofilm/microalgae thickness, submersed macrophyte cover, filamentous algae cover, substrate composition, and sediment film thickness on substrate are several metrics that were found to be responsive to TP enrichment by King et al. (2009; Appendix B). However, these variables were assessed using a whole-reach zig-zag transect with 100 points of measurement, which provided a more comprehensive characterization of these often patchy variables than the TCEQ cross-section approach. We compared similar metrics used in the HQI survey (June-August 2008) to those of King et al. (June 2009) and found relatively weak correspondence between the two protocols (Figure 40, next page). However, some of the variance could have been due to differences in the day of sampling (protocols were not compared on the same day at each site). Nevertheless, we recommend that the TCEQ physical habitat assessment and associated HQI consider incorporate more direct measures of these variables into their assessments and consider a more extensive coverage of the reach, either by adding more cross-section transects for certain variables (e.g., EMAP uses 21 for substrate characterization) or adopting the 100-point zig-zag approach used by King et al. 2009. Once investigators are adequately trained, the 100-point method is relatively rapid to employ (1 investigator can complete the 100-point counts in 1-2 hours).

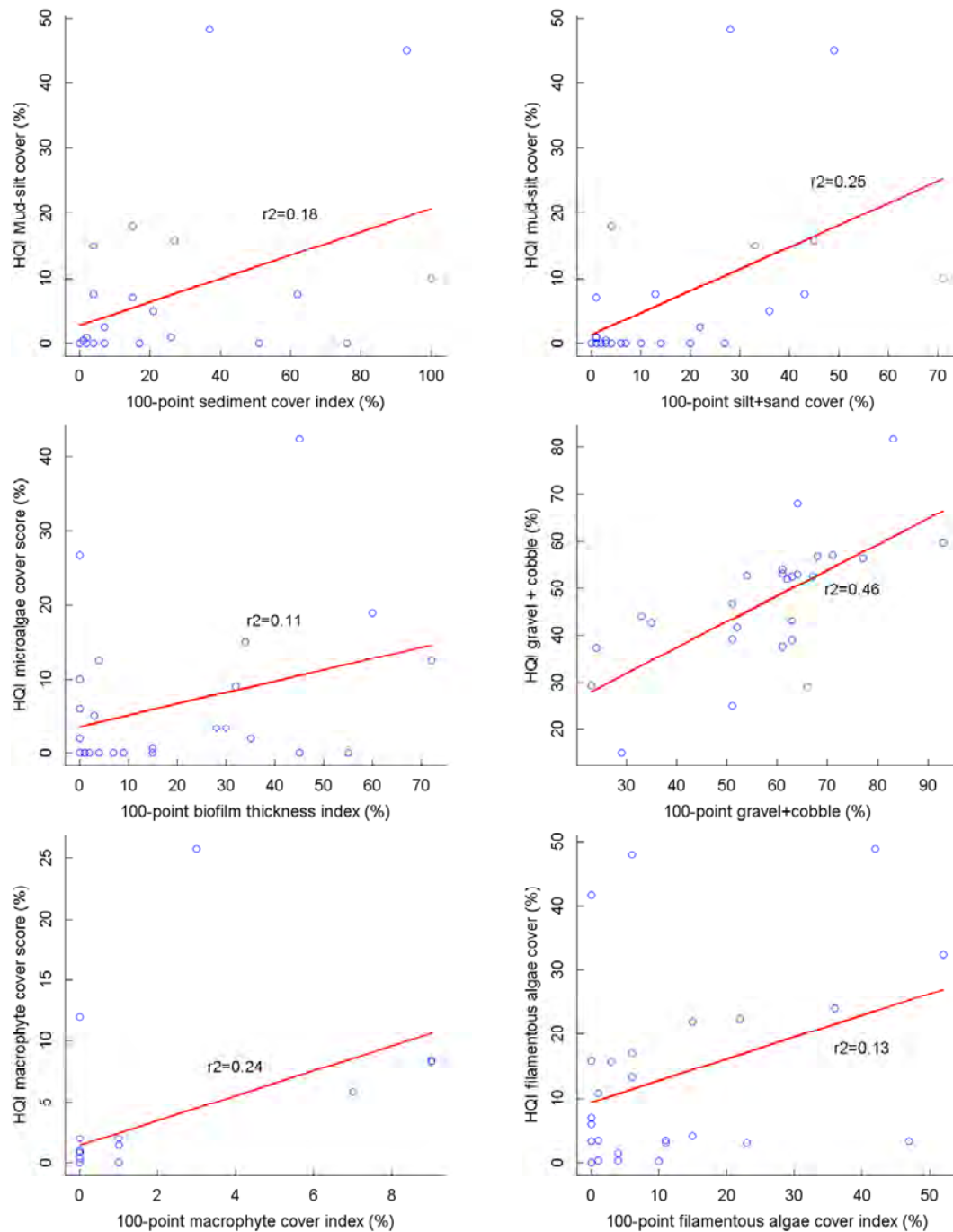


Figure 40. Comparison of fine sediment, gravel+cobble, filamentous algae, biofilm/microalgae, and macrophyte cover (% of reach) using the 100-point zig-zag transect method described in King et al. (2009) versus comparable metrics included in the TCEQ HQI method. Comparisons were made using the 26 stream locations sampled by King et al (2009) in June 2008 and the same locations sampled again in late June-August 2008 for the TCEQ HQI and Nutrient Indicators studies (Winemiller et al. 2009 and this report).

- As a practical compromise to adding some new physical habitat or algal/macrophyte metrics, we suggest that TCEQ consider reducing or eliminating habitat measurements that are either never used in the existing HQI or were not shown to correspond to any biological changes in the 3 ecoregions, as recommended by Winemiller et al (2009).
- Periphyton chlorophyll-a and ash-free dry mass (AFDM) were not reliable indicators of nutrient enrichment in any ecoregion. This is not surprising given the shift from thick, calcareous periphyton comprised of cyanobacteria, diatoms, fungi, and bacteria to a community of pollution-tolerant diatoms and colonial/filamentous green algae consistently reported by King et al. (2009; Appendix B). Periphyton biomass is high in all of these streams, but the structure and function of the periphyton is very different in response to nutrient enrichment. The ratio of chlorophyll a to AFDM (CHLA:AFDM) did show a moderately strong response to TP enrichment in Ecoregion 29, and may be an indicator of significant functional changes in the periphyton as non-chlorophyll bearing organisms decline and are replaced by algae. This metric also consistently increased in response to TP in King et al. (2009; Appendix B).
- Surface-water variables related to particulates (chlorophyll-a, nonfiltrable and filtrable residue) also significantly increased in response to nutrients and may be useful indicators of nutrient-related degradation if found to exceed the reported thresholds in this report. However, some sites had low values for these variables even though sites had high nutrients and substantial changes in biological indicators, thus surface-water measures alone are not adequate for characterizing biological condition.
- Algal species composition was very strongly linked to surface water nutrients, particularly phosphorus, in Ecoregion 29, but was noisy and not related to any nutrient or nutrient related variables among the sand/mud algal samples from Ecoregions 32 and 33. We suggest that algal species composition may provide the most sensitive and direct measure of biological integrity in streams of Ecoregion 29. However, given the strong relationship to less costly and more easily measured predictors (surface-water nutrients,

C:P content of periphyton, etc), these measures are likely to be strong surrogate variables for screening sites for potential biological degradation.

- Numerous algal species declined sharply in Ecoregion 29 in response to surface-water TP between 15 and 25 ug/L. Many other tolerant algae increased at TP between 20 and 50 ug/L. The significant threshold indicator species reported in this document, coupled with species lists provided in King et al. (2009; Appendix B) could be used in developing univariate metrics of nutrient enrichment for Ecoregion 29 streams.
- Fish communities were tightly coupled to the lower-trophic-level biological changes in streams of Ecoregion 29. Based on these and results described in Winemiller et al. (2009), we recommend five potential new fish metrics of nutrient or nutrient-related problems in streams of Ecoregion 29:
 - Fish community index (nMDS Axis 1). Site values are scores along the primary axis of variation in fish community structure from non-metric multidimensional scaling ordination of the 38 sites in Ecoregion 29 during summer 2008. Low (negative) scores represent sites that are most dissimilar from sites with high levels of outfalls, pasture, nutrients, chloride, and sediment (high, positive scores on axis 1).
 - Percent grazing herbivore abundance (*Campostoma anomalum*, central stoneroller). *Campostoma* was found to decline significantly in response to pasture, outfalls, embeddedness, and mud-silt in Winemiller et al. (2009), and additionally to chloride, TP, and C:P periphyton in this study. *Campostoma* plays a fundamental role in stream ecosystem processes in these streams by grazing on periphyton, recycling nutrients, exporting sediment, and as a primary food resource for native predator fishes such as spotted bass (*Micropterus punctulatus*).
 - Percent abundance of darters (*Etheostoma*). *Etheostoma spectabile* was the dominant benthic invertivore in clear-water, low nutrient streams in Ecoregion 29, but rapidly declined with increasing nutrient enrichment, sedimentation, chloride, and drivers of these stressors (outfalls, pasture). Other related species (*Percina* spp.) were too infrequently collected to determine statistical significance but

likely were negatively affected by these stressors as well and could be combined with this metric. Primarily riffle, crevice-dwelling fish, these fish are mechanistically linked to benthic processes in streams and are another key indicator of biological integrity in these ecosystems.

- o Percent abundance of nutrient-intolerant cyprinids (*Cyprinella venusta*, *Notropis volucellus*). Blacktail shiners are common in most streams in Ecoregion 29, but their percent contribution to community structure clearly declined as nutrient enrichment and sedimentation increased. Mimic shiner was also sensitive to these stressors.
- o Percent abundance of nutrient-tolerant cyprinids (*Cyprinella lutrensis*, *Pimephales vigilax*). Both of these species showed sharp increases in abundance with nutrient enrichment, as indicated by TITAN. Although these are native species and contribute positively to “number of native cyprinids”, a metric used in the TCEQ IBI, these species are in fact very tolerant of pollution and benefit from human alterations to streams. Red shiners have been shown through historical analysis of Brazos River seine data (T. Bonner, unpublished data) to have markedly increased in abundance in the past 30-50 years while other native cyprinids have declined, a phenomenon coincident with dam construction and water quality declines in the mainstem Brazos. Red shiner was found to be particularly prolific at sites below outfalls in this study. *Pimephales vigilax*, or bullhead minnow, is a close relative to the toxicological test organism *Pimephales promelas*, or fathead minnow, used because of its ease in reproduction and resistance to physiological stress. Bullhead minnows were only occasionally collected in low-nutrient streams, but were dominant in enriched streams.

In summary, when coupling results of this study with findings of King et al. (2009; Appendix B), there is a very high probability that streams in Ecoregion 29 exposed to surface-water TP levels exceeding 20 ug/L, and possibly 15 ug/L, will experience a strong biological response needing further investigation to establish thresholds for nutrient management, including loss of characteristic structure (periphyton and macrophytes), loss of numerous species (algae, macroinvertebrates (King et al. 2009), and fish), additions of species that are associated with

eutrophication or disturbance, minimum dissolved oxygen levels unsuitable for supporting native fauna during low flows (King et al. 2009), and increase likelihood of nuisance algal growth that could limit the recreational use of streams (King et al. 2009). Streams exceeding 200-500 ug/L may represent another threshold of biological response, with more consistent nuisance algal growth and additional losses of algal, macroinvertebrate and fish species and replacement with species associated with poor water quality.

Additional research on algae and fish community responses to nutrient enrichment and sedimentation is needed in Ecoregions 32 and 33. Insufficient numbers of sites coupled with the poor quality of the sand/mud samples renders these results too uncertain for definitive recommendations. Future studies need to target a minimum of 30 sites per ecoregion and use a reconnaissance approach before selecting sites to ensure that enough sites with both very low and high nutrient levels are represented in the data set to allow indicator development and threshold detection.

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Appendix A1. Key to water chemistry and periphyton variable short names used throughout this document.

Variable	Description
TKN	Total Kjehldal Nitrogen, ug/L
NH3-N	Ammonia-nitrogen, surface water, ug/L
NO2NO3-N	Nitrite + nitrate-nitrogen, surface water, ug/L
PO4-P	Orthophosphate, surface water, ug/L
TN_TCEQ	Total nitrogen, TCEQ lab, (NH3-N + TKN + NO2NO3N)
TN_BU	Total nitrogen, Baylor lab surface water, ug/L
TP_TCEQ	Total phosphorus, TCEQ lab, surface water, ug/L
TP_BU	Total phosphorus, Baylor lab, surface water, ug/L
ALKALIN	Alkalinity, total, surface water, mg/L
CHLORIDE	Chloride, surface water, mg/L
FLOURIDE	Flouride, surface water, mg/L
TNONRESI	Total nonfiltrable residue, mg/L
VNONRESI	Volatile nonfiltrable residue, mg/L
TFILRESI	Total filterable residue, mg/L
CHLA_UGL	Chlorophyll-a, surface water, ug/L
C_ALG	Total carbon, organic fraction of periphyton, %
C_BULK	Total carbon, bulk periphyton, %
N_ALG	Total nitrogen, organic fraction of periphyton, %
N_BULK	Total nitrogen, bulk periphyton, %
P_ALG	Total phosphorus, organic fraction of periphyton, %
P_BULK	Total phosphorus, bulk periphyton, %
CN_ALG	Carbon:nitrogen ratio, OM fraction of periphyton
CN_BULK	Carbon:nitrogen ratio, bulk periphyton
CP_ALG	Carbon:phosphorus ratio, OM fraction of periphyton
CP_BULK	Carbon:phosphorus ratio, bulk periphyton
CP_SED	Carbon:phosphorus ratio, sed fraction of periphyton
NP_ALG	Nitrogen:phosphorus ratio, OM fraction of periphyton
NP_BULK	Nitrogen:phosphorus ratio, bulk periphyton
CHLA_M2	Chloophyll a, periphyton, mg/m2 (rock surface area)
AFDM_M2	Ash-free dry mass, periphyton, g/m2
CHL_AFDM	Chlorophyll-a:AFDM ratio, periphyton, mg/g

Appendix A2. Local-scale environmental variables measured in the HQI component of the study.

Category	Abbreviation	Variable
Habitat type	HAB_TYPE	Habitat type score (riffle, run, pool, or glide) averaged across transects
	NO_RIFF	Number of riffles in study reach
Substrate	BEDROCK	Percent of substrate that is bedrock
	LG_BLDR	Percent of substrate that is large boulders (>45 cm)
	SM_BLDR	Percent of substrate that is small boulders (25-45 cm)
	COBBLE	Percent of substrate that is cobble (6-25 cm)
	GRAVEL	Percent of substrate that is gravel (2-60 mm)
	SAND	Percent of substrate that is sand (0.06-2 mm)
	MUDSILT	Percent of substrate that is mud or silt (<0.06 mm)
	GRV_LRG	Percent of substrate that is gravel or larger
	EMBEDDED	Substrate embeddedness (percent of boulders and cobble covered in fine sediment)
Algae/macrophytes	ALGAE_AB	Abundance of algae in study reach (scored as abundant, common, rare, or absent)
	MCRPH_AB	Abundance of aquatic macrophytes in study reach (scored as abundant, common, rare, or absent)
Instream cover	STRM_COV	Visually estimated percent cover
	FILA_ALG	Percent of instream cover provided by filamentous algae
	MICRALG	Percent of instream cover provided by microalgae and biofilms
	MACRPHYT	Percent of instream cover provided by aquatic macrophytes
	LWD	Percent of instream cover provided by large woody debris
	SWD	Percent of instream cover provided by small woody debris
	ROOTS	Percent of instream cover provided by submerged roots
	OVR_VEG	Percent of instream cover provided by overhanging terrestrial vegetation
	UNDERCUT	Percent of instream cover provided by undercut banks
	LEAFPACK	Percent of instream cover provided by leaf packs
	BOULDER	Percent of instream cover provided by boulders and other large substrates
	ARTIFICL	Percent of instream cover provided by artificial objects (e.g., tires, cement blocks)
	COV_TYPE	Number of the above cover types present

Appendix A2, continued. Local-scale environmental variables used in this study.

Category	Abbreviation	Variable
Stream morphology	STRMBEND	Number of stream bends in study reach
	WELLBEND	Number of well-defined stream bends in study reach
	MODBEND	Number of moderately-defined stream bends in study reach
	POORBEND	Number of poorly-defined stream bends in study reach
	WETWIDTH	Wetted width of stream (averaged across transects)
	AVG_DEP	Average stream depth
	THAL_DEP	Thalweg depth (averaged across transects)
	POOL_WID	Maximum pool width
	POOL_DEP	Maximum pool depth
Flow	VELDEPTH	Velocity/depth regime score (optimal, suboptimal, marginal, or poor)
	FLOWSTAT	Flow status score (high, moderate, low, or no flow)
Roots/woody debris	DISCHARG	Discharge (instantaneous stream flow in ft ³ /s)
	CWD_WET	Count of wetted coarse woody debris in study reach
	CWD_BKF	Count of dry coarse woody debris within bank-full stream width
	ROOT_WET	Count of wetted root wads in study reach
Riparian buffer	ROOT_BKF	Count of dry root wads within bank-full stream width
	BUFFER	Width of riparian buffer (averaged across transects)
	RIP_TREE	Percent of riparian vegetation consisting of trees
	RIP_SHRB	Percent of riparian vegetation consisting of shrubs
	RIP_GRAS	Percent of riparian vegetation consisting of grasses/forbs
	RIP_CULT	Percent of riparian vegetation consisting of cultivated fields
	OTHER CANOPY	Percent of riparian vegetation consisting of other types Percent of stream shaded by tree canopy (measured with densitometer)
Aesthetics	AESTHET	Aesthetics score (wilderness, natural area, common setting, or offensive)
Bank characteristics	BNK_SLOP	Bank slope (averaged across transects)
	EROSION	Percentage of bank with evident or potential erosion
	SOIL_EXP	Percentage of exposed soil on banks
Water parameters	DO	Dissolved oxygen (mg/L)
	PH	pH
	SPCOND	Specific conductivity (μs)
	TEMP	Water temperature (°C)

Appendix A3. Watershed physiographic variables used in this study

Variable	Description
LAT_DS	Latitude, decimal degrees
LONG_DS	Longitude, decimal degrees
EcoLev3	Level 3 ecoregion
PRECIP	Mean annual precipitation, calculated for watershed
ELEV_M	Mean elevation
WSLOPE	Mean watershed slope
WSHEDKM2	Watershed area
DAMS_CT	Number of dams in watershed
OUT_MGD	Cumulative permitted outfall discharge rate within watershed (million gallons per day)
OUT_CT	Number of outfalls
RESV_CT	Number of reservoirs within watershed
RESV_PCT	% of land covered by reservoirs within watershed
WATER	% of land covered by water within watershed
DEV_TOT	% developed land
FOR_TOT	% forested land, including forested wetlands
SHRUB	% shrubland
GRASS	% grassland
PASTURE	% pasture
ROWCROP	% rowcrop
WET_TOT	% wetland
AG_TOT	% agriculture (crop + pasture)
IMP_PCT	% impervious cover
CNPY_PCT	% canopy cover

Appendix A4. Species codes for fish collected among the 64 stream sites in 2008.

