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