CODE	SPECIES	FAMILY	ORDER
AMEIMELA	<i>Ameiurus melas</i>	Ictaluridae	Siluriformes
AMEINATA	<i>Ameiurus natalis</i>	Ictaluridae	Siluriformes
APHRSAJA	<i>Aphredoderus sayanus</i>	Aphredoderidae	Percopsiformes
APLOGRUN	<i>Aplodinotus grunniens</i>	Sciaenidae	Perciformes
ASTYMEXI	<i>Astyanax mexicanus</i>	Characidae	Cypriniformes
ATRASPAT	<i>Atractosteus spatula</i>	Lepisosteidae	Semionotiformes
CAMPANOM	<i>Campostoma anomalum</i>	Cyprinidae	Cypriniformes
CARPCARP	<i>Carpionodes carpio</i>	Catostomidae	Cypriniformes
CYPRCARP	<i>Cyprinus carpio</i>	Cyprinidae	Cypriniformes
CYPRLUTR	<i>Cyprinella lutrensis</i>	Cyprinidae	Cypriniformes
CYPRVENU	<i>Cyprinella venusta</i>	Cyprinidae	Cypriniformes
DOROCEPI	<i>Dorosoma cepedianum</i>	Clupeidae	Clupeiformes
DOROPETE	<i>Dorosoma petenense</i>	Clupeidae	Clupeiformes
ERIMSUCE	<i>Erimyzon sucetta</i>	Catostomidae	Cypriniformes
ESOXAMER	<i>Esox americanus vermiculatus</i>	Esocidae	Esociformes
ETHECHLO	<i>Etheostoma chlorosomum</i>	Percidae	Perciformes
ETHEGRAC	<i>Etheostoma gracile</i>	Percidae	Perciformes
ETHESPEC	<i>Etheostoma spectabile</i>	Percidae	Perciformes
FUNDNOTA	<i>Fundulus notatus</i>	Fundulidae	Cyprinodontiformes
FUNDZEBR	<i>Fundulus zebrinus</i>	Fundulidae	Cyprinodontiformes
GAMBAFFI	<i>Gambusia affinis</i>	Poeciliidae	Cyprinodontiformes
HYBONUCH	<i>Hybognathus nuchalis</i>	Cyprinidae	Cypriniformes
ICTAPUNC	<i>Ictalurus punctatus</i>	Ictaluridae	Siluriformes
ICTIBUBA	<i>Ictiobus bubalus</i>	Catostomidae	Cypriniformes
LABISICC	<i>Labidesthes sicculus</i>	Atherinidae	Atheriniformes
LEPIOCUL	<i>Lepisosteus oculatus</i>	Lepisosteidae	Semionotiformes
LEPIOSSE	<i>Lepisosteus osseus</i>	Lepisosteidae	Semionotiformes
LEPOAURI	<i>Lepomis auritus</i>	Centrarchidae	Perciformes
LEPOCYAN	<i>Lepomis cyanellus</i>	Centrarchidae	Perciformes
LEPOGULO	<i>Lepomis gulosus</i>	Centrarchidae	Perciformes
LEPOHUMI	<i>Lepomis humilus</i>	Centrarchidae	Perciformes
LEPOMACR	<i>Lepomis macrochirus</i>	Centrarchidae	Perciformes
LEPOMEGA	<i>Lepomis megalotis</i>	Centrarchidae	Perciformes
LEPOMICR	<i>Lepomis microlophus</i>	Centrarchidae	Perciformes
LEPOMINI	<i>Lepomis miniatus</i>	Centrarchidae	Perciformes
LEPOSPP	<i>Lepomis spp.</i>	Centrarchidae	Perciformes
LYTHFUME	<i>Lythrurus fumeus</i>	Cyprinidae	Cypriniformes
LYTHUMBR	<i>Lythrurus umbratilis</i>	Cyprinidae	Cypriniformes
MENIBERY	<i>Menidia beryllina</i>	Atherinidae	Atheriniformes
MICRPUNC	<i>Micropterus punctatus</i>	Centrarchidae	Perciformes
MICRSALM	<i>Micropterus salmoides</i>	Centrarchidae	Perciformes
MINYMELA	<i>Minytrema melanops</i>	Catostomidae	Cypriniformes
MOROCHRY	<i>Morone chrysops</i>	Moronidae	Perciformes
MOXOCONG	<i>Moxostoma congestum</i>	Catostomidae	Cypriniformes
MOXOPOEC	<i>Moxostoma poecilurum</i>	Catostomidae	Cypriniformes
MUGICEPH	<i>Mugil cephalus</i>	Mugilidae	Perciformes
NOTECRYS	<i>Notemigonus crysoleucas</i>	Cyprinidae	Cypriniformes

NOTRATRO	<i>Notropis atrocaudalis</i>	Cyprinidae	Cypriniformes
NOTRBUCH	<i>Notropis buchanani</i>	Cyprinidae	Cypriniformes
NOTRTEXA	<i>Notropis texanus</i>	Cyprinidae	Cypriniformes
NOTRVOLU	<i>Notropis volucellus</i>	Cyprinidae	Cypriniformes
NOTUGYRI	<i>Noturus gyrinus</i>	Ictaluridae	Siluriformes
NOTUNOCT	<i>Noturus nocturnus</i>	Ictaluridae	Siluriformes
OPSOEMIL	<i>Opsopoeodus emiliae</i>	Cyprinidae	Cypriniformes
PERCCARB	<i>Percina carbonaria</i>	Percidae	Perciformes
PERCMACR	<i>Percina macrolepida</i>	Percidae	Perciformes
PERCSCIE	<i>Percina sciera</i>	Percidae	Perciformes
PIMEVIGI	<i>Pimephales vigilax</i>	Cyprinidae	Cypriniformes
POMOANNU	<i>Pomoxis annularis</i>	Centrarchidae	Perciformes
POMONIGR	<i>Pomoxis nigromaculatus</i>	Centrarchidae	Perciformes
PYLOOLIV	<i>Pylodictis olivaris</i>	Ictaluridae	Siluriformes

Appendix A5. Species codes for algae collected among the 64 stream sites in 2008.

Type	TAXON_ID	SPECIES
Diatom	ACbiasol	<i>Achnanthes biassolettiana</i>
Diatom	ACcoarct	<i>Achnanthes coarctata</i>
Diatom	AClanapi	<i>Achnanthes lanceolata</i> var. <i>apiculata</i>
Diatom	ACploens	<i>Achnanthes ploenensis</i>
Diatom	ADbryoph	<i>Adlafia bryophila</i>
Diatom	AHexigum	<i>Achnanthidium exiguum</i>
Diatom	AHminuti	<i>Achnanthidium minutissimum</i>
Diatom	ALpelluc	<i>Amphipleura pellucida</i>
Diatom	AMbullat	<i>Amphora bullatoides</i>
Diatom	AMcoffea	<i>Amphora coffeaeformis</i>
Diatom	AMinarie	<i>Amphora inariensis</i>
Diatom	AMlibyca	<i>Amphora libyca</i>
Diatom	AMmontan	<i>Amphora montana</i>
Diatom	AMovalis	<i>Amphora ovalis</i>
Diatom	AMpedcls	<i>Amphora pediculus</i>
Diatom	AMsabina	<i>Amphora sabiniana</i>
Diatom	AMveneta	<i>Amphora veneta</i>
Diatom	ANcostat	<i>Anomoeoneis costata</i>
Diatom	ANSphaer	<i>Anomoeoneis sphaerophora</i>
Diatom	ANSphcos	<i>Anomoeoneis sphaerophora</i> cf. <i>costata</i>
Diatom	ATnorman	<i>Actinocyclus normanii</i>
Diatom	AUalpige	<i>Aulacoseira alpigena</i>
Diatom	AUambig	<i>Aulacoseira ambigua</i>
Diatom	AUgranlt	<i>Aulacoseira granulata</i>
Diatom	AUgrnang	<i>Aulacoseira granulata</i> var. <i>angustissima</i>
Diatom	AUsp	<i>Aulacoseira</i> sp.
Diatom	BApardxa	<i>Bacillaria paradoxa</i>
Diatom	BMcircum	<i>Biremis circumtexta</i>
Diatom	BMLucns	<i>Biremis lucens</i>
Diatom	BRvitrea	<i>Brachyseira vitrea</i>
Diatom	CAaeroph	<i>Caloneis aerophila</i>
Diatom	CABacill	<i>Caloneis bacillum</i>
Diatom	CASchuma	<i>Caloneis schumanniana</i>
Diatom	CAsilicu	<i>Caloneis silicula</i>
Diatom	CCpedcls	<i>Cocconeis pediculus</i>
Diatom	CCplacen	<i>Cocconeis placentula</i>
Diatom	CCplapse	<i>Cocconeis placentula</i> var. <i>pseudolineata</i>
Diatom	CMaffins	<i>Cymbella affinis</i>
Diatom	CMamphic	<i>Cymbella amphicephala</i>
Diatom	CMcistul	<i>Cymbella neocistula</i>
Diatom	CMcymbif	<i>Cymbella cymbiformis</i>
Diatom	CMdelcat	<i>Cymbella delicatula</i>
Diatom	CMdelcat	<i>Encyonema delicatula</i>
Diatom	CMelgine	<i>Encyonema elginense</i>
Diatom	CMhusted	<i>Cymbella hustedtii</i>
Diatom	CMkolbei	<i>Cymbella kolbei</i>
Diatom	CMlaevis	<i>Cymbella laevis</i>
Diatom	CMnavfrm	<i>Cymbella naviculiformis</i>

Diatom	CMpaucst	<i>Encyonema (Cymbella) paucistriata</i>
Diatom	CMpusill	<i>Cymbella pusilla</i>
Diatom	CMtriang	<i>Cymbella triangulum</i>
Diatom	CMtumida	<i>Cymbella tumida</i>
Diatom	CPcrucic	<i>Capartogramma crucicula</i>
Diatom	CQsoehas	<i>Chamaepinnularia soehrensii</i> var. <i>hassiac</i>
Diatom	CSdubius	<i>Cyclostephanos dubius</i>
Diatom	CSinvisi	<i>Cyclostephanos invisitatus</i>
Diatom	CStholif	<i>Cyclostephanos tholiformis</i>
Diatom	CTellipt	<i>Cymatopleura elliptica</i>
Diatom	CTsolea	<i>Cymatopleura solea</i>
Diatom	CYatomus	<i>Cyclotella atomus</i>
Diatom	CYdisuni	<i>Cyclotella distinguenda</i> var. <i>unipunctata</i>
Diatom	CYmenegh	<i>Cyclotella meneghiniana</i>
Diatom	CYmichig	<i>Cyclotella michiganiana</i>
Diatom	CYocella	<i>Cyclotella ocellata</i>
Diatom	CYstelli	<i>Cyclotella stelligera</i>
Diatom	DEkuetzi	<i>Denticula kuetzingii</i>
Diatom	DEsubtil	<i>Denticula subtilis</i>
Diatom	DIconfer	<i>Diadisma confervacea</i>
Diatom	DIconten	<i>Diadisma contenta</i>
Diatom	DPellipt	<i>Diploneis elliptica</i>
Diatom	DPmargin	<i>Diploneis marginestriata</i>
Diatom	DPoblong	<i>Diploneis oblongella</i>
Diatom	DPpsudov	<i>Diploneis pseudovalis</i>
Diatom	DPpuella	<i>Diploneis puella</i>
Diatom	ECminutu	<i>Encyonema minutum</i>
Diatom	ECneomul*	<i>Encyonema neomuelleri</i>
Diatom	ECprostr	<i>Encyonema prostratum</i>
Diatom	ECsilesi	<i>Encyonema silesiacum</i>
Diatom	ECtriang	<i>Encyonema triangulum</i>
Diatom	EPadnata	<i>Epithemia adnata</i>
Diatom	EPsorex	<i>Epithemia sorex</i>
Diatom	EPturgid	<i>Epithemia turgida</i>
Diatom	ESflexel	<i>Eucocconeis flexella</i>
Diatom	EUbilun	<i>Eunotia bilunaris</i>
Diatom	EUpectin	<i>Eunotia pectinalis</i>
Diatom	EYevergl	<i>Encyonopsis evergladianum</i>
Diatom	EYmicroc	<i>Encyonopsis microcephala</i>
Diatom	FAinsoc	<i>Fallacia insociabilis</i>
Diatom	FASubham	<i>Fallacia subhamulata</i>
Diatom	FAtener2	<i>Fallatia tenera</i>
Diatom	FRcapuci	<i>Fragilaria capucina</i>
Diatom	FRelliptc	<i>Fragilaria elliptica</i>
Diatom	FRfascic	<i>Fragilaria fasciculata</i>
Diatom	FRleptos	<i>Fragilaria leptostauron</i>
Diatom	FRnanan	<i>Fragilaria nanana</i>
Diatom	FRtenera	<i>Fragilaria tenera</i>
Diatom	FSrhoCAF	<i>Frustulia rhomboides</i>
Diatom	FSvulgar	<i>Frustulia vulgaris</i>

Diatom	GEaiken	<i>Geissleria aikenensis</i>
Diatom	GEdecu	<i>Geisleria decussis</i>
Diatom	GEthingv	<i>Geisleria thingvallae</i>
Diatom	GMgrovei	<i>Gomposphenia grovei</i>
Diatom	GMlinfor	<i>Gomphosphenia lingulatiformis</i>
Diatom	GNexigua	<i>Gomphonitzschia exigua</i>
Diatom	GOacumin	<i>Gomphonema acuminatum</i>
Diatom	GOaffine	<i>Gomphonema affine</i>
Diatom	GOangstt	<i>Gomphonema angustatum</i>
Diatom	GOangust	<i>Gomphonema angustum</i>
Diatom	GOclavat	<i>Gomphonema clavatum</i>
Diatom	GOgracil	<i>Gomphonema gracile</i>
Diatom	GOinsign	<i>Gomphonema insigne</i>
Diatom	GOintvib	<i>Gomphonema intricatum var vibrio</i>
Diatom	GOMaclau	<i>Gomphonema macLaughlinii</i>
Diatom	GOMexica	<i>Gomphonema mexicanum</i>
Diatom	GOParvul	<i>Gomphonema parvulum</i>
Diatom	GOPumilu	<i>Gomphonema pumilum</i>
Diatom	GOTrunca	<i>Gomphonema truncatum</i>
Diatom	GYacumin	<i>Gyrosigma acuminatum</i>
Diatom	GYeximum	<i>Gyrosigma eximium</i>
Diatom	Gynodfrm	<i>Gyrosigma nodiferum</i>
Diatom	GYobtusa	<i>Gyrosigma obtusatum</i>
Diatom	HAamphio	<i>Hantzschia amphioxys</i>
Diatom	HAcapita	<i>Hippodonta capitata</i>
Diatom	HAdist	<i>Hantzschia distinctepunctata</i>
Diatom	HIhunga	<i>Hippodonta hungarica</i>
Diatom	KCambig	<i>Craticula ambigua</i>
Diatom	KCbude	<i>Craticula buderi</i>
Diatom	KCcuspid	<i>Craticula cuspidata</i>
Diatom	LUgoepp2	<i>Luticola goeppertiana</i>
Diatom	LUmutica	<i>Luticola mutica</i>
Diatom	LUundula	<i>Luticola undulata</i>
Diatom	MDCircul	<i>Meridion circulare</i>
Diatom	MEvarian	<i>Melosira varians</i>
Diatom	MSellipt	<i>Mastogloia elliptica</i>
Diatom	MSsmithi	<i>Mastogloia smithii</i>
Diatom	MYatomus	<i>Mayamaea atomus</i>
Diatom	NACaprad	<i>Navicula capitatoradiata</i>
Diatom	NACarioc	<i>Navicula cariocincta</i>
Diatom	NACfstr	<i>Navicula cf. striolata</i>
Diatom	NACircum	<i>Navicula circumtexta</i>
Diatom	NACrypto	<i>Navicula cryptocephala</i>
Diatom	NACryten	<i>Navicula cryptotenella</i>
Diatom	NAerifga	<i>Navicula erifuga</i>
Diatom	NAgermii	<i>Navicula germanii</i>
Diatom	NAingua	<i>Navicula ingenua</i>
Diatom	NAkotsch	<i>Navicula kotschy</i>
Diatom	NAlancel	<i>Navicula lanceolata</i>
Diatom	NAlatrpn	<i>Navicula(Geisleria) lateropunctata</i>

Diatom	NAlatrpn	<i>Navicula lateropunctata</i>
Diatom	NAlibone	<i>Navicula libonensis</i>
Diatom	NAlucdia	<i>Navicula sublucidula</i>
Diatom	NAmenscl	<i>Navicula menisculus</i>
Diatom	EOminima	<i>Eolima minima</i>
Diatom	NAoblong	<i>Navicula oblonga</i>
Diatom	NAorangi	<i>Navicula orangiana</i>
Diatom	NAphylpt	<i>Navicula phyllepta</i>
Diatom	NAradios	<i>Navicula radiosa</i>
Diatom	NArecons	<i>Navicula recens</i>
Diatom	NAreichd	<i>Navicula reichardtiana</i>
Diatom	NAreichd	<i>Navicula reichardtiana</i>
Diatom	NArhynch	<i>Navicula rhynchocephala</i>
Diatom	NArostel	<i>Navicula rostellata</i>
Diatom	NAsancru	<i>Navicula sanctaecrucis</i>
Diatom	NAschdei	<i>Navicula schadei</i>
Diatom	NAschroe	<i>Navicula schroeterii</i>
Diatom	NAstroem	<i>Sellaphora stroemii</i>
Diatom	NAsubmin	<i>Fallacia subminuscula</i>
Diatom	NEOsubmin	<i>Fallatia (Eolima) subminuscula</i>
Diatom	NAsubpla	<i>Navicula (Placoneis) subplacentula</i>
Diatom	NAsubrhy	<i>Navicula subrhynchocephala</i>
Diatom	NAsuec	<i>Navicula suecorum</i>
Diatom	NAsymtrc	<i>Navicula symmetrica</i>
Diatom	NAtenell	<i>Navicula tenelloides</i>
Diatom	NAtripun	<i>Navicula tripunctata</i>
Diatom	NAttrivis	<i>Navicula trivialis</i>
Diatom	NAveneta	<i>Navicula veneta</i>
Diatom	NEamplia	<i>Neidium ampliatum</i>
Diatom	NEbisulc	<i>Neidium bisulcatum</i>
Diatom	NEdubium	<i>Neidium dubium</i>
Diatom	NIaeroph	<i>Nitzschia aerophila</i>
Diatom	NIamphib	<i>Nitzschia amphibia</i>
Diatom	NIampoid	<i>Nitzschia amphibioides</i>
Diatom	NIangtu	<i>Nitzschia angustatula</i>
Diatom	NIangust	<i>Nitzschia angustata</i>
Diatom	NIbremen	<i>Nitzschia bremensis</i>
Diatom	NIbrevis	<i>Nitzschia brevissima</i>
Diatom	NIcapite	<i>Nitzschia capitellata</i>
Diatom	NIclausi	<i>Nitzschia clausii</i>
Diatom	NIcoarct	<i>Nitzschia coarctata</i>
Diatom	NIcombal	<i>Nitzschia compressa var. balatonis</i>
Diatom	NIcompre	<i>Nitzschia compressa</i>
Diatom	NIdebili	<i>Nitzschia debilis</i>
Diatom	NIidentic	<i>Nitzschia denticula</i>
Diatom	NIidissip	<i>Nitzschia dissipata</i>
Diatom	NIfilifr	<i>Nitzschia filiformis</i>
Diatom	NIfrustu	<i>Nitzschia frustulum</i>
Diatom	NIgeitlr	<i>Nitzschia geitleri</i>
Diatom	NIincons	<i>Nitzschia inconspicua</i>

Diatom	NIliebrt	<i>Nitzschia liebetruthii</i>
Diatom	NIlinear	<i>Nitzschia linearis</i>
Diatom	NIlorenz	<i>Nitzschia lorenziana</i>
Diatom	NImicroc	<i>Nitzschia microcephala</i>
Diatom	NIana	<i>Nitzschia nana</i>
Diatom	NIobtusa	<i>Nitzschia obtusa</i>
Diatom	NIpalea	<i>Nitzschia palea</i>
Diatom	NIrecta	<i>Nitzschia recta</i>
Diatom	NIrevers	<i>Nitzschia reversa</i>
Diatom	NIscalpe	<i>Nitzschia scalpelliformis</i>
Diatom	NIsigma	<i>Nitzschia sigma</i>
Diatom	NIshintab	<i>Nitzschia sinuata</i> var. <i>tabellaria</i>
Diatom	NIsolita	<i>Nitzschia solita</i>
Diatom	NItropi	<i>Nitzschia tropica</i>
Diatom	NIvaldec	<i>Nitzschia valdecostata</i>
Diatom	NIvitrea	<i>Nitzschia vitrea</i>
Diatom	ORdendro	<i>Orthoseira dentroteres</i>
Diatom	PBprotr	<i>Parlibellus protracta</i>
Diatom	PCclemto	<i>Placoneis clementioides</i>
Diatom	PCconst	<i>Placoneis constans</i>
Diatom	PCparel	<i>Placoneis paraelginensis</i>
Diatom	PCplacen	<i>Placoneis placentula</i>
Diatom	PCseudo	<i>Placoneis pseudanglica</i>
Diatom	PDbrevis	<i>Pseudostaurosira brevistriata</i>
Diatom	PGlepidp	<i>Plagiotropis lepidoptera</i>
Diatom	PIappend	<i>Pinnularia appendiculata</i>
Diatom	PIboreal	<i>Pinnularia borealis</i>
Diatom	PIgibba	<i>Pinnularia gibba</i>
Diatom	PInterr	<i>Pinnularia interrupta</i>
Diatom	PIlundii	<i>Pinnularia lundii</i>
Diatom	PImicros	<i>Pinnularia microstauron</i>
Diatom	PIobscur	<i>Pinnularia obscura</i>
Diatom	PIsubcap	<i>Pinnularia subcapitata</i>
Diatom	PIviridi	<i>Pinnularia viridis</i>
Diatom	PLdelica	<i>Pleurosigma delicatulum</i>
Diatom	PRlaevis	<i>Pleurosira laevis</i>
Diatom	PTlanapi* AClanapi	<i>Planothidium (Achnanthes) lanceolatum</i> var. <i>apiculata</i>
Diatom	PTlanceo	<i>Planothidium lanceolata</i>
Diatom	RESinuta	<i>Reimeria sinuata</i>
Diatom	ROabbre	<i>Rhoicosphenia abbreviata</i>
Diatom	RPbrebsn	<i>Rhopalodia brebissonii</i>
Diatom	RPgibba	<i>Rhopalodia gibba</i>
Diatom	RPgibbrl	<i>Rhopalodia gibberula</i>
Diatom	RPmuscul	<i>Rhopalodia musculus</i>
Diatom	RPoprta	<i>Rhopalodia operculata</i>
Diatom	SFlaevis	<i>Sellaphora laevis</i>
Diatom	SFpupula	<i>Sellaphora pupula</i>
Diatom	SFseminu	<i>Sellaphora seminulum</i>
Diatom	SNstrigos	<i>Seminavis strigosa</i>
Diatom	SRconstr	<i>Staurosira construens</i>

Diatom	SRconven	<i>Staurosira construens</i> var. <i>venter</i>
Diatom	SSanceps	<i>Stauroneis anceps</i>
Diatom	SSobtusa	<i>Stauroneis obtusa</i>
Diatom	SSphoeni	<i>Stauroneis phoenicentron</i>
Diatom	SSpssbob	<i>Stauroneis pseudosubobtusoides</i>
Diatom	SSsmithi	<i>Stauroneis smithii</i>
Diatom	STmedius	<i>Stephanodiscus medius</i>
Diatom	SUangust	<i>Surirella angusta</i>
Diatom	SUbreb	<i>Surirella brebissonii</i>
Diatom	SUElegan	<i>Surirella elegans</i>
Diatom	SUminuta	<i>Surirella minuta</i>
Diatom	SUovalis	<i>Surirella ovalis</i>
Diatom	SUspiral	<i>Surirella spiralis</i>
Diatom	SUsplen	<i>Surirella splendida</i>
Diatom	SUtenera	<i>Surirella tenera</i>
Diatom	SYacus	<i>Synedra acus</i>
Diatom	SYgoular	<i>Synedra goulardi</i>
Diatom	SYulna	<i>Synedra ulna</i>
Diatom	TEmusica	<i>Terpsinoe musica</i>
Diatom	THbrampt	<i>Thalassiosira bramaputrae</i>
Diatom	THnorden	<i>Thalassiosira nordenskioldii</i> Cleve
Diatom	THsp	<i>Thalassiosira</i> sp.
Diatom	THvisurg	<i>Thalassiosira visurgis</i>
Diatom	TYacumin	<i>Tryblionella acuminata</i>
Diatom	TYaeroph	<i>Tryblionella aerophila</i>
Diatom	TYapicul	<i>Tryblionella apiculata</i>
Diatom	TYcaldid	<i>Tryblionella calida</i>
Diatom	TYcfmarg	<i>Tryblionella</i> cf. <i>marginulata</i>
Diatom	TYdebili	<i>Tryblionella debilis</i>
Diatom	TYhungar	<i>Nitzschia hungarica</i>
Diatom	TYhungar	<i>Tryblionella hungarica</i>
Diatom	TYlevide	<i>Tryblionella levidensis</i>
Diatom	TYlittor	<i>Tryblionella littoralis</i>
Soft	AFCsp	<i>Aphanothece</i> sp.
Soft	ANBsp	<i>Anabaena</i> sp.
Soft	ANKfalca	<i>Ankistrodesmus falcatus</i>
Soft	ANKsp	<i>Ankistrodesmus</i> sp
Soft	CALsp	<i>Calothrix</i> sp.
Soft	CHC0AUL	<i>Chlorococcum</i> sp.
Soft	CHLsp	<i>Chlamydomonas</i> sp.
Soft	CHOsp	<i>Chroococcus</i> sp.
Soft	CHRsp	<i>Characium</i> sp.
Soft	CLAgglomer	<i>Cladophora glomerata</i>
Soft	CLAsp	<i>Cladophora</i> sp.
Soft	CLOsp2	<i>Closterium</i> sp.
Soft	COEsp	<i>Coelastrum</i> sp.
Soft	COHsp	<i>Coelosphaerium</i> sp.
Soft	COSsp	<i>Cosmarium</i> sp.
Soft	CRUsp	<i>Crucigenia</i> sp
Soft	DESsp	<i>Desmidium</i> sp.

Soft	EUGacus	<i>Euglena acus</i>
Soft	EUGsp	<i>Euglena sp.</i>
Soft	EUTsp	<i>Eutreptia sp</i>
Soft	GLCsp	<i>Gloeocystis sp.</i>
Soft	GLHsp	<i>Glothece sp.</i>
Soft	GLKturf	<i>Gloeoskene turfosa</i>
Soft	KIRobesa	<i>Kirchneriella obesa</i>
Soft	KIRsp	<i>Kirchneriella sp.</i>
Soft	MERconvl	<i>Merismopedia convoluta</i>
Soft	MERglauc	<i>Merismopedia glauca</i>
Soft	MICsp	<i>Microcystis sp</i>
Soft	MOUsp	<i>Mougeotia sp.</i>
Soft	OEDsp	<i>Oedogonium sp.</i>
Soft	OOCsp	<i>Oocystis sp.</i>
Soft	OSCsp	<i>Oscillatoria sp.</i>
Soft	PEDboryn	<i>Pediastrum boryanum</i>
Soft	PEDsp	<i>Pediastrum sp</i>
Soft	PHAsp	<i>Phacus sp.</i>
Soft	RIVsp	Unknown Rivulariaceae
Soft	SCEabund	<i>Scenedesmus abundans</i>
Soft	SCEbijug	<i>Scenedesmus bijuga</i>
Soft	SCEdimor	<i>Scenedesmus dimorphus</i>
Soft	SCEquadr	<i>Scenedesmus quadricauda</i>
Soft	SCEsp	<i>Scenedesmus sp.</i>
Soft	SCRsetig	<i>Schroderia setigera</i>
Soft	SCZsp	<i>Schizothrix sp.</i>
Soft	SPHsp	<i>Sphaerocystis sp.</i>
Soft	SPIsp	<i>Spirogyra sp.</i>
Soft	SPLsp	<i>Spirulina sp.</i>
Soft	STAsp	<i>Staurastrum sp.</i>
Soft	SYCsp	<i>Synechococcus sp.</i>
Soft	TETminum	<i>Tetraedron minimum</i>
Soft	TETregul	<i>Tetraedron regulare</i>
Soft	TETsp	<i>Tetraedron sp.</i>
Soft	TRAsp	<i>Trachelomonas sp.</i>
Soft	TRIs	<i>Tribonema sp.</i>
Soft	UNcent	Centric diatoms
Soft	UNpennte	Pennate diatoms
Soft	XCLalga	<i>Cladophoraceae</i>
Soft	XDFalga	Unidentified dinoflagellates
Soft	XEUsp	Unknown Euglenophyte sp.
Soft	XXAsp	Unknown alga sp.

Appendix A6. Taxa-specific results from Threshold Indicator Taxa Analysis (TITAN) on **algal species composition** in response to nutrient and nutrient-related stressors among 38 sites in **Ecoregion 29** during summer 2008. Only species that showed significant threshold declines or increases in response to predictors are included in this table. The observed (*Obs*) threshold value of predictors for each taxon is shown in bold, whereas lower (10%), middle (50%), and upper (90%) quantiles of 1,000 bootstraps represent measures of uncertainty around the observed threshold. *Z* represents the standardized indicator score from TITAN (larger numbers = stronger threshold response), *IndVal* is the unstandardized indicator score (scaled from 0-100%, with 100=perfect indicator). *Purity* is the relative consistency of the response direction among the 1,000 bootstraps (*purity* > 0.95 is significant). *P-value* is the likelihood of getting an equal or larger *IndVal* if the score were computed with random shuffling of the observed data (*P*<0.05 is significant). See Appendix A5 for full species names corresponding to *Taxon IDs*.

Predictor	Taxon ID	Threshold (obs)	Response > obs.	z	IndVal	P	Purity	Bootstrap threshold quantiles		
								10%	50%	90%
TP (ug/L)	COSsp	19.68	Decline	3.24	60.83	0.004	1.000	16.18	22.82	1069.33
TP (ug/L)	KIRsp	18.23	Decline	3.25	25.00	0.036	0.968	10.89	17.03	24.22
TP (ug/L)	MERglauc	21.43	Decline	6.79	75.84	0.004	1.000	16.18	19.68	28.95
TP (ug/L)	OSCsp	16.18	Decline	5.31	72.66	0.004	1.000	14.27	17.03	34.18
TP (ug/L)	PEDboryn	18.23	Decline	3.94	38.60	0.008	0.970	14.27	17.03	24.22
TP (ug/L)	SCZsp	125.08	Decline	3.41	53.13	0.004	0.968	30.18	125.08	1069.33
TP (ug/L)	GOMaclau	15.30	Decline	5.54	42.22	0.004	0.996	14.27	16.61	21.43
TP (ug/L)	GOintvib	17.03	Decline	6.04	51.61	0.004	1.000	12.44	17.03	24.22
TP (ug/L)	NAstroem	21.43	Decline	5.64	55.04	0.004	1.000	12.44	19.68	28.95
TP (ug/L)	BRvitrea	16.18	Decline	5.13	52.22	0.004	0.998	10.89	16.18	30.18
TP (ug/L)	CMlaevis	13.42	Decline	4.04	43.37	0.020	0.988	10.89	14.27	30.18
TP (ug/L)	NIampoid	15.30	Decline	3.11	37.15	0.016	0.978	14.27	19.05	52.08
TP (ug/L)	ALpelluc	34.18	Decline	3.46	28.57	0.012	0.982	13.42	24.22	44.68
TP (ug/L)	HAamphio	18.23	Decline	3.66	25.00	0.028	0.960	10.89	17.03	24.22
TP (ug/L)	NACrypto	14.27	Decline	3.09	36.67	0.016	0.962	12.44	17.03	40.73
TP (ug/L)	CMdelcat	17.03	Decline	8.00	83.62	0.004	1.000	14.62	18.23	24.22
TP (ug/L)	SYacus	16.18	Decline	6.34	65.13	0.004	0.998	12.44	16.18	19.68

TP (ug/L)	GOclavat	24.22	Decline	6.56	63.98	0.004	1.000	14.62	19.68	28.95
TP (ug/L)	GOgracil	26.78	Decline	3.92	51.69	0.004	0.996	18.23	28.95	77.03
TP (ug/L)	NAradios	27.77	Decline	3.64	33.33	0.024	0.990	14.62	26.78	40.73
TP (ug/L)	FRcapuci	24.22	Decline	4.01	53.37	0.004	0.978	15.30	24.22	44.68
TP (ug/L)	CMaffins	34.18	Decline	4.31	48.22	0.004	0.972	16.18	28.95	52.08
TP (ug/L)	GOangstt	21.43	Decline	4.94	53.50	0.004	0.998	15.30	27.77	77.03
TP (ug/L)	CMkolbei	40.73	Decline	4.91	54.55	0.012	0.996	24.22	40.73	69.78
TP (ug/L)	EYevergl	21.43	Decline	6.45	73.80	0.004	1.000	19.05	26.78	40.73
TP (ug/L)	EYmicroc	21.43	Decline	6.24	66.95	0.004	1.000	16.10	19.68	40.73
TP (ug/L)	ACbiasol	21.43	Decline	4.44	62.28	0.004	0.980	17.03	26.78	52.08
TP (ug/L)	DEkuetzi	21.43	Decline	5.82	67.48	0.004	1.000	14.27	19.05	34.18
TP (ug/L)	AHminuti	52.08	Decline	6.40	76.47	0.004	1.000	19.05	44.68	125.08
TP (ug/L)	ECsilesi	10.89	Decline	2.91	71.27	0.004	0.958	12.44	34.18	770.33
TP (ug/L)	SYulna	69.78	Decline	3.78	65.87	0.004	0.958	28.95	77.03	1069.33
TP (ug/L)	ANKsp	44.68	Increase	4.06	46.27	0.004	0.960	28.95	44.68	932.17
TP (ug/L)	CHRsp	30.18	Increase	4.98	44.44	0.004	1.000	26.78	34.18	598.33
TP (ug/L)	SCEquadr	932.17	Increase	8.93	60.00	0.004	0.954	368.33	1069.33	1235.00
TP (ug/L)	XXAsp	10.89	Increase	2.80	70.59	0.020	0.970	12.44	17.03	932.17
TP (ug/L)	NAsubmin	1235.00	Increase	5.45	63.31	0.012	0.958	40.73	1069.33	1235.00
TP (ug/L)	AMveneta	125.08	Increase	6.36	50.00	0.004	0.996	61.90	368.33	1069.33
TP (ug/L)	GMgrovei	125.08	Increase	3.12	26.09	0.024	0.968	28.95	125.08	1235.00
TP (ug/L)	ROabbre	69.78	Increase	4.98	33.33	0.004	0.986	44.68	77.03	598.33
TP (ug/L)	FRellptc	52.08	Increase	4.99	40.94	0.004	0.988	28.95	69.78	1069.33
TP (ug/L)	TEmusica	26.78	Increase	2.83	28.57	0.028	0.964	19.05	27.77	187.50
TP (ug/L)	NItropic	44.68	Increase	3.64	26.67	0.024	0.980	28.95	52.08	1235.00
TP (ug/L)	NIcombal	44.68	Increase	6.02	46.67	0.004	0.996	30.18	52.08	125.08
TP (ug/L)	NIangtu	30.18	Increase	3.39	27.78	0.016	0.986	26.78	52.08	1069.33
TP (ug/L)	AMSabina	44.68	Increase	3.97	46.37	0.004	0.982	21.43	52.08	1235.00
TP (ug/L)	GMlinfor	26.78	Increase	4.88	42.86	0.004	1.000	23.94	44.68	368.33
TP (ug/L)	TYapicul	26.78	Increase	2.86	35.81	0.016	0.968	19.05	28.95	69.78

TP (ug/L)	FAtener2	52.08	Increase	5.35	42.86	0.004	1.000	30.18	52.08	125.08
TP (ug/L)	NIsolita	1069.33	Increase	3.49	58.80	0.020	0.990	19.05	40.73	1235.00
TP (ug/L)	PCplacen	1235.00	Increase	5.51	89.33	0.008	0.998	30.06	125.08	1235.00
TP (ug/L)	PRlaevis	125.08	Increase	7.53	83.50	0.004	1.000	44.68	77.03	598.33
TP (ug/L)	AMpedcls	69.78	Increase	5.20	66.56	0.004	1.000	18.23	44.68	187.50
TP (ug/L)	AHexigum	40.73	Increase	4.99	60.69	0.004	1.000	18.23	28.95	598.33
TP (ug/L)	HIhunga	44.68	Increase	6.55	59.75	0.004	0.994	30.18	52.08	187.50
TP (ug/L)	NAsancru	34.18	Increase	5.90	50.29	0.004	1.000	26.78	40.73	77.03
TP (ug/L)	DIconfer	21.43	Increase	7.60	81.10	0.004	1.000	18.23	24.22	77.03
TP (ug/L)	NIincons	30.18	Increase	6.46	69.20	0.004	1.000	16.18	21.43	34.18
TP (ug/L)	NIfrustu	40.73	Increase	4.46	59.56	0.004	0.992	18.23	30.18	598.33
TP (ug/L)	GOpumilu	16.18	Increase	3.58	56.89	0.008	0.964	13.42	17.03	69.78
TP (ug/L)	NArecons	24.22	Increase	6.16	68.71	0.004	1.000	19.05	26.78	40.73
TP (ug/L)	REsinuta	69.78	Increase	3.22	59.41	0.020	0.990	14.62	34.18	131.32
TP (ug/L)	NAkotsch	28.95	Increase	2.86	48.89	0.012	0.988	17.03	34.18	1069.33
TP (ug/L)	CYmenegh	19.05	Increase	3.95	63.65	0.004	0.990	16.18	24.22	125.08
TP (ug/L)	CCplacen	17.03	Increase	6.34	76.98	0.004	0.996	13.42	17.03	21.43
TP (ug/L)	AMlibyca	30.18	Increase	3.14	57.13	0.008	0.972	17.03	27.77	77.03
TP (ug/L)	GOparvul	14.27	Increase	6.10	87.47	0.004	1.000	10.89	14.62	24.22
TP (ug/L)	NIamphib	10.89	Increase	3.01	65.58	0.024	0.984	10.89	12.44	77.03
TN (ug/L)	MERglauc	271.00	Decline	3.62	60.35	0.012	0.998	261.83	295.67	490.67
TN (ug/L)	OSCsp	490.67	Decline	4.60	61.48	0.004	0.994	280.17	440.83	633.33
TN (ug/L)	NAstroem	271.00	Decline	4.70	55.80	0.008	1.000	249.67	295.67	525.83
TN (ug/L)	BRvitrea	266.00	Decline	4.18	51.54	0.008	0.996	238.83	280.17	490.67
TN (ug/L)	ALpelluc	249.67	Decline	3.74	44.66	0.008	0.992	225.33	266.00	525.83
TN (ug/L)	CMdelcat	328.17	Decline	5.17	54.22	0.004	0.982	266.00	328.17	525.83
TN (ug/L)	SYacus	362.00	Decline	4.72	46.18	0.008	0.990	261.83	328.17	455.45
TN (ug/L)	RPgibba	362.00	Decline	2.91	43.12	0.016	0.986	266.00	402.50	918.17
TN (ug/L)	GOangstt	546.17	Decline	3.27	42.21	0.012	0.996	238.83	454.67	918.17
TN (ug/L)	EYevergl	384.67	Decline	5.56	68.35	0.004	0.994	294.12	402.50	918.17

TN (ug/L)	EYmicroc	328.17	Decline	5.84	67.33	0.004	0.996	261.83	328.17	440.83
TN (ug/L)	ACbiasol	295.67	Decline	5.65	68.51	0.004	1.000	249.67	280.17	494.18
TN (ug/L)	DEkuetzi	261.83	Decline	4.92	72.56	0.004	0.998	248.58	271.00	800.17
TN (ug/L)	AHminuti	328.17	Decline	5.75	71.94	0.004	1.000	295.67	420.17	1195.50
TN (ug/L)	ECsilesi	249.67	Decline	3.17	68.76	0.012	0.982	238.83	280.17	1891.67
TN (ug/L)	CHRsp	420.17	Increase	4.01	36.36	0.012	0.998	384.67	462.50	918.17
TN (ug/L)	AMveneta	5723.33	Increase	4.87	63.03	0.004	0.992	462.50	1891.67	5723.33
TN (ug/L)	NIcombal	440.83	Increase	3.48	33.33	0.020	0.994	402.50	462.50	1016.00
TN (ug/L)	GMlinfor	384.67	Increase	2.99	37.50	0.016	0.988	328.17	440.83	2393.33
TN (ug/L)	NIsolita	454.67	Increase	3.79	41.80	0.004	0.994	362.00	458.58	2393.33
TN (ug/L)	PCplacen	5723.33	Increase	2.75	55.35	0.024	0.986	384.67	1195.50	5723.33
TN (ug/L)	PRlaevis	1195.50	Increase	6.81	68.44	0.004	1.000	633.33	1891.67	3603.33
TN (ug/L)	AMpedcls	462.50	Increase	4.75	60.12	0.004	0.998	384.67	462.50	918.17
TN (ug/L)	NAsancru	440.83	Increase	3.70	39.66	0.004	0.980	295.67	420.17	633.33
TN (ug/L)	DIconfer	1891.67	Increase	5.29	77.39	0.004	1.000	271.00	867.83	2393.33
TN (ug/L)	NIincons	440.83	Increase	5.21	63.99	0.004	1.000	271.00	420.17	806.93
TN (ug/L)	NArecons	328.17	Increase	3.61	56.35	0.008	0.990	271.00	384.67	1016.00
TN (ug/L)	GOparvul	261.83	Increase	3.59	68.08	0.004	1.000	238.83	295.67	867.83
C:P (bulk)	CHRsp	183.72	Decline	5.21	44.44	0.004	1.000	133.26	182.03	312.80
C:P (bulk)	SCEquadr	124.70	Decline	5.59	33.33	0.008	0.950	95.50	118.40	147.69
C:P (bulk)	AMveneta	159.00	Decline	5.09	38.46	0.008	0.996	100.55	124.70	170.90
C:P (bulk)	GMgrovei	178.01	Decline	2.79	25.00	0.040	0.976	110.49	134.04	183.72
C:P (bulk)	ROabbre	165.95	Decline	3.98	28.57	0.020	0.976	95.50	147.69	182.03
C:P (bulk)	FRellptc	159.00	Decline	4.78	44.31	0.004	1.000	95.50	147.69	191.55
C:P (bulk)	NIcombal	170.90	Decline	4.20	35.51	0.008	1.000	100.55	170.90	245.12
C:P (bulk)	THsp	191.55	Decline	3.13	26.32	0.044	0.990	118.40	182.03	334.10
C:P (bulk)	NIangtu	183.72	Decline	3.22	27.78	0.020	0.990	95.50	134.04	245.12
C:P (bulk)	GMlinfor	182.03	Decline	6.08	52.94	0.004	1.000	124.70	170.90	245.12

C:P (bulk)	FAtener2	165.95	Decline	3.96	32.68	0.008	0.996	95.50	165.95	245.12
C:P (bulk)	PCplacen	126.22	Decline	3.84	41.51	0.020	0.974	100.55	126.22	183.72
C:P (bulk)	PRlaevis	159.00	Decline	8.13	73.53	0.004	1.000	118.40	134.04	170.90
C:P (bulk)	AMpedcls	334.10	Decline	5.43	65.98	0.004	1.000	178.01	312.80	341.09
C:P (bulk)	AHexigum	134.04	Decline	2.78	51.31	0.024	0.960	95.50	159.00	350.49
C:P (bulk)	HIhunga	165.95	Decline	5.49	54.83	0.004	1.000	100.55	134.04	183.72
C:P (bulk)	NAsancru	159.00	Decline	4.34	44.46	0.004	1.000	110.49	182.03	338.25
C:P (bulk)	DIconfer	134.04	Decline	6.80	80.28	0.004	1.000	121.16	159.00	183.72
C:P (bulk)	NIincons	334.10	Decline	6.22	73.56	0.004	1.000	178.01	334.10	350.49
C:P (bulk)	NIfrustu	334.10	Decline	3.25	51.91	0.004	0.986	100.55	170.90	363.95
C:P (bulk)	NArecens	183.72	Decline	5.09	60.14	0.008	0.996	110.49	178.01	334.27
C:P (bulk)	REsinuta	338.25	Decline	5.28	69.94	0.004	0.998	331.97	350.49	406.22
C:P (bulk)	CYmenegh	462.85	Decline	2.78	63.64	0.020	0.994	110.49	191.55	406.22
C:P (bulk)	CCplacen	335.81	Decline	6.15	73.01	0.004	1.000	245.12	340.04	438.57
C:P (bulk)	GOparvul	312.80	Decline	5.42	67.53	0.004	1.000	191.55	338.25	462.85
C:P (bulk)	NIamphib	406.22	Decline	2.79	60.55	0.004	0.950	118.40	390.09	562.94
C:P (bulk)	KIRsp	363.95	Increase	3.68	27.27	0.028	0.950	191.55	363.95	462.85
C:P (bulk)	MERglauc	312.80	Increase	5.04	62.86	0.004	1.000	182.03	335.81	390.09
C:P (bulk)	OSCsp	245.12	Increase	3.71	54.74	0.004	0.958	159.00	191.55	368.01
C:P (bulk)	SCZsp	191.55	Increase	3.79	52.76	0.004	1.000	126.22	183.72	390.09
C:P (bulk)	GOMaclau	462.85	Increase	5.09	55.01	0.008	0.996	334.10	390.09	562.94
C:P (bulk)	GOintvib	462.85	Increase	5.70	71.27	0.004	0.998	334.10	438.57	562.94
C:P (bulk)	NAstroem	368.01	Increase	4.54	47.86	0.008	1.000	165.95	312.80	390.09
C:P (bulk)	BRvitrea	245.12	Increase	5.50	50.00	0.004	1.000	182.03	323.45	406.22
C:P (bulk)	CMlaevis	562.94	Increase	4.36	61.04	0.020	0.982	182.03	498.33	562.94
C:P (bulk)	NIampoid	183.72	Increase	2.26	28.06	0.048	0.952	165.26	323.45	562.94
C:P (bulk)	ALpelluc	350.49	Increase	2.46	27.93	0.016	0.972	165.26	334.10	376.04
C:P (bulk)	HAamphio	350.49	Increase	3.27	25.00	0.040	0.956	334.10	363.95	498.33
C:P (bulk)	CMdelcat	334.10	Increase	4.95	54.08	0.004	0.996	245.12	368.01	462.85
C:P (bulk)	SYacus	245.12	Increase	6.59	50.00	0.004	1.000	183.72	335.81	406.22

C:P (bulk)	GOclavat	562.94	Increase	2.79	74.97	0.036	0.980	134.04	334.10	498.33
C:P (bulk)	GOgracil	390.09	Increase	3.86	56.62	0.004	0.996	147.69	312.80	438.57
C:P (bulk)	CMaffins	165.95	Increase	3.47	40.94	0.012	0.960	134.04	182.03	462.85
C:P (bulk)	CMkolbei	165.95	Increase	3.31	41.87	0.012	0.986	126.22	170.90	334.10
C:P (bulk)	EYevergl	182.03	Increase	6.58	72.79	0.004	1.000	147.69	178.01	312.80
C:P (bulk)	EYmicroc	312.80	Increase	6.00	67.01	0.004	1.000	182.03	334.10	376.04
C:P (bulk)	PIgibba	170.90	Increase	4.00	39.13	0.008	0.996	165.26	191.55	438.57
C:P (bulk)	NACryten	340.04	Increase	3.44	42.54	0.008	0.992	159.00	350.49	562.94
C:P (bulk)	DEkuetzi	335.81	Increase	7.49	75.32	0.004	1.000	182.03	335.81	363.95
C:P (bulk)	GOaffine	390.09	Increase	2.32	60.46	0.032	0.954	95.50	334.10	406.22
C:P (bulk)	AHminuti	165.95	Increase	6.18	74.83	0.004	0.996	124.70	165.95	183.72
C:P (OM)	CHRsp	165.20	Decline	7.50	54.90	0.004	0.996	153.44	165.20	177.05
C:P (OM)	GLKturf	153.44	Decline	3.53	33.49	0.036	0.984	137.69	155.31	180.64
C:P (OM)	SCEquadr	147.31	Decline	3.68	25.76	0.024	0.968	131.40	156.76	177.05
C:P (OM)	AMveneta	147.31	Decline	4.22	38.61	0.012	0.968	131.40	153.44	185.75
C:P (OM)	FRellptc	147.31	Decline	4.49	49.84	0.008	0.996	137.69	155.31	183.77
C:P (OM)	NIcombal	137.69	Decline	3.41	51.99	0.016	0.998	131.40	155.31	193.47
C:P (OM)	NAtrivis	156.76	Decline	4.21	35.64	0.008	0.978	147.31	161.63	182.03
C:P (OM)	NIangtu	166.54	Decline	4.89	35.71	0.004	0.992	147.31	165.20	177.05
C:P (OM)	GMlinfor	216.60	Decline	3.25	37.50	0.024	0.986	141.18	182.03	227.15
C:P (OM)	FAtener2	156.76	Decline	4.57	46.88	0.012	0.998	131.40	156.76	180.64
C:P (OM)	NIsolita	141.57	Decline	3.60	53.05	0.012	0.996	131.40	156.76	225.08
C:P (OM)	PRlaevis	137.69	Decline	4.32	66.41	0.012	0.996	137.69	165.37	193.47
C:P (OM)	AMpedcls	225.08	Decline	4.01	57.32	0.008	0.988	176.64	205.26	228.73
C:P (OM)	AHexigum	156.76	Decline	4.86	67.63	0.004	0.998	153.44	166.54	227.15
C:P (OM)	HIhunga	169.17	Decline	4.62	50.60	0.008	0.970	131.40	166.54	183.77
C:P (OM)	NAsancru	182.03	Decline	3.22	37.69	0.008	0.970	172.88	193.47	227.99
C:P (OM)	DIconfer	225.08	Decline	5.33	64.66	0.004	0.996	155.31	193.47	228.73
C:P (OM)	NIincons	225.08	Decline	6.11	73.68	0.004	1.000	177.05	216.60	244.77
C:P (OM)	NIfrustu	225.08	Decline	3.71	59.62	0.008	0.966	180.64	216.60	244.77

C:P (OM)	NArecens	161.63	Decline	4.61	61.85	0.004	1.000	156.76	182.03	227.99
C:P (OM)	CYmenegh	216.60	Decline	3.56	54.86	0.004	0.962	147.31	193.47	244.77
C:P (OM)	CCplacen	225.08	Decline	5.24	71.99	0.004	0.986	182.03	205.26	246.50
C:P (OM)	GOparvul	227.15	Decline	6.03	77.60	0.004	1.000	193.47	227.99	288.83
C:P (OM)	NIamphib	288.83	Decline	3.45	64.03	0.008	0.980	155.12	273.82	298.29
C:P (OM)	MERglauc	225.08	Increase	6.17	71.16	0.004	1.000	166.54	193.47	227.99
C:P (OM)	OSCsp	244.77	Increase	4.99	69.93	0.004	0.988	193.47	227.99	264.51
C:P (OM)	PEDboryn	183.77	Increase	4.20	33.33	0.012	0.998	182.03	216.60	264.51
C:P (OM)	SCZsp	185.75	Increase	2.92	52.32	0.004	0.970	155.12	185.75	227.15
C:P (OM)	GOmaclau	216.60	Increase	5.11	35.71	0.004	0.996	193.47	244.77	298.29
C:P (OM)	NAstroem	227.99	Increase	3.19	43.86	0.012	0.984	165.20	227.15	288.83
C:P (OM)	BRvitrea	216.60	Increase	4.27	41.35	0.008	0.998	166.54	216.60	288.83
C:P (OM)	CMdelcat	225.08	Increase	7.02	69.18	0.004	0.994	185.75	225.08	262.07
C:P (OM)	SYacus	193.47	Increase	6.66	56.25	0.004	1.000	182.03	216.60	288.83
C:P (OM)	GOclavat	244.77	Increase	4.36	59.30	0.004	0.964	172.88	227.15	273.82
C:P (OM)	GOgracil	180.64	Increase	6.28	64.96	0.004	1.000	166.54	182.03	216.60
C:P (OM)	NAradios	182.03	Increase	4.29	31.58	0.004	0.992	172.88	183.77	244.77
C:P (OM)	FRcapuci	165.20	Increase	4.09	56.33	0.004	0.974	155.31	166.54	227.99
C:P (OM)	EYevergl	216.60	Increase	4.32	61.76	0.004	0.988	155.31	193.47	262.07
C:P (OM)	EYmicroc	216.60	Increase	4.67	55.91	0.004	0.994	168.91	227.15	273.82
C:P (OM)	PIgibba	225.08	Increase	2.68	34.61	0.024	0.976	169.17	205.26	273.82
C:P (OM)	ACbiasol	165.20	Increase	3.37	54.25	0.016	0.990	161.63	227.15	295.89
C:P (OM)	NACryten	166.54	Increase	3.41	41.95	0.012	0.978	156.76	169.17	288.83
C:P (OM)	DEkuetzi	227.15	Increase	4.01	59.99	0.008	0.994	152.83	205.26	244.77
C:P (OM)	AHminuti	244.77	Increase	4.67	68.79	0.004	1.000	141.57	205.26	262.07
C:P (OM)	ECsilesi	273.82	Increase	3.36	68.76	0.008	0.990	165.55	262.07	288.83
Pasture (%)	GLHsp	3.08	Decline	3.11	58.21	0.004	0.996	2.11	4.47	13.17
Pasture (%)	MERglauc	2.58	Decline	3.29	53.58	0.012	0.990	0.77	2.11	7.76

Pasture (%)	SCEbijug	2.81	Decline	4.17	43.30	0.004	0.982	1.58	2.81	4.47
Pasture (%)	SPHsp	8.61	Decline	3.59	53.94	0.004	0.956	2.81	8.61	11.53
Pasture (%)	GOmaclau	2.11	Decline	4.77	35.71	0.004	0.992	0.68	1.54	2.81
Pasture (%)	NAstroem	5.83	Decline	3.27	38.49	0.004	0.982	0.68	2.11	7.05
Pasture (%)	BRvitrea	5.83	Decline	4.25	40.91	0.004	1.000	0.68	1.58	7.05
Pasture (%)	NIampoid	1.79	Decline	5.75	42.01	0.004	1.000	0.77	1.54	3.08
Pasture (%)	CMdelcat	0.77	Decline	5.58	84.70	0.004	0.976	0.68	0.93	2.58
Pasture (%)	SYacus	1.79	Decline	5.29	49.49	0.004	1.000	0.68	1.52	3.75
Pasture (%)	GOclavat	2.11	Decline	5.29	57.64	0.004	0.998	0.77	1.54	3.08
Pasture (%)	GOgracil	4.47	Decline	4.44	53.63	0.004	0.996	1.54	3.26	8.42
Pasture (%)	CMkolbei	3.08	Decline	4.93	49.71	0.004	0.974	1.58	3.08	6.89
Pasture (%)	EYevergl	0.77	Decline	3.66	75.83	0.004	0.986	0.77	1.54	5.83
Pasture (%)	EYmicroc	1.46	Decline	6.14	72.39	0.004	0.994	0.77	1.46	2.58
Pasture (%)	NAcryten	1.58	Decline	3.32	45.34	0.016	0.990	1.39	2.11	7.05
Pasture (%)	DEkuetzi	3.75	Decline	3.54	54.69	0.012	0.990	0.77	2.81	8.42
Pasture (%)	AHminuti	1.79	Decline	4.89	65.57	0.004	0.986	1.46	2.42	5.83
Pasture (%)										
Pasture (%)	ROabbre	7.76	Increase	4.40	30.77	0.008	0.992	3.75	7.76	9.79
Pasture (%)	TEmusica	12.64	Increase	6.39	67.16	0.008	0.996	8.42	11.53	12.64
Pasture (%)	NIcombal	7.76	Increase	6.39	53.85	0.004	1.000	4.47	8.42	10.87
Pasture (%)	NIangtu	11.53	Increase	5.69	54.42	0.008	0.996	7.05	10.87	12.64
Pasture (%)	GMlinfor	8.42	Increase	6.86	60.67	0.004	0.996	6.89	8.61	12.33
Pasture (%)	TYapicul	6.89	Increase	5.48	48.99	0.004	0.998	3.75	8.42	12.36
Pasture (%)	NIangust	9.79	Increase	4.61	55.07	0.008	0.998	3.75	8.82	12.64
Pasture (%)	FAtener2	6.89	Increase	5.41	40.00	0.004	0.998	3.75	7.05	10.87
Pasture (%)	NIsolita	12.64	Increase	3.11	61.67	0.020	0.994	1.58	8.61	12.64
Pasture (%)	NAm minima	9.79	Increase	4.54	53.49	0.008	0.994	1.58	8.42	12.07
Pasture (%)	PRlaevis	5.83	Increase	4.10	50.23	0.008	0.992	2.58	6.89	8.82
Pasture (%)	AMpedcls	2.58	Increase	4.21	51.80	0.004	1.000	1.79	3.75	11.53
Pasture (%)	TYlevide	7.05	Increase	3.96	38.56	0.012	0.988	2.58	7.05	10.87
Pasture (%)	AHexigum	2.58	Increase	3.20	51.42	0.016	0.992	0.93	2.42	9.79

Pasture (%)	HIhunga	1.54	Increase	3.67	44.44	0.008	1.000	1.58	6.89	12.07
Pasture (%)	NAsancru	4.47	Increase	5.54	50.29	0.004	1.000	2.79	5.83	9.79
Pasture (%)	DIconfer	12.07	Increase	4.37	74.93	0.004	1.000	1.46	4.47	12.07
Pasture (%)	NIincons	10.87	Increase	4.88	71.94	0.004	1.000	2.58	8.42	12.07
Pasture (%)	GOpumilu	1.58	Increase	3.11	52.52	0.012	0.988	1.39	2.58	9.79
Pasture (%)	DPellipt	12.64	Increase	3.21	68.42	0.020	0.960	1.54	6.89	12.64
Pasture (%)	NArecens	10.87	Increase	6.53	80.05	0.004	1.000	5.83	8.82	12.07
Pasture (%)	REsinuta	3.75	Increase	3.76	58.41	0.004	0.974	2.42	5.83	8.82
Pasture (%)	CCplacen	4.47	Increase	5.21	67.39	0.004	1.000	0.93	3.26	6.89
Pasture (%)	GOpavul	1.21	Increase	3.65	71.18	0.004	0.982	0.68	1.21	6.89
Pasture (%)	NIamphib	0.93	Increase	6.44	70.59	0.004	0.988	0.68	0.93	1.52
Mud-silt (%)	MERglauc	0.00	Decline	3.87	55.10	0.008	0.966	0.00	1.50	10.00
Mud-silt (%)	PEDboryn	0.00	Decline	3.74	37.91	0.008	0.986	0.00	2.20	7.50
Mud-silt (%)	SCEbijug	0.00	Decline	4.07	42.49	0.004	0.996	0.00	0.00	7.28
Mud-silt (%)	CMdelcat	0.20	Decline	4.42	48.20	0.004	0.994	0.00	0.92	7.50
Mud-silt (%)	SYacus	0.62	Decline	4.16	38.60	0.008	0.992	0.00	0.62	7.25
Mud-silt (%)	GOclavat	7.25	Decline	5.17	55.64	0.004	0.994	0.00	2.45	8.88
Mud-silt (%)	GOgracil	8.75	Decline	2.75	44.76	0.032	0.958	0.00	6.00	15.00
Mud-silt (%)	EYevergl	6.00	Decline	3.62	57.37	0.008	0.966	0.00	3.75	15.00
Mud-silt (%)	DEkuetzi	7.25	Decline	3.69	53.15	0.008	0.988	0.00	2.45	12.50
Mud-silt (%)	SYulna	8.75	Decline	3.71	63.68	0.004	0.968	0.00	7.50	15.42
Mud-silt (%)	GLKturf	17.75	Increase	6.32	47.83	0.004	0.952	3.75	17.75	27.42
Mud-silt (%)	CSdubius	16.04	Increase	5.61	37.50	0.008	0.950	10.00	16.04	18.17
Mud-silt (%)	NIangtu	3.75	Increase	3.65	29.41	0.004	0.996	2.20	7.50	15.42
Mud-silt (%)	TYapicul	15.42	Increase	2.97	44.99	0.028	0.950	2.20	16.04	21.17
Mud-silt (%)	CAsilicu	18.17	Increase	3.09	48.70	0.020	0.990	1.50	16.88	27.42
Mud-silt (%)	NAMinima	15.42	Increase	4.32	56.09	0.004	0.962	3.75	15.00	17.75
Mud-silt (%)	AMpedcls	8.75	Increase	5.35	61.76	0.004	1.000	2.20	8.75	18.17
Mud-silt (%)	NAsancru	21.17	Increase	2.82	59.91	0.020	0.978	1.50	15.00	27.42

Mud-silt (%)	NIincons	8.75	Increase	5.18	68.54	0.008	1.000	0.92	7.25	15.00
Mud-silt (%)	GOpumilu	3.75	Increase	5.20	59.50	0.004	0.966	0.62	3.75	10.00
Mud-silt (%)	NArecens	10.00	Increase	6.15	71.83	0.008	0.996	3.75	10.00	16.04
Mud-silt (%)	REsinuta	12.50	Increase	5.09	70.16	0.004	0.996	6.00	12.50	16.04
Mud-silt (%)	CAbacill	17.75	Increase	4.27	72.70	0.008	0.984	7.13	16.88	21.17
Mud-silt (%)	CCplacen	0.00	Increase	4.46	63.69	0.008	1.000	0.00	7.25	15.00
Mud-silt (%)	GOparvul	8.75	Increase	3.75	61.63	0.012	0.990	0.00	7.25	15.00
Outfalls (MGD)	SCZsp	5.69	Decline	3.95	55.45	0.004	0.994	0.01	4.55	6.41
Outfalls (MGD)	UNpennte	4.92	Decline	3.89	59.63	0.004	0.968	1.22	4.15	5.69
Outfalls (MGD)	GOmaclau	0.32	Decline	3.12	26.32	0.036	0.968	0.01	0.18	0.94
Outfalls (MGD)	CTellipt	0.01	Decline	3.86	31.83	0.016	0.966	0.01	0.06	0.44
Outfalls (MGD)	FRtenera	0.08	Decline	3.84	31.25	0.008	0.996	0.01	0.03	0.32
Outfalls (MGD)	GOintvib	0.94	Decline	2.85	30.43	0.016	0.982	0.01	0.44	1.22
Outfalls (MGD)	EPturgid	0.94	Decline	2.71	30.43	0.044	0.992	0.01	0.44	1.22
Outfalls (MGD)	NAstroem	0.08	Decline	5.41	51.11	0.004	1.000	0.01	0.08	0.81
Outfalls (MGD)	BRvitrea	0.08	Decline	3.03	36.83	0.016	0.958	0.01	0.06	1.03
Outfalls (MGD)	CMlaevis	0.01	Decline	6.17	41.67	0.004	0.992	0.01	0.01	0.10
Outfalls (MGD)	ALpelluc	0.01	Decline	3.30	33.65	0.024	0.990	0.01	0.06	0.94
Outfalls (MGD)	CMdelcat	0.58	Decline	4.49	51.23	0.004	1.000	0.06	0.69	3.26
Outfalls (MGD)	SYacus	0.32	Decline	3.77	40.29	0.012	0.990	0.01	0.14	0.81
Outfalls (MGD)	GOclavat	0.58	Decline	4.63	52.23	0.004	0.994	0.06	0.44	3.51
Outfalls (MGD)	NAradios	0.06	Decline	3.32	30.31	0.012	0.992	0.01	0.08	0.81
Outfalls (MGD)	FRcapuci	0.44	Decline	3.70	52.92	0.008	0.980	0.01	0.18	1.03
Outfalls (MGD)	BApardxa	0.01	Decline	3.07	23.08	0.036	0.960	0.01	0.01	0.08
Outfalls (MGD)	CMaffins	0.18	Decline	4.36	47.62	0.004	0.990	0.01	0.10	0.94
Outfalls (MGD)	GOangstt	0.94	Decline	3.93	50.14	0.012	0.994	0.08	0.58	3.26
Outfalls (MGD)	CMkolbei	0.94	Decline	3.31	45.56	0.012	0.986	0.01	0.58	2.17
Outfalls (MGD)	EYevergl	0.06	Decline	5.35	64.86	0.004	1.000	0.01	0.18	1.22
Outfalls (MGD)	EYmicroc	1.22	Decline	4.22	58.09	0.004	0.994	0.03	0.81	3.26
Outfalls (MGD)	ACbiasol	0.01	Decline	4.95	67.93	0.004	0.998	0.01	0.10	3.26

Outfalls (MGD)	NAcryten	0.01	Decline	4.42	52.57	0.004	0.982	0.01	0.01	0.32
Outfalls (MGD)	DEkuetzi	0.08	Decline	6.02	71.15	0.004	0.998	0.01	0.08	0.58
Outfalls (MGD)	AHminuti	0.08	Decline	7.17	77.47	0.004	1.000	0.01	0.18	2.28
Outfalls (MGD)	ECsilesi	3.51	Decline	3.56	64.13	0.008	0.984	0.32	2.17	4.15
Outfalls (MGD)	CHRsp	5.69	Increase	6.83	88.44	0.004	1.000	0.44	4.55	6.41
Outfalls (MGD)	SCEquadr	4.15	Increase	8.62	42.86	0.008	0.964	3.51	4.55	6.41
Outfalls (MGD)	AMveneta	6.41	Increase	7.35	93.02	0.004	0.994	1.22	4.92	6.41
Outfalls (MGD)	GMgrovei	6.41	Increase	4.32	61.00	0.024	0.976	0.44	5.69	6.41
Outfalls (MGD)	ROabbre	2.17	Increase	4.51	33.33	0.012	0.986	0.58	2.17	4.55
Outfalls (MGD)	FRellptc	3.26	Increase	3.48	39.10	0.012	0.958	0.32	3.26	6.41
Outfalls (MGD)	TEmusica	0.32	Increase	3.44	31.58	0.024	0.992	0.08	0.44	4.92
Outfalls (MGD)	NIcombal	0.32	Increase	4.44	36.84	0.004	0.998	0.10	0.58	2.17
Outfalls (MGD)	NIangtu	0.81	Increase	4.03	31.25	0.004	0.994	0.18	0.87	3.51
Outfalls (MGD)	GMlinfor	0.32	Increase	5.33	47.37	0.004	1.000	0.08	0.81	6.41
Outfalls (MGD)	NIangust	0.06	Increase	4.00	39.13	0.008	0.992	0.01	0.18	1.03
Outfalls (MGD)	FAtener2	1.22	Increase	4.36	35.64	0.008	0.982	0.18	0.87	3.51
Outfalls (MGD)	NIsolita	0.08	Increase	4.36	45.45	0.008	1.000	0.01	0.10	1.22
Outfalls (MGD)	PRlaevis	5.69	Increase	4.38	80.36	0.004	0.994	0.44	3.89	6.41
Outfalls (MGD)	AMpedcls	0.81	Increase	5.75	57.62	0.004	0.996	0.03	0.44	1.22
Outfalls (MGD)	HIhunga	1.22	Increase	4.02	47.88	0.004	0.968	0.01	0.94	3.51
Outfalls (MGD)	NAsancru	0.44	Increase	6.47	55.56	0.004	1.000	0.10	0.58	2.17
Outfalls (MGD)	DIconfer	3.26	Increase	5.61	76.18	0.004	1.000	0.18	2.17	3.63
Outfalls (MGD)	GOpumilu	1.22	Increase	5.18	63.21	0.004	0.980	0.32	1.03	3.51
Outfalls (MGD)	NArecons	0.32	Increase	5.97	62.84	0.004	0.980	0.06	0.32	1.03
Outfalls (MGD)	REsinuta	0.58	Increase	3.53	59.25	0.004	0.994	0.01	0.32	2.17
Outfalls (MGD)	CCplacen	0.18	Increase	5.72	69.16	0.004	1.000	0.06	0.32	0.94
Outfalls (MGD)	GOparvul	0.18	Increase	3.42	58.98	0.012	0.972	0.01	0.06	0.58
Chloride (mg/L)	GLHsp	18.50	Decline	2.41	57.17	0.004	0.982	13.50	18.00	35.00
Chloride (mg/L)	MERglauc	19.50	Decline	7.94	77.50	0.004	0.998	18.00	20.50	26.50

Chloride (mg/L)	OSCsp	21.50	Decline	3.29	55.89	0.004	0.968	18.00	24.00	72.50
Chloride (mg/L)	GOMaclau	20.50	Decline	3.21	29.41	0.020	0.994	11.00	18.50	24.00
Chloride (mg/L)	GOintvib	26.00	Decline	3.87	35.00	0.012	0.992	18.00	21.50	30.00
Chloride (mg/L)	NAstroem	20.50	Decline	6.70	58.82	0.004	1.000	17.00	19.50	26.00
Chloride (mg/L)	BRvitrea	20.50	Decline	4.83	45.89	0.004	0.998	18.00	20.50	28.50
Chloride (mg/L)	CMlaevis	18.00	Decline	5.33	41.67	0.004	0.998	11.50	15.00	19.50
Chloride (mg/L)	NIampoid	20.50	Decline	3.12	33.60	0.012	0.992	13.50	19.00	28.50
Chloride (mg/L)	ALpelluc	26.00	Decline	3.32	30.00	0.004	0.956	10.50	24.00	30.10
Chloride (mg/L)	NACrypto	30.00	Decline	2.41	26.09	0.020	0.964	13.50	24.00	31.00
Chloride (mg/L)	CMdelcat	20.50	Decline	5.18	57.82	0.004	1.000	15.00	20.50	30.00
Chloride (mg/L)	SYacus	20.50	Decline	2.75	35.21	0.020	0.984	18.00	24.00	60.50
Chloride (mg/L)	GOclavat	24.00	Decline	4.53	51.07	0.008	0.990	13.50	18.50	26.00
Chloride (mg/L)	GOgracil	69.00	Decline	3.72	53.57	0.004	0.998	13.50	31.00	73.50
Chloride (mg/L)	NAradios	12.50	Decline	4.30	45.70	0.004	0.998	11.00	15.00	21.50
Chloride (mg/L)	FRcapuci	26.00	Decline	5.01	59.78	0.004	0.994	13.50	21.50	31.00
Chloride (mg/L)	CMaffins	18.00	Decline	5.90	56.70	0.004	0.996	11.50	17.00	26.00
Chloride (mg/L)	GOangstt	12.50	Decline	5.08	70.05	0.004	1.000	11.50	17.00	26.05
Chloride (mg/L)	CMkolbei	31.00	Decline	4.88	50.00	0.008	1.000	11.00	20.50	35.00
Chloride (mg/L)	EYevergl	19.00	Decline	6.64	75.32	0.004	1.000	15.00	19.00	28.65
Chloride (mg/L)	EYmicroc	26.00	Decline	5.56	62.75	0.004	0.996	18.00	21.50	30.00
Chloride (mg/L)	PIgibba	35.00	Decline	2.71	36.00	0.040	0.976	11.00	28.50	60.50
Chloride (mg/L)	ACbiasol	26.00	Decline	5.57	62.60	0.004	0.992	15.00	20.50	28.50
Chloride (mg/L)	DEkuetzi	21.50	Decline	4.18	60.35	0.004	0.996	18.00	21.50	60.50
Chloride (mg/L)	AHminuti	24.00	Decline	6.05	69.10	0.004	1.000	19.00	24.00	46.50
Chloride (mg/L)	ECsilesi	17.00	Decline	4.26	66.01	0.004	0.998	13.50	18.50	35.00
Chloride (mg/L)	SYulna	94.50	Decline	6.56	94.12	0.004	1.000	69.00	92.50	100.50
Chloride (mg/L)	ANBsp	31.00	Increase	2.83	21.43	0.048	0.964	24.00	31.00	69.00
Chloride (mg/L)	CHRsp	24.00	Increase	4.69	42.11	0.004	1.000	19.50	26.00	46.50
Chloride (mg/L)	SCEquadr	69.00	Increase	4.52	30.00	0.016	0.968	31.00	72.50	92.50
Chloride (mg/L)	NASubmin	31.00	Increase	3.01	21.43	0.032	0.966	26.50	35.00	92.50

Chloride (mg/L)	AMveneta	60.50	Increase	6.55	45.45	0.004	0.994	31.00	72.50	92.50
Chloride (mg/L)	ROabbre	92.50	Increase	8.96	59.02	0.004	0.982	69.00	86.00	94.50
Chloride (mg/L)	FRellptc	46.50	Increase	5.30	48.22	0.004	1.000	26.00	46.50	86.00
Chloride (mg/L)	NIotropic	26.50	Increase	2.95	23.53	0.036	0.978	20.50	28.50	73.85
Chloride (mg/L)	NIcombal	26.50	Increase	4.81	41.18	0.004	1.000	24.00	46.50	94.50
Chloride (mg/L)	NIangtu	94.50	Increase	4.28	43.76	0.012	0.982	20.50	69.00	100.50
Chloride (mg/L)	SUbreb	94.50	Increase	5.49	45.30	0.008	0.960	26.00	77.00	100.50
Chloride (mg/L)	AMSabina	18.50	Increase	4.14	45.83	0.008	0.996	18.00	20.50	35.00
Chloride (mg/L)	GMlinfor	35.00	Increase	2.96	37.33	0.020	0.972	18.00	28.50	72.50
Chloride (mg/L)	TYapicul	31.00	Increase	4.05	43.11	0.008	0.994	20.50	35.00	94.50
Chloride (mg/L)	FAtener2	92.50	Increase	5.01	53.29	0.012	1.000	26.00	69.00	100.50
Chloride (mg/L)	PCplacen	26.00	Increase	5.31	44.44	0.008	1.000	21.50	30.00	86.00
Chloride (mg/L)	PRlaevis	46.50	Increase	7.64	69.18	0.004	1.000	26.50	35.00	72.50
Chloride (mg/L)	AMPedcls	69.00	Increase	3.50	58.66	0.012	0.986	17.00	46.50	86.00
Chloride (mg/L)	AHexigum	21.50	Increase	4.87	62.19	0.004	0.998	18.50	26.00	60.50
Chloride (mg/L)	HIhunga	92.50	Increase	6.46	87.18	0.004	1.000	19.00	73.50	94.50
Chloride (mg/L)	PTlanceo	31.00	Increase	3.65	40.25	0.008	0.956	19.00	30.00	72.50
Chloride (mg/L)	NAsancru	20.50	Increase	2.97	37.20	0.032	0.974	17.00	24.00	72.50
Chloride (mg/L)	DIconfer	28.50	Increase	6.31	71.71	0.004	1.000	18.50	26.00	35.00
Chloride (mg/L)	NIincons	20.50	Increase	5.16	67.15	0.004	1.000	18.00	21.50	73.85
Chloride (mg/L)	NIfrustu	31.00	Increase	3.18	53.80	0.020	0.954	20.50	35.00	94.50
Chloride (mg/L)	NARostel	26.00	Increase	2.81	39.65	0.028	0.986	17.00	26.00	86.00
Chloride (mg/L)	NAREcens	24.00	Increase	4.54	55.41	0.008	0.978	18.00	24.00	77.00
Chloride (mg/L)	CYmenegh	92.50	Increase	4.04	75.49	0.004	0.986	20.50	35.00	94.50
Chloride (mg/L)	GOParvul	19.50	Increase	2.73	56.39	0.008	0.964	18.00	30.00	86.00

Appendix A7. Taxa-specific results from Threshold Indicator Taxa Analysis (TITAN) on **fish species composition** in response to nutrient and nutrient-related stressors among 38 sites in **Ecoregion 29** during summer 2008. Only fish species that showed significant threshold declines or increases in response to predictors are included in this table. The observed (*Obs*) threshold value of the predictor for each taxon is shown in bold, whereas lower (10%), middle (50%), and upper (90%) quantiles of 1,000 bootstraps represent measures of uncertainty around the observed threshold. *Z* represents the standardized indicator score from TITAN (larger numbers = stronger threshold response), *IndVal* is the unstandardized indicator score (scaled from 0-100%, with 100=perfect indicator). *Purity* is the relative consistency of the response direction among the 1,000 bootstraps (*purity* > 0.95 is significant). *P-value* is the likelihood of getting an equal or larger *IndVal* if the score were computed with random shuffling of the observed data (*P*<0.05 is significant). See Appendix A6 for full species names corresponding to *Taxon IDs*.

Predictor	Taxon ID	Threshold (obs)	Response > obs.	z	IndVal	P	Purity	Bootstrap threshold quantiles		
								10%	50%	90%
TP (ug/L)	CAMPANOM	24.2	Decline	4.6	66.6	0.004	0.980	19.7	27.8	44.7
TP (ug/L)	CYPRVENU	17.0	Decline	2.5	59.0	0.016	0.972	14.6	18.2	368.3
TP (ug/L)	ETHESPEC	19.7	Decline	5.8	73.8	0.004	1.000	17.0	26.8	44.7
TP (ug/L)	LEPOGULO	14.3	Decline	2.2	49.8	0.044	0.940	12.4	19.7	52.1
TP (ug/L)	LEPOMACR	61.9	Decline	4.0	61.4	0.004	0.982	34.2	69.8	598.3
TP (ug/L)	CARPCARP	52.1	Increase	3.8	33.6	0.008	0.980	28.9	52.1	770.3
TP (ug/L)	CYPRCARP	187.5	Increase	5.7	59.1	0.004	0.990	34.2	125.1	932.2
TP (ug/L)	CYPRLUTR	21.4	Increase	4.9	67.0	0.004	0.996	16.9	27.8	52.1
TP (ug/L)	LEPIOSSE	34.2	Increase	3.9	40.2	0.004	0.962	19.7	40.7	187.5
TP (ug/L)	PIMEVIGI	21.4	Increase	4.2	63.3	0.004	0.986	14.6	26.8	44.7
Pasture (%)	CYPRVENU	12.3	Decline	4.4	68.0	0.004	1.000	3.8	11.5	13.2
Pasture (%)	CAMPANOM	7.1	Decline	3.8	65.7	0.004	0.998	2.4	6.9	12.3
Pasture (%)	ETHESPEC	4.5	Decline	5.2	71.8	0.004	0.998	2.8	4.5	7.8
Pasture (%)	MOXOCONG	8.4	Decline	4.0	50.0	0.004	0.996	3.1	7.1	8.8
Pasture (%)	NOTRVOLU	8.6	Decline	4.1	51.9	0.008	0.990	2.6	8.4	9.8
Pasture (%)	ICTAPUNC	10.9	Decline	4.4	72.3	0.004	0.956	7.8	9.8	12.6

Pasture (%)	CYPRLUTR	1.6	Increase	6.1	74.5	0.004	1.000	0.8	1.8	3.3
Pasture (%)	DOROCEPE	8.8	Increase	5.6	55.8	0.004	1.000	3.1	8.6	11.5
Pasture (%)	PIMEVIGI	3.3	Increase	5.2	66.7	0.004	1.000	0.9	2.8	5.8
Pasture (%)	CARPCARP	3.8	Increase	4.4	33.3	0.008	0.996	3.1	5.8	12.3
Pasture (%)	POMOANNU	4.5	Increase	3.8	29.4	0.008	0.994	2.8	4.5	8.6
Pasture (%)	LYTHUMBR	9.8	Increase	6.5	44.4	0.004	0.980	8.6	10.9	13.2
Pasture (%)	NOTUGYRI	6.9	Increase	2.7	20.0	0.056	0.952	3.3	7.1	8.8
Outfalls (MGD)	CAMPANOM	0.3	Decline	4.9	67.9	0.004	0.984	0.0	0.3	0.9
Outfalls (MGD)	ETHESPEC	0.3	Decline	5.3	71.4	0.004	0.990	0.0	0.3	0.9
Outfalls (MGD)	FUNDNOTA	2.2	Decline	4.3	64.0	0.004	0.986	0.8	2.2	4.1
Outfalls (MGD)	LEPOCYAN	0.8	Decline	3.5	56.1	0.004	0.948	0.2	1.2	5.7
Outfalls (MGD)	LEPOMACR	2.2	Decline	3.8	60.7	0.004	0.982	0.4	1.2	3.6
Outfalls (MGD)	CYPRCARP	4.1	Increase	3.1	46.2	0.008	0.966	0.1	3.5	5.7
Outfalls (MGD)	CYPRLUTR	0.4	Increase	4.5	66.4	0.004	0.996	0.0	0.2	1.0
Outfalls (MGD)	DOROCEPE	0.4	Increase	2.8	30.7	0.024	0.910	0.0	0.9	5.7
Outfalls (MGD)	ICTAPUNC	3.5	Increase	3.2	64.5	0.008	0.902	0.8	3.5	4.5
Outfalls (MGD)	LEPIOSSE	0.6	Increase	3.8	40.2	0.016	0.944	0.1	0.8	3.2
Outfalls (MGD)	PIMEVIGI	0.4	Increase	4.7	66.4	0.004	0.952	0.0	0.3	1.0
Outfalls (MGD)	PYLOOLIV	3.5	Increase	5.0	72.9	0.004	0.998	0.8	2.2	3.6
Mud-silt (%)	CAMPANOM	0.0	Decline	4.0	64.6	0.008	0.942	0.0	3.8	12.5
Mud-silt (%)	CYPRVENU	1.5	Decline	3.1	58.6	0.012	0.996	0.0	8.8	17.8
Mud-silt (%)	ICTAPUNC	15.0	Decline	3.9	67.4	0.004	0.976	7.3	15.0	17.0
Mud-silt (%)	LEPOCYAN	7.3	Decline	3.5	56.4	0.012	0.970	0.9	8.8	18.2
Mud-silt (%)	NOTRVOLU	7.5	Decline	2.5	43.5	0.032	0.950	0.0	6.0	15.4
Mud-silt (%)	CYPRLUTR	6.0	Increase	3.8	64.1	0.004	0.982	0.2	3.8	8.8
Mud-silt (%)	LEPIOSSE	15.0	Increase	3.8	47.9	0.012	0.926	2.2	15.0	18.2

Mud-silt (%)	LYTHUMBR	16.0	Increase	6.6	50.0	0.004	0.992	12.5	16.0	18.2
Mud-silt (%)	PIMEVIGI	1.5	Increase	3.7	63.1	0.004	0.992	0.2	2.5	12.5
Chloride (mg/L)	AMEINATA	12.0	Decline	3.3	68.4	0.004	0.980	11.0	17.0	26.5
Chloride (mg/L)	CAMPANOM	17.0	Decline	4.5	68.7	0.004	1.000	15.0	19.5	86.0
Chloride (mg/L)	ETHESPEC	30.0	Decline	4.6	69.2	0.004	1.000	17.0	24.0	60.5
Chloride (mg/L)	FUNDNOTA	60.5	Decline	3.7	61.0	0.004	0.974	19.0	46.5	73.5
Chloride (mg/L)	LEPOGULO	19.5	Decline	3.5	49.5	0.016	0.962	15.0	19.0	28.5
Chloride (mg/L)	LEPOMACR	92.5	Decline	4.6	66.4	0.004	0.998	30.0	77.0	100.5
Chloride (mg/L)	CARPCARP	46.5	Increase	6.2	50.0	0.004	1.000	28.5	46.5	73.5
Chloride (mg/L)	CYPRCARP	30.0	Increase	3.9	40.6	0.008	0.990	18.5	35.0	94.5
Chloride (mg/L)	CYPRLUTR	13.5	Increase	7.9	88.1	0.004	1.000	11.5	13.5	18.0
Chloride (mg/L)	PIMEVIGI	13.5	Increase	6.9	86.3	0.004	1.000	11.5	13.5	19.0
Chloride (mg/L)	PYLOOLIV	19.5	Increase	3.5	58.5	0.004	0.936	17.0	20.5	61.4

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 *Campos-toma 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 N₂, 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,

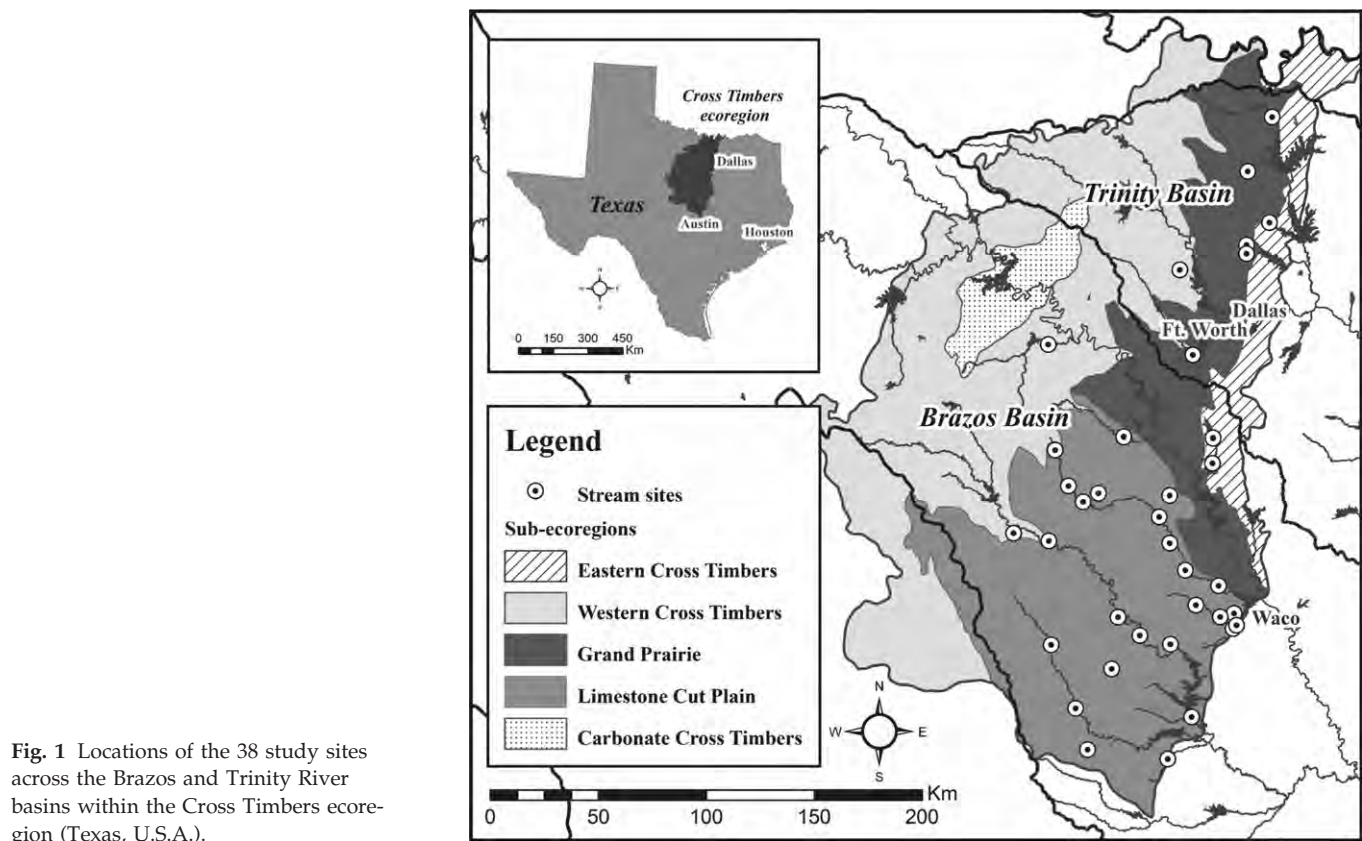


Fig. 1 Locations of the 38 study sites across the Brazos and Trinity River basins within the Cross Timbers ecoregion (Texas, U.S.A.).

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 ²)	68	6112	845	404
WWTP (1 × 10 ⁶ L d ⁻¹ km ⁻²)	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 ³ s ⁻¹)	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 (μS cm ⁻¹)	184	1225	646	604
Cl (mg L ⁻¹)	7	140	41	24
TSS (mg L ⁻¹)	1	105	14	7
Surface water TP (μg L ⁻¹)	7	2380	296	29
Surface water TN (μg L ⁻¹)	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 EATM 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⁻¹ K₂S₂O₈), boric acid (50 g L⁻¹ H₃BO₃) and sodium hydroxide (15 g L⁻¹ 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 CI despite strong relationships with surface water TP because we wanted to contrast assemblage responses to both nutrients, whereas CI 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 CI concentrations (see Table S3). In general, sites with high TP also had high CI, indicating a wastewater effluent

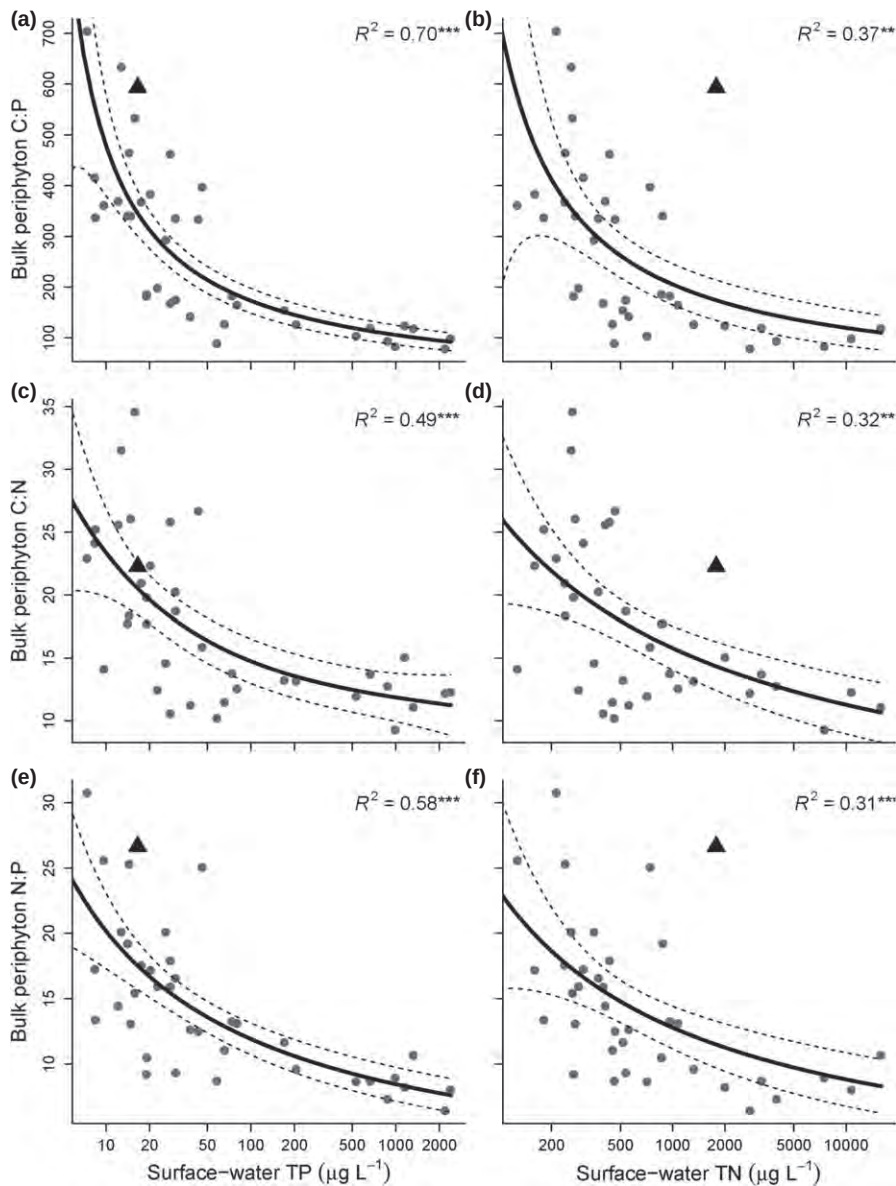


Fig. 2 GAM smoothers for periphyton C : P (a, b), C : N (c, d) and N : P (e, f) in response to surface water TP and TN. Solid lines represent predicted ratios with 95% CI (dashed lines). R^2 = null deviance – residual deviance/null deviance, and $P < 0.001$ is indicated by ***. Black triangle represents 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-

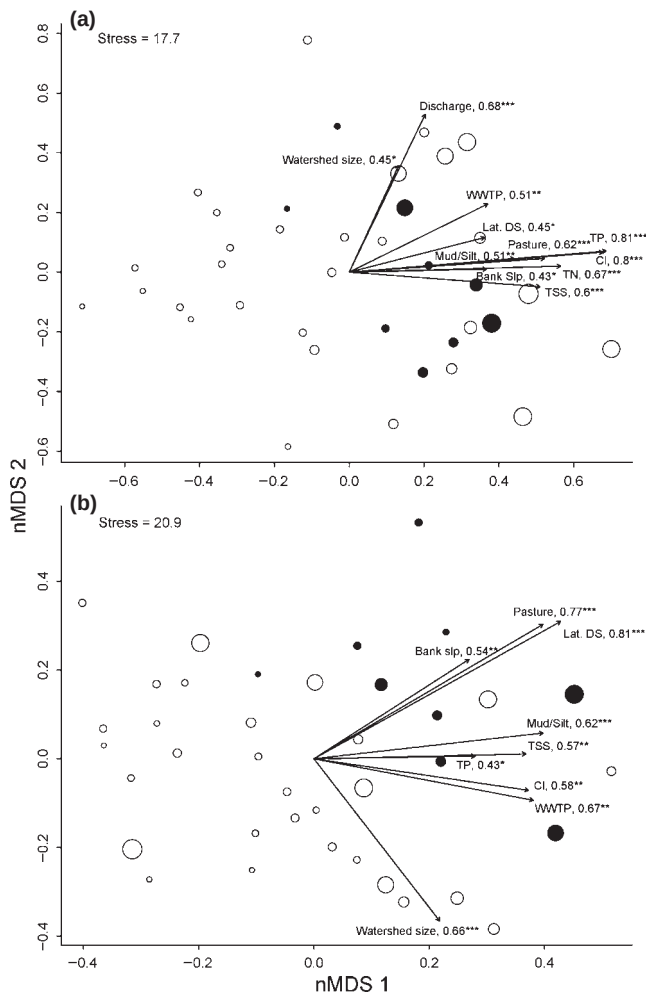


Fig. 3 Non-metric multidimensional scaling (NMS) ordination of sites based on periphyton (a) and fish (b) assemblage data. Symbol shading indicates membership among the Brazos (open) and Trinity (filled) river basins. Environmental vectors show the direction and magnitude of significant correlations 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 km^2 ($1 \times 10^6 \text{ L d}^{-1}$); Bank Slop, bank slope (%); TSS, total suspended solids (mg L^{-1}); Cl, chloride (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, $W = 251$, $n_1 = 9$, $n_2 = 26$, $P < 0.001$).

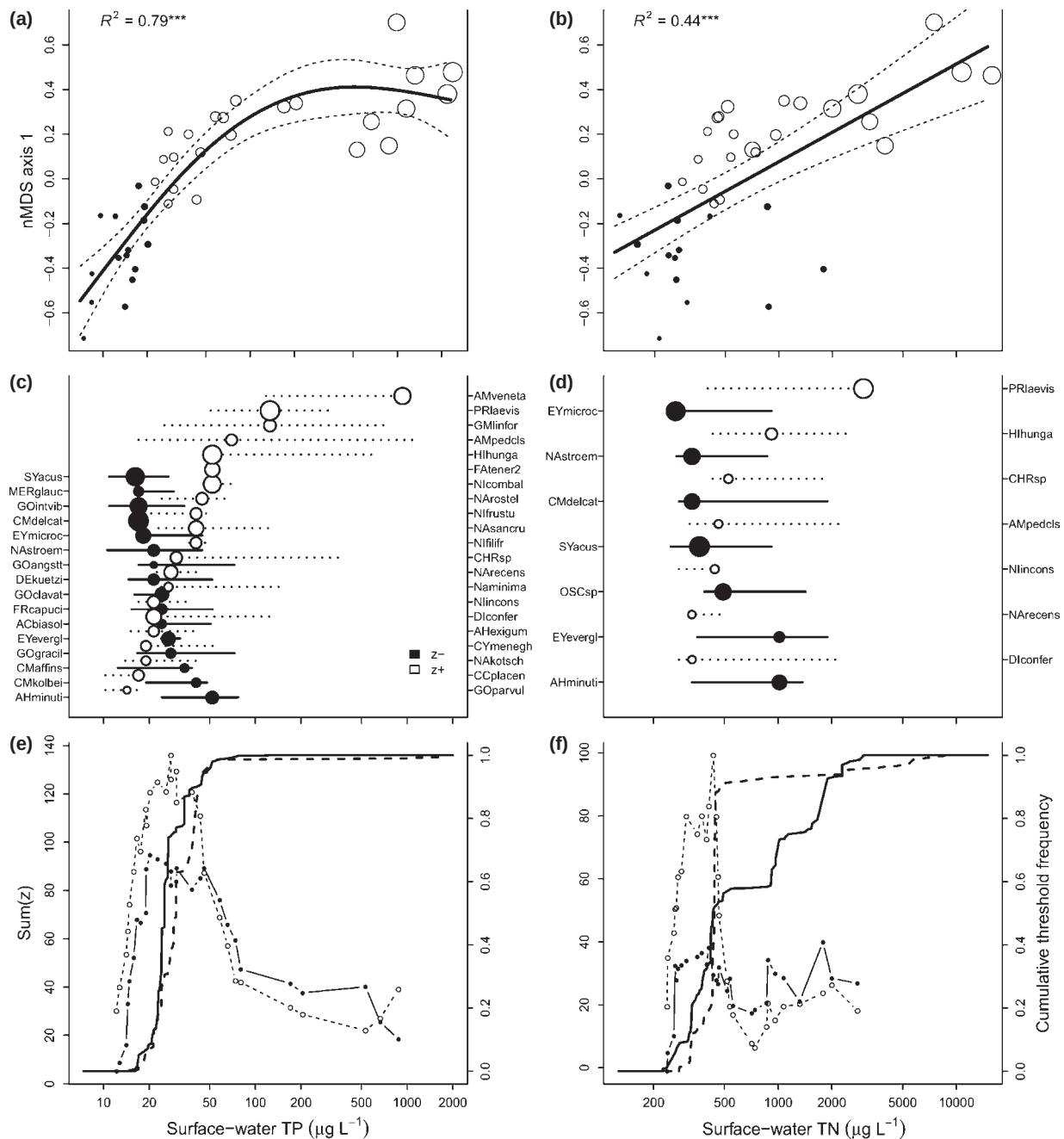


Fig. 4 Change in periphyton assemblage structure (nMDS axis 1 from Fig. 3a) across surface water TP and TN ($\mu\text{g L}^{-1}$) gradients spanning 38 sample sites (a, b). Solid lines represent predicted abundance with 95% CI (dashed lines). Solid symbols represent sites below the surface water TP ($\mu\text{g L}^{-1}$), 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 = null deviance – residual deviance/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 ≥ 0.95 , reliability ≥ 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 ($10\text{--}50\ \mu\text{g L}^{-1}$) 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 $16\text{ and }52\ \mu\text{g L}^{-1}$ (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, $18\text{--}48\ \mu\text{g L}^{-1}$) (Table 2, Fig. 4e). There was also an assemblage-level threshold identified at $28\ \mu\text{g L}^{-1}$ (90% CI, $19\text{ and }48\ \mu\text{g L}^{-1}$) 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 (*Campostoma 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, $24\text{--}56\ \mu\text{g L}^{-1}$) and $34\ \mu\text{g L}^{-1}$ (90% CI, $21\text{--}56\ \mu\text{g L}^{-1}$), 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, $13\text{--}24\ \mu\text{g L}^{-1}$). 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 $33\text{--}1069\ \mu\text{g L}^{-1}$) (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 $27\text{--}598\ \mu\text{g L}^{-1}$) than the increasing species component (90% CI of $13\text{--}52\ \mu\text{g L}^{-1}$) (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)

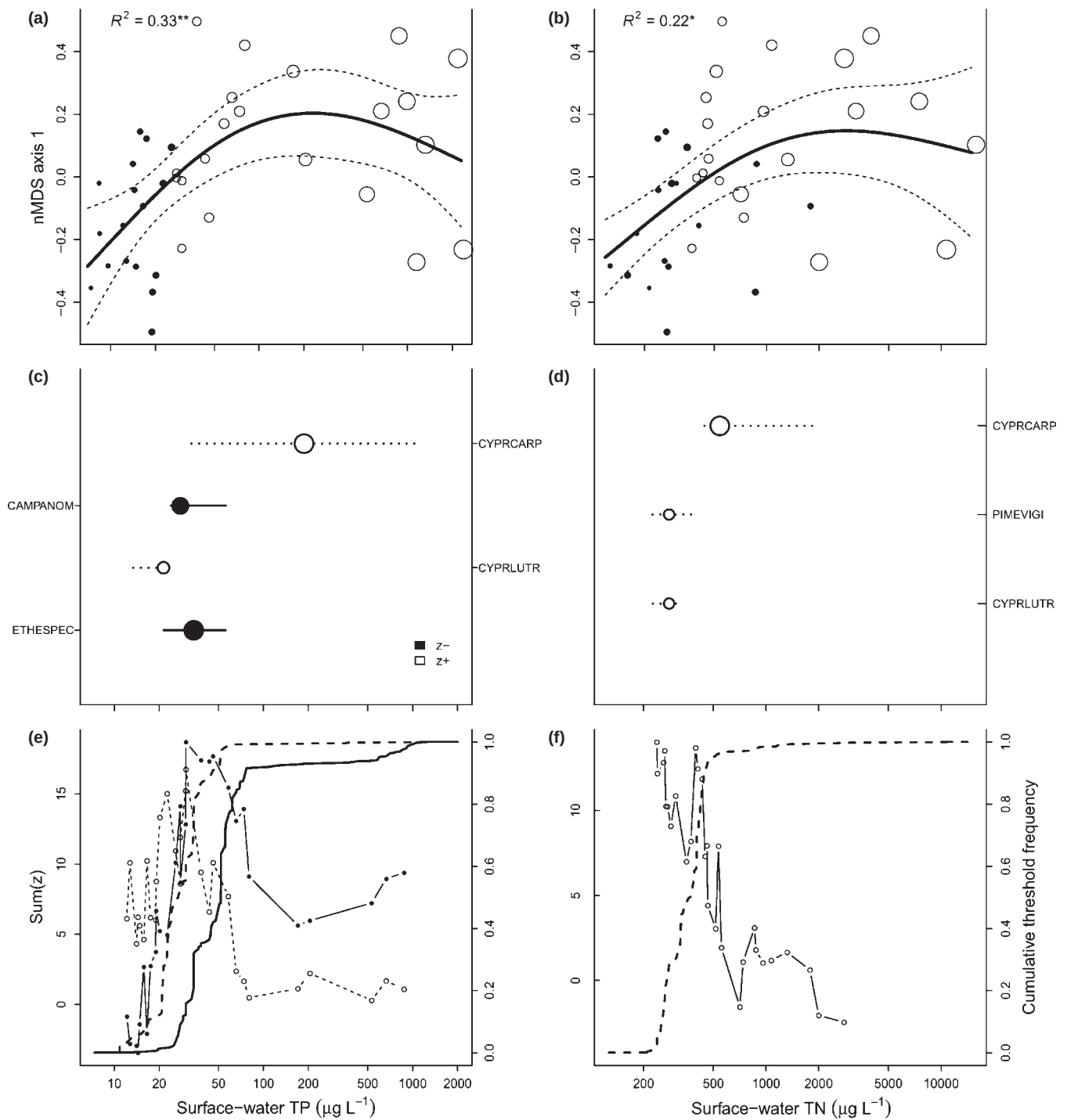


Fig. 5 Change in fish assemblage structure (nMDS axis 1 from Fig. 3a) across surface water TP and TN ($\mu\text{g L}^{-1}$) gradients spanning 38 sample sites (a, b). Significant fish indicator taxa identified in threshold indicator taxa analysis (TITAN) across surface water TP and TN ($\mu\text{g L}^{-1}$) 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

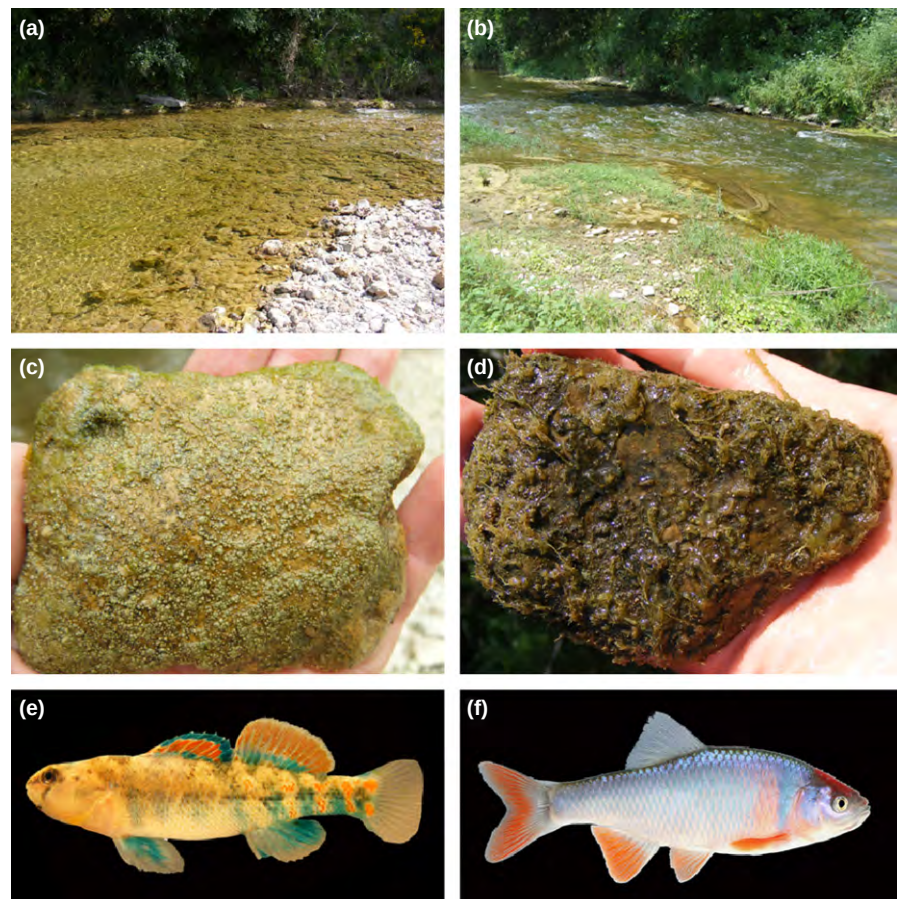


Fig. 6 Representative differences in stream habitat (a, b), periphyton assemblages (c, d) and fish indicator species (e, f) between low nutrient (TP = $7 \mu\text{g L}^{-1}$; TN = $213 \mu\text{g L}^{-1}$) (a, c, e) and slightly enriched (TP = $66 \mu\text{g L}^{-1}$; TN = $450 \mu\text{g L}^{-1}$) (b,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

Spatial, temporal and experimental: Three study design cornerstones for establishing defensible numeric criteria in freshwater ecosystems

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Abstract

1. Nutrient over-enrichment increasingly threatens global water resources. Stressor-response studies specifically designed to identify levels of nutrients strongly associated with undesirable ecological conditions are needed to inform numeric nutrient criteria that protect inland waters.
2. Diatoms are important components of aquatic life, which support higher trophic levels and are sensitive to nutrient enrichment. We tested a framework that relies on stressor-response modelling of phosphorus (P) enrichment and stream diatom assemblages across many field locations, multiple years and seasons within years, and under controlled experimental conditions to inform nutrient criteria development.
3. Diatom species composition was nonlinearly correlated with total phosphorus (TP) throughout the 2-year field study. This occurred despite temporal shifts in species composition between two hydrologically distinct years and over eight seasons.
4. Species assemblages on rocks transplanted from a low P stream to mesocosms representing a P enrichment gradient (8, 20 and 100 µg/L) shifted into two groups over time. Species composition on rocks in low (20 µg/L) and high (100 µg/L) P mesocosms was consistent with assemblages at P-enriched field sites, whereas rocks in control (8 µg/L) mesocosms had significantly different species composition, consistent with low P field sites. Species composition on rocks transplanted from high P streams did not shift as dramatically and were not significantly different after exposure to different P treatments in mesocosms.
5. Threshold Indicator Taxa Analysis identified synchronous declines in several diatom species that culminated in assemblage thresholds associated with TP concentrations >20 and 25 µg/L for 2006 and 2007 respectively.
6. *Synthesis and applications.* Diatom assemblages show consistent responses to nutrient enrichment despite temporal shifts associated with confounding factors common in stream ecosystems. Regulators should include diatom assemblage responses when developing numeric nutrient criteria. We present a framework that includes spatial, temporal and experimental components, and has broad applicability for use in different ecological settings to evaluate ecological endpoints and

set limits for a variety of contaminants threatening freshwater ecosystems throughout the world.

KEYWORDS

diatom, Eutrophication, freshwater ecosystems, manyglm, mesocosm, phosphorus, threshold, TITAN

1 | INTRODUCTION

Cultural inputs of excess phosphorus (P) and nitrogen (N) are a leading cause of stream impairment around the world (Carpenter et al., 1998; Dodds & Smith, 2016; Smith, Joye, & Howarth, 2006). To address this persistent threat to aquatic ecosystems, regulatory agencies seek to establish numeric criteria or standards for nutrients that protect the physical, chemical and biological integrity of aquatic ecosystems (Hering et al., 2010; U.S. Environmental Protection Agency, 1998). Development has progressed slowly due in part to a specific lack of understanding of what policies or practices will help meet potential criteria (McDowell et al., 2014), but also largely to the need for robust stressor-response relationships to inform numeric criteria (U.S. Environmental Protection Agency, 2010). This is troubling because despite efforts to develop and implement nutrient controls, national datasets indicate that nutrient concentrations continue to increase in inland waters and eutrophication associated with non-point sources of P remains a widespread problem (Dodds & Smith, 2016; Stoddard et al., 2016).

Phosphorus is a limiting nutrient of temperate inland waters (Schindler, 1977). This observation is particularly apparent in watersheds draining alkaline regions (Noe, Childers, & Jones, 2001), where calcium carbonate (CaCO_3) deposition can lower phosphate concentrations, thereby decreasing P availability relative to N and fundamentally shifting streams to P limitation (Corman, Moody, & Elser, 2015). In addition to point and non-point source P inputs in impacted watersheds, larger-scale changes in run-off patterns, forest cover and atmospheric contribution are also shifting P dynamics in relatively pristine watersheds. Extensive loss of naturally oligotrophic streams and lakes could have widespread ecosystem consequences including increased incidence of harmful algal blooms, altered habitat for aquatic organisms and unforeseen impacts (Stoddard et al., 2016). Thus, developing measures that detect early changes in ecosystem structure is a fundamental component of developing defensible nutrient criteria.

While well-established in lakes, the concept of oligotrophy and eutrophy is difficult to apply in streams due to the fact that flow velocity, shading and a reliance on allochthonous energy all influence primary production (Schneider & Lindstrøm, 2011). However, recognizing river eutrophication can also be represented by shifts in benthic algal species composition associated with increasing nutrient availability, support the use of algal assemblage measures as response variables for developing defensible nutrient criteria and standards (Hering et al., 2006; United States Environmental

Protection Agency, 2014). Spatial studies that rely on regional enrichment gradients suggest that relatively low levels of P enrichment can induce major changes in algal species composition in streams (Schneider & Lindstrøm, 2011; Smucker, Becker, Detenbeck, & Morrison, 2013; Stevenson et al., 2008; Taylor, King, Pease, & Winemiller, 2014). However, the inherent stochastic nature of riverine ecosystems introduces appreciable uncertainty in the reliability of these measures (Biggs, 2000; Larned, 2010), and potential flow induced assemblage variation is of particular concern given that climate change projections predict streams will become flashier with larger swings between drought and flood conditions on seasonal and annual time-scales (Grimm et al., 2013). Shifts in hydrology may influence benthic assemblages by altering nutrient delivery or increasing scouring and dewatering of habitat (Biggs, Nikora, & Snelder, 2005; Biggs & Thomsen, 1995; Blinn, Shannon, Stevens, & Carder, 1995).

Seasonal patterns in canopy cover, nutrients and herbivory can also influence algal assemblage responses to nutrient enrichment (DeNicola, McIntire, Lamberti, Gregory, & Ashkenas, 1990; Rosemond, Mulholland, & Brawley, 2000). Thus, quantifying how seasonal and annual variation influences benthic algal assemblage response to nutrient enrichment promotes an advanced understanding of how nutrient subsidies alter stream ecosystem structure within the context of a changing climate. Finally, given the diverse confounding factors that may affect nutrient-benthic algal relationships, conducting mesocosm experiments provide additional empirical evidence to evaluate field observations and increase defensibility of criteria (King & Richardson, 2003; Townsend, Uhlmann, & Matthaei, 2008).

Here, we illustrate a framework for modelling stressor-response relationships between benthic diatom assemblages and P enrichment that relies on a coupled observational and experimental approach. We focused on diatoms because taxonomic resolution for soft alga and measuring biomass of macroscopic species at microscopic scales can limit non-diatom taxa usefulness in interpreting assemblage responses. Our observational approach relies on repeated sampling seasonally ($n = 8$ events) across two hydrologically dynamic and distinct years to assess seasonal and annual variation in diatom assemblage structure among sampling sites representing a strong total P gradient (4–4,600 $\mu\text{g/L}$). Severe drought conditions with minimal precipitation and low stream flow during the summer and fall of 2006 resulted in most streams becoming dewatered with disconnected pools. In contrast, 2007 was an exceptionally wet year with periodic spring flooding and uncharacteristic sustained high flows

throughout the summer (Stanley, Taylor, & King, 2012; Appendix S1, Figure S1). We sampled nutrients and periphyton during a 2-year period to examine: (1) the consistency of assemblage responses to spatial gradients in enrichment from data collected repeatedly from many field locations over a wide range of seasonal and annual hydrological conditions; and (2) the utility of diatom assemblage structure for identifying thresholds that can inform scientifically defensible regional nutrient criteria. Our experimental approach consisted of a transplant experiment in large-scale outdoor stream mesocosms to assess a third objective of verifying field observed diatom assemblage responses with experimental data. This coupling of field and experimental approaches can be applied as a framework for identifying assemblage responses to nutrient enrichment or other stream perturbations in other regions and using different taxonomic groups. Based on our previous work (Taylor et al., 2014), we hypothesized that benthic diatom assemblages would respond strongly to surface water P enrichment and exhibit consistent threshold responses to enrichment. Alternatively, we hypothesized that extreme hydrological conditions across seasons and between climatically distinct years would influence diatom assemblage structure and its response to enrichment.

2 | MATERIALS AND METHODS

2.1 | Field study design

Study sites were selected using a targeted approach to provide broad geographical coverage and a range of landscape features that drive nutrient enrichment (pasture and wastewater treatment plant [WWTP] effluent discharge), which resulted in a wide range of stream nutrient conditions (Appendix S1, Table S1 and Figure S2). We collected physical, chemical and periphyton data from 26 stream sampling locations within a region delineated by the intersection of the Brazos River basin and the Cross Timbers ecoregion in Texas (Figure S1, Figure 1) on a quarterly basis from July 2006 through June 2008 following American Public Health Association (1998) and Texas Commission on Environmental Quality (2005) methods. We collected triplicate surface water samples from the thalweg of each stream reach to determine ambient total and dissolved nutrient concentrations and collected reach-scale composite samples of epilithic periphyton for the analysis of diatom assemblage structure by removing material from the surface of at least 25 rocks for a composite periphyton slurry for laboratory subsampling (Taylor et al., 2014; See Appendix S1 for details).

2.2 | Mesocosm study experimental design

We conducted the stream mesocosm experiment in 12 outdoor flow-through stream mesocosms at the Baylor Experimental Aquatic Research (BEAR) facility in McLennan County, Texas, to test field observations (Figure 1a,b). Details of the design and set up of the experimental streams have been previously described (Taylor, Back, & King, 2012) and are also presented in Supporting information. We



FIGURE 1 (a) Baylor Experimental Aquatic Research (BEAR) stream facility; (b) Experimental mesocosm riffle; (c) Habitat at Rocky Creek

initiated experimental nutrient addition on 11 March 2008 using peristaltic pumps calibrated to deliver solutions of sodium nitrate (NaNO_3) and dibasic sodium phosphate (NaH_2PO_4) from 50 L carboys to mixing tanks before discharging water at concentrations indicative of background $\text{NO}_3\text{-N}$ ($300 \mu\text{g/L}$) in central Texas streams in

combination with experimentally manipulated control (background wetland water, 8 µg/L), low (20 µg/L) and high (100 µg/L) $\text{PO}_4\text{-P}$ concentrations into mesocosms. The lower nominal P concentration selected for the experiment (20 µg/L P) corresponded to the concentration associated with sharp, nonlinear changes in periphyton taxonomic composition, biomass and nutrient content observed in a previous study (Taylor et al., 2014). We selected the upper nominal concentration (100 µg/L P) to test the hypothesis that higher P concentrations would induce changes in diatom species composition, but after sufficient exposure to both enriched levels of P, diatom assemblages would not differ between the two treatments based on previous observed threshold assemblage responses to P enrichment (13). We replicated each P treatment in four streams. We were interested in how established diatom assemblages that had developed under different nutrient regimes would respond to P enrichment, so we transplanted 300 cobbles with established periphyton from Rocky Creek, Texas (TP, total phosphorus = 8.82 ± 1.41 µg/L; $\text{PO}_4\text{-P}$ = 6.2 ± 0.9 µg/L; Figure 1c) and 300 cobbles from the North Bosque River (TP = 125.71 ± 41.45 µg/L; $\text{PO}_4\text{-P}$ = 79.4 ± 38.1 µg/L) to experimental streams. Cobbles from both streams were placed in random cross-sections throughout the reach of each experimental stream a few days prior to initiation of nutrient treatment dosing. We dosed the streams continuously for 28 days, ending on 8 April 2008. We collected triplicate samples for dissolved nutrients from each mesocosm twice weekly and collected composite periphyton samples from each experimental stream mesocosm on day 0 and 28 by scraping periphyton from the upper surface of one rock randomly selected from four different riffle transects and compositing material (See Appendix S1 for details).

2.3 | Statistical analysis

Gradients of diatom species composition in relation to P enrichment and sample timing factors were identified with non-metric multidimensional scaling (nMDS) using $\log_{10}(x)$ -transformed abundance of diatom species and Bray–Curtis dissimilarity as the distance measure (McCune & Grace, 2002). We conducted three different nMDS analyses: (1) an ordination comparing two dates (August 2006, September 2007) that represented widely different hydrological conditions (drought, flood) where we had periphyton species composition data for all 26 sites; (2) an ordination of eight sampling events from six sites to assess how variability in species composition within sites over the study period may influence relationships with P enrichment; and (3) an ordination assessing the effects of experimental P enrichment on diatom assemblage structure succession on rocks transplanted from a low P (Rocky Creek) and a high P (North Bosque River) streams in the 28-day mesocosm study. Ordination of experimental data included field data collected before and after the experiment from our six intensive field sites to assess magnitude and direction of mesocosm changes in assemblage structure within the context of field species turnover driven by P enrichment. We performed nMDS and related analyses using the vegan package (Oksanen et al., 2010) in the open source statistical environment R

(R Development Core Team, 2017). We used the *ordisurf* function in the vegan package to build two-parameter generalized additive models (GAM) and plot 2-D contour lines on nMDS plots to visually assess nonlinear relationships between diatom assemblage structure and log transformed TP and Chl *a* data (Oksanen et al., 2010). We tested the statistical significance of TP and benthic Chl *a* biomass (which was identified by 2-D GAMs as an important secondary predictor), and their interaction on field diatom assemblage structure using multivariate GLMs (Wang, Naumann, Wright, & Warton, 2012). We fit all multivariate GLM models using the *manyglm* function with the negative binomial distribution and an offset ($\log(\text{area})$) in the *mvabund* package in R (Wang et al., 2012). We limited the multivariate GLM analysis to taxa with $\geq 20\%$ occurrence (Wang et al., 2012). Finally, we analysed the magnitude, direction and uncertainty of individual taxa and whole assemblage threshold responses to P-enrichment gradients for each year separately using Threshold Indicator Taxa Analysis (TITAN; Baker & King, 2010). We performed TITAN with the *TITAN2* package (Baker, King, & Kahle, 2015) in R using un-transformed taxa abundances (King & Baker, 2014) for taxa with ≥ 3 occurrences. All partitions required a minimum number of three observations on both sides.

3 | RESULTS

3.1 | Annual assemblage responses

Non-metric dimensional scaling ordination (2-D stress = 14.9) explained 84% of the variance in diatom assemblage data representing summer sampling events from two hydrologically distinct years and sorted streams along axis 1 according to increasing P enrichment (Figure 2a). Graphical overlays of TP combined with contour plots of predicted TP concentrations on the nMDS plot based on 2-D GAM surface smoothers ($R^2 = .58$, $p < .001$) indicated a nonlinear relationship with sites representing roughly half of species space within the nMDS plot predicted TP concentrations < 33 µg/L, whereas sites representing the other half represented a wide range of concentrations (Figure 2a). A second 2-D GAM surface smoother was orthogonal to TP contour lines and indicated that significant variation in ordination space on nMDS axis 2 was associated with differences in Chl *a* biomass between the 2 years ($R^2 = .68$, $p < .001$). The multivariate GLM interaction term was not significant (deviance test statistic = 59.2, $p = .100$) confirming that gradients in diatom assemblage structure associated with TP and annual differences in Chl *a* were independent. Diatom assemblage structure was influenced by TP concentrations (deviance test statistic = 150.1, $p = .001$) at stream sites in both years, despite significant relationships between diatom assemblage structure and Chl *a* biomass between years (deviance test statistic = 68.6, $p = .014$).

3.2 | Seasonal assemblage responses

Diatom assemblage structure corresponded to stream sampling events from eight seasons at six sites (80% of variance in original

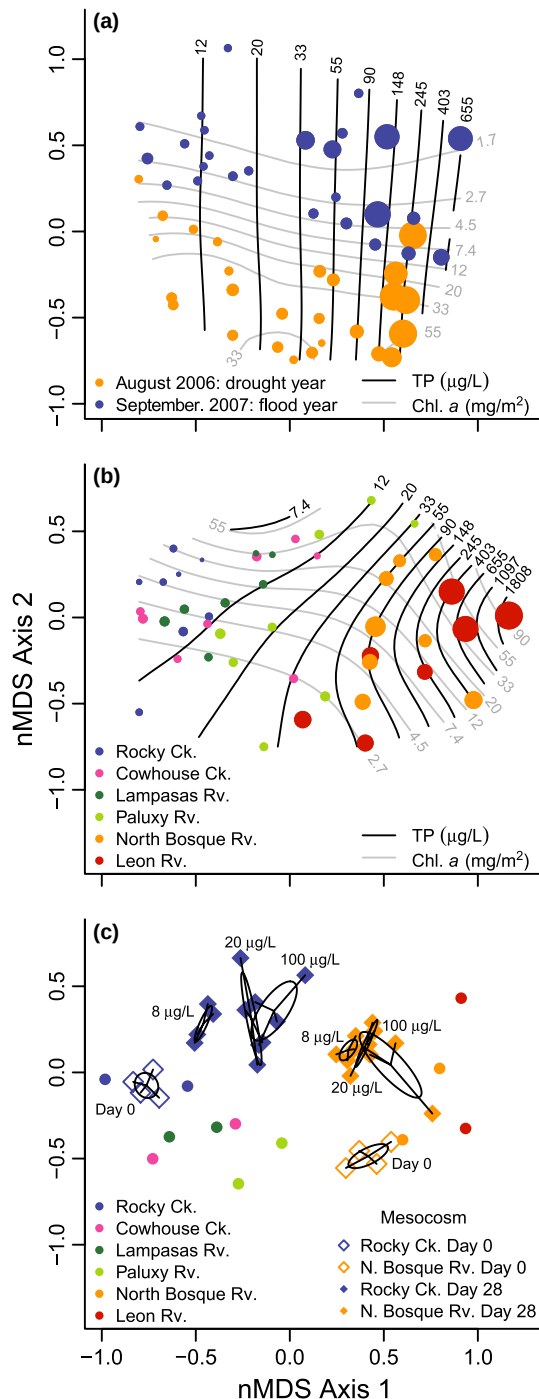


FIGURE 2 Diatom assemblage composition in relation to field and experimental total phosphorus (TP) gradients: (a) Non-metric multidimensional scaling (nMDS) ordination of summer diatom assemblage structure across 26 sites over two distinct hydrological years. Symbol shading indicates membership among 2006 (drought year) or 2007 (flood year); (b) Diatom assemblage structure at six intensive sites over eight seasons are plotted in nMDS; (c) Diatom assemblage data from ROCK-01 (low TP) and NBOS-03 (high TP) transplant rocks incubated across 3 PO₄-P treatments are plotted in nMDS with diatom assemblage data from six intensive field sites collected before and after mesocosm experiment. All ordinations are rotated on axis 1 to correlate with surface water TP. Trends between diatom assemblage structure and surface water TP (black) and benthic Chl *a* (grey) are represented by contour lines showing 2 dimensional generalized additive model (GAM) predictive surfaces in panels a and b. Site specific surface water TP concentrations (µg/L) are represented by size of circles in (a) and (b)

that was orthogonal for low P sites but increasingly became parallel to the strong TP gradient at high P sites (2-D GAM, $R^2 = .61$, $p < .001$). **This consistent sorting of sites along a strong P gradient was confirmed by multivariate GLM.** Diatom assemblage structure was strongly predicted by TP across combined seasonal sampling events (deviance statistic = 192, $p = .001$), with marginal evidence for the influence of Chl *a* on diatom assemblage structure changing with TP (TP × Chl *a*: Deviance statistic = 45.3, $p = .07$).

3.3 | Experimental assemblage responses

Species composition on Rocky Creek transplant rocks on day 0 was intermediate in ordination position between field samples collected at Rocky Creek from February 2008 (before mesocosm study) and June 2008 (after mesocosm study; Figure 2c). On day 28, all Rocky Creek transplant rock samples moved in the direction of sites with species composition indicative of higher TP concentrations within ordination space, but diatom assemblages from low (20 µg/L) and high (100 µg/L) P mesocosms shifted further than control (8 µg/L) streams on P induced species gradients (Figure 2c). In contrast, species composition on transplant rocks from the North Bosque River was very similar to field samples from February 2008 and diatom assemblages shifted during the 28-day experiment in the same direction as natural field species turnover trajectories (February to June). There was significant overlap between assemblage centroids between the three experimental P treatments, indicating that diatom species composition on North Bosque River transplanted rocks was not changing in response to P enrichment (Figure 2c). Comparison of multivariate GLMs confirmed a significant TP × Site interaction, indicating that the response of diatom species composition to experimental P enrichment varied with site origin for transplant rocks (deviance statistic = 191.7, $p < .001$). A separate multivariate GLM model examining the effect of experimental P enrichment on diatom species composition did not detect a significant difference in assemblage structure among North Bosque River rocks distributed across the three experimental treatments (deviance statistic = 144.8, $p = .176$). Differences in diatom assemblage structure among Rocky

distance matrix; 2-D stress = 18.1). Sites sorted primarily along nMDS axis 1 according to their TP concentrations and mimicked patterns of association identified in the 2 year × 26 site dataset (2-D GAM, $R^2 = .81$, $p < .001$; Figure 2b). Sampling events conducted at sites representing low to moderately enriched sites (<33 µg/L) occupied approximately half of the species space in the nMDS plot. In contrast, North Bosque and Leon River sampling events were primarily restricted to the other half of the ordination plot representing the area of species space predicted to be associated with a wide range of high TP concentrations (33–2,000 µg/L) at field sites. Chlorophyll *a* explained additional variation associated with seasonal differences

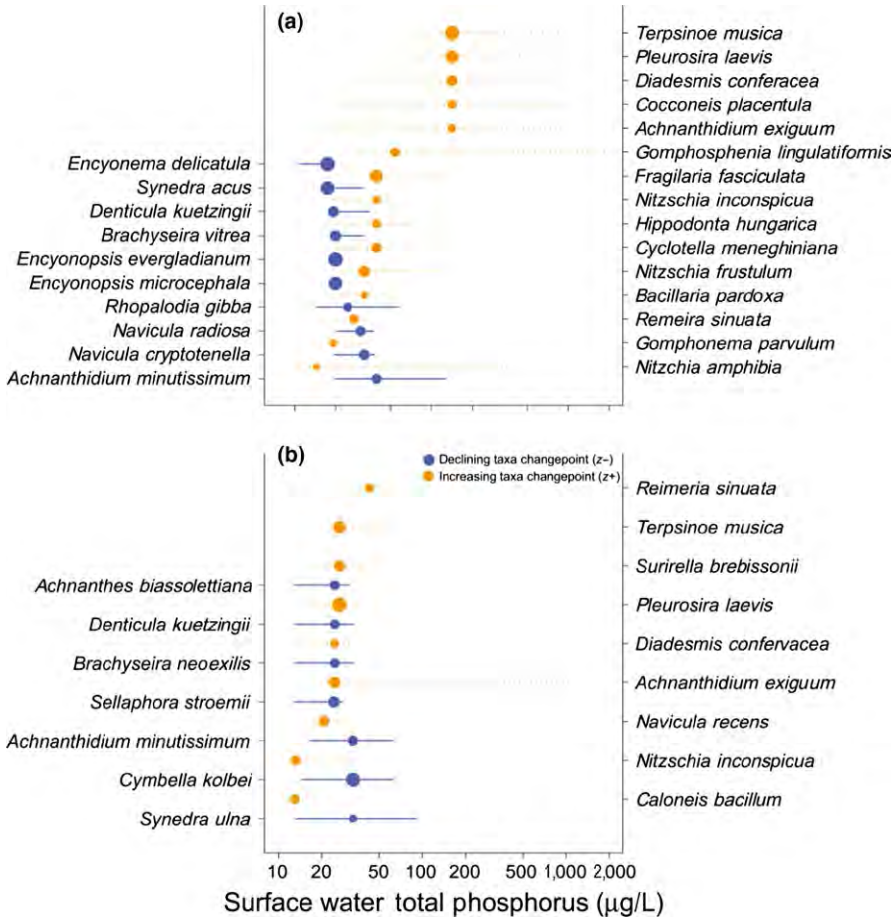


FIGURE 3 Significant diatom indicator taxa identified in threshold indicator taxa analysis (TITAN) across TP gradients spanning 26 sites sampled in (a) 2006 and (b) 2007. Significant (purity ≥ 0.95 , reliability ≥ 0.95 , $p < .05$) indicator taxa are plotted in increasing order with respect to observed change points. Blue symbols correspond to negative (z-) indicator taxa, whereas orange symbols correspond to positive (z+) taxa. Symbols are sized in proportion to magnitude of the response (z scores). Horizontal lines overlapping each symbol represent 5th and 95th percentiles among 1,000 bootstrap replicates

Creek rocks were significantly influenced by experimental P enrichment (deviance statistic = 187.7, $p = .031$).

3.4 | Observed species and assemblage level threshold responses in two hydrologically distinct years

Threshold Indicator Taxa Analysis cumulatively identified 15 diatom taxa that sharply declined in response to increasing TP with all observed negative change points occurring between 18 and 33 $\mu\text{g/L}$ (Figure 3). About 10 out of 110 taxa (9%) exhibited significant negative change points indicating threshold reductions in abundance in response to increasing TP concentrations during the low flow conditions of 2006 (Figure 3a). A distinct suite of 5 out of 73 taxa (7%) responded negatively to increasing TP concentrations in 2007 (Figure 3b). In contrast, positive change points were observed for 18 taxa when TP concentrations exceeded a wide range of concentrations from 12 to 142 $\mu\text{g/L}$ during the 2-year study (Figure 3). There was considerable overlap in significant increasing species between the 2 years despite differences in flow conditions. Six of these 18 taxa consistently increased in response to higher TP concentrations in both years (Figure 3). The range of TP resulting in sharp synchronous taxa declines was similar between 2006 (19–32 $\mu\text{g/L}$) and 2007 (13–37 $\mu\text{g/L}$) with maximum assemblage level changes occurring at relatively low TP concentrations for both years (2006 = 20 $\mu\text{g/L}$;

2007 = 25 $\mu\text{g/L}$; Table 1 and Appendix S1, Figure S3). Despite low numbers of individual species exhibiting negative (z-) change points, declining species made up a considerable proportion of total diatom abundance when surface water TP at field sites was below 20 $\mu\text{g/L}$ in 2006 (44%) and 25 $\mu\text{g/L}$ in 2007 (66%) but declined (<10%) at sites that exceeded observed thresholds in both years (Table 1). There was a more gradual shift in assemblage structure associated with taxa that increased in abundance with P enrichment in 2006. Increasing taxa (z+) responded to increasing TP at concentrations ranging from 29 to 142 $\mu\text{g/L}$ with a maximum aggregate taxa response observed at 40 $\mu\text{g/L}$. In contrast, positive responding taxa exhibited a nearly synchronous increase in abundance at much lower concentrations (13–37 $\mu\text{g/L}$, maximum change at 25 $\mu\text{g/L}$) in 2007 (Table 1). Significant increasing species made up a 58% and 54% of the total abundance of diatoms at sampling sites that exceeded observed assemblage thresholds for increasing species in 2006 and 2007 respectively (Table 1).

4 | DISCUSSION

Water quality practitioners increasingly recognize that algal assemblage response measures are sensitive to nutrient pollution in streams and should be incorporated in studies designed to establish regional criteria for nutrients in streams (United States

TABLE 1 Community-level thresholds to increasing stream total phosphorus concentrations ($\mu\text{g/L}$) for years representing contrasting drought (2006) and high flow (2007) conditions in central Texas streams. Threshold estimates were computed using Threshold Indicator Taxa Analysis (TITAN). Observed sum (z-) and (z+) thresholds represent the aggregate response of negative and positive indicator taxa (Figure 3) and correspond to the maximum sum(z-), whereas the confidence intervals represent the 5th and 95th percentiles (in parentheses) observed from the frequency distribution of thresholds of 1,000 bootstrap replicates. Sum(z-) and sum(z+) rel. abundance represent the per cent relative abundance of that subset of the assemblage that responded negatively or positively (± 1 SE) to increasing TP concentrations respectively

Year	TITAN sum(z-) changepoint	Mean z-taxa % rel. abund.		TITAN sum(z+) changepoint	Mean z+ taxa % rel. abund.	
		Left	Right		Left	Right
2006	19.9 (19.0–31.9)	0.44 $\pm$ 0.08	0.07 $\pm$ 0.03	39.7 (29.2–141.7)	0.15 $\pm$ 0.03	0.58 $\pm$ 0.07
2007	24.5 (13.0–37.4)	0.66 $\pm$ 0.04	0.09 $\pm$ 0.03	26.5 (13.1–36.4)	0.08 $\pm$ 0.03	0.54 $\pm$ 0.07

Environmental Protection Agency, 2014). Yet, considerable debate remains on how to efficiently design and analyse comprehensive datasets for identifying algal and other biological responses to nutrient stressor gradients (Chambers et al., 2012; Dodds, Clements, Gido, Hilderbrand, & King, 2010). In particular, single-stressor models that are commonly used to establish criteria have the significant shortcoming of potentially confounding interactions among nutrient enrichment and natural environmental variables or other stressors (Leps, Leisner, Haase, & Sundermann, 2016; Smucker et al., 2013; Wagenhoff, Clapcott, Lau, Lewis, & Young, 2017). However, study designs that adequately represent regional gradients in enrichment and potentially confounding factors can quickly become prohibitively expensive. The strength of our study design framework is that it addresses these limitations by including: (1) targeted site selection to represent a strong P enrichment gradient across sites within the same regional context; (2) repeated sampling that characterized temporal variation in overall environmental conditions, including dramatic changes in instream flows, that may confound effects of P enrichment on diatom assemblage structure at annual and seasonal time-scales; and (3) a large-scale outdoor mesocosm study that experimentally isolated the influence of P enrichment on diatom assemblage structure. **Our results demonstrate consistent shifts in diatom assemblage structure in response to low levels of P enrichment,** despite seasonal variation in stream conditions and major differences in hydrology that led to distinct differences in benthic algal biomass (Chl *a*) observed between summer seasons of two different years. Additionally, TITAN identified threshold changes in assemblage structure associated with similar surface water P concentrations (20 and 25 $\mu\text{g/L}$) in both years. We supported our observed threshold responses of diatom assemblage structure to P enrichment with stream mesocosm results that confirmed similar responses of diatom assemblages transplanted from a low P field site to an experimental P gradient representing concentrations below (8 $\mu\text{g/L}$), within (20 $\mu\text{g/L}$) and above (100 $\mu\text{g/L}$) the cumulative probability of negative threshold changes identified with field data.

A wide range of studies has established the utility of diatom-based metrics and species composition as a sensitive response measure to nutrient pollution in streams but few studies have addressed temporal variability of such relationships (Hering et al., 2006; Smucker et al., 2013; Stevenson et al., 2008; Taylor et al.,

2014). Multivariate GLM models supported our first hypothesis by demonstrating that diatom assemblage structure was significantly related to P enrichment despite significant differences in assemblage structure associated with changes in benthic algal biomass over both annual (between 2 years for 26 sites) and seasonal (over eight seasons for six sites) time scales. Additionally, TITAN indicated that diatom assemblage responses during these 2 years were driven by synchronous declines in abundance of several sensitive taxa that exhibited similar probabilities of an assemblage threshold response at low TP concentrations during the summer in 2006 (20 $\mu\text{g/L}$) and 2007 (25 $\mu\text{g/L}$), despite such pronounced hydrological differences. These responses correspond well with a previous study in central Texas streams, where we demonstrated that changes in algal assemblage structure were associated with **synchronous declines of several sensitive taxa at TP concentrations around 21 $\mu\text{g/L}$** (Taylor et al., 2014). Our observed threshold responses also correspond well with other regional studies from North America and Europe that reported significant changes in stream algal assemblage structure at TP concentrations ranging between 10 and 32 $\mu\text{g/L}$ (Chambers et al., 2012; Schneider & Lindström, 2011; Smucker et al., 2013).

Despite strong evidence for threshold responses of diatom assemblage structure to P enrichment over three different years (this study; Taylor et al., 2014), our results were based on a single-stressor model potentially confounded by interactions between nutrient enrichment and natural environmental variables or other stressors (Smucker et al., 2013; Wagenhoff, Clapcott, et al., 2017; Wagenhoff, Liess, et al., 2017). Stream classification based on local and regional ecohydrological context can reduce uncertainty associated with underlying natural gradients when assessing stream assemblage responses to single-stressor gradients (King, Baker, Kazyak, & Weller, 2011; King, Scoggins, & Porras, 2016). Our field study sites were located within a region representing the intersection of a single ecoregion (Cross Timbers) and river basin (Brazos River) to insure all streams had a similar watershed context and regional diatom species pool so as to avoid confounding underlying natural gradients (King et al., 2005). We also characterized the influence of temporal variation in overall environmental conditions on stressor-response relationships by testing for interactions between P enrichment and Chl *a* on diatom assemblage structure at annual and seasonal time-scales. A surprising finding of this study was that

despite significant seasonal shifts in diatom assemblage structure presumably associated with streamflow impacts on periphyton biomass and succession, overall consistent relationships between diatom species composition and P enrichment remained robust. Few studies have examined fine-scale temporal patterns in diatom species composition in relation to nutrient enrichment. Our results suggest that while there is significant seasonal diatom species turnover in streams, P enrichment remains an important driver of regional patterns in diatom assemblage structure.

Clear separation of control streams from systems enriched with $\geq 20 \mu\text{g/L}$ P in species space based on diatom assemblage structure provide additional experimental mesocosm evidence that confirm our observed threshold responses in the field. Interestingly, we only observed experimental P enrichment effects on diatom assemblage structure from rocks transplanted from our low P field site (Rocky Creek) but not our enriched site (North Bosque River). These novel experimental results suggest that oligotrophic streams rapidly respond to increased nutrient availability whereas eutrophic streams appear more resilient to short-term reductions in nutrient availability. Coupling of algal and heterotrophic production through efficient cycling of limiting nutrients in periphyton mats increases with reduced nutrient availability in streams across our region (Scott, Back, Taylor, & King, 2007). Luxury uptake of P in the field and potential increased rates of internal nutrient cycling during the 28-day transplant study could have met P requirements and maintained the diatom assemblage adapted to high P conditions in the North Bosque River despite low P availability in our experimental control streams. Longer term experimental studies are needed to better understand potential legacy effects of P and N enrichment on diatom assemblage structure in streams undergoing nutrient reductions.

4.1 | Implications for management

Developing nutrient criteria or standards that protect aquatic life in flowing waters is a difficult endeavour. Human populations continue to concentrate in urban areas, yet 80% of the global sewage production goes untreated before being discharged to surface waters. Fertilizer also continues to be an important tool used by agricultural producers to meet growing global food and fibre demands. Alternative criteria for assessing overall water quality and ecological health of rivers often rely on biotic indices rather than numerical values for contaminants (Hering et al., 2010). Given strong shifts in assemblage structure between years, our results highlight potential confounding factors if temporal variability is not accounted for when using biotic indices in lieu of numeric criteria given the predicted variable hydrological conditions under future climate scenarios. Our results demonstrate that significant threshold shifts in diatom assemblage structure occur based on declines in relatively few taxa and corresponding increases in species tolerant to P enrichment in streams associated with intensive agriculture and urban effluent gradients. These results were consistent across multiple years and seasons, and confirmed with experimental manipulations, demonstrating the utility of

coupling experimental outdoor mesocosm studies with repeated field sampling to identify assemblage level responses to nutrient enrichment in streams. We found that limiting surface-water TP concentrations to $<20 \mu\text{g/L}$ is necessary to ensure that natural diatom assemblages persist within regional stream networks. While this study focused on diatom assemblage structure as the environmental response, a similar approach could be used to identify additional ecological responses to nutrient enrichment. Identifying environmental changes based on additional measures representing different functional and trophic levels (Hering et al., 2006; Taylor et al., 2014; Wagenhoff, Clapcott, et al., 2017; Wagenhoff, Liess, et al., 2017) is important to understand the full context of ecological degradation associated with nutrient enrichment. Our ecological thresholds based on diatom assemblage response to TP likely represent the beginning of a gradient in declining ecological condition and therefore may represent criteria that indicate initiation of ecological degradation. More studies that include repeated sampling events, an experimental mesocosm component and focus on a range of ecological responses representative of stream ecosystems (benthic Chl *a*, macroinvertebrates, fish, leaf pack breakdown, etc.) are needed to fully understand stream ecosystem degradation associated with increasing eutrophication and to support development and implementation of nutrient standards that protect stream ecosystems.

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AUTHORS' CONTRIBUTIONS

R.S.K., B.W.B., J.M.T. and J.A.B. conceived ideas and designed methodology; J.M.T., J.A.B. and R.S.K. collected field and mesocosm data; R.S.K. led mesocosm study; J.M.T. and R.S.K. analysed the data; J.M.T. led the writing of the manuscript. All authors

contributed critically to the manuscript and gave final approval for publication.

DATA ACCESSIBILITY

Data available from the Dryad Digital Repository <https://doi.org/10.5061/dryad.2mn480b> (Taylor, Back, Brooks, & King, 2018).

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SUPPORTING INFORMATION

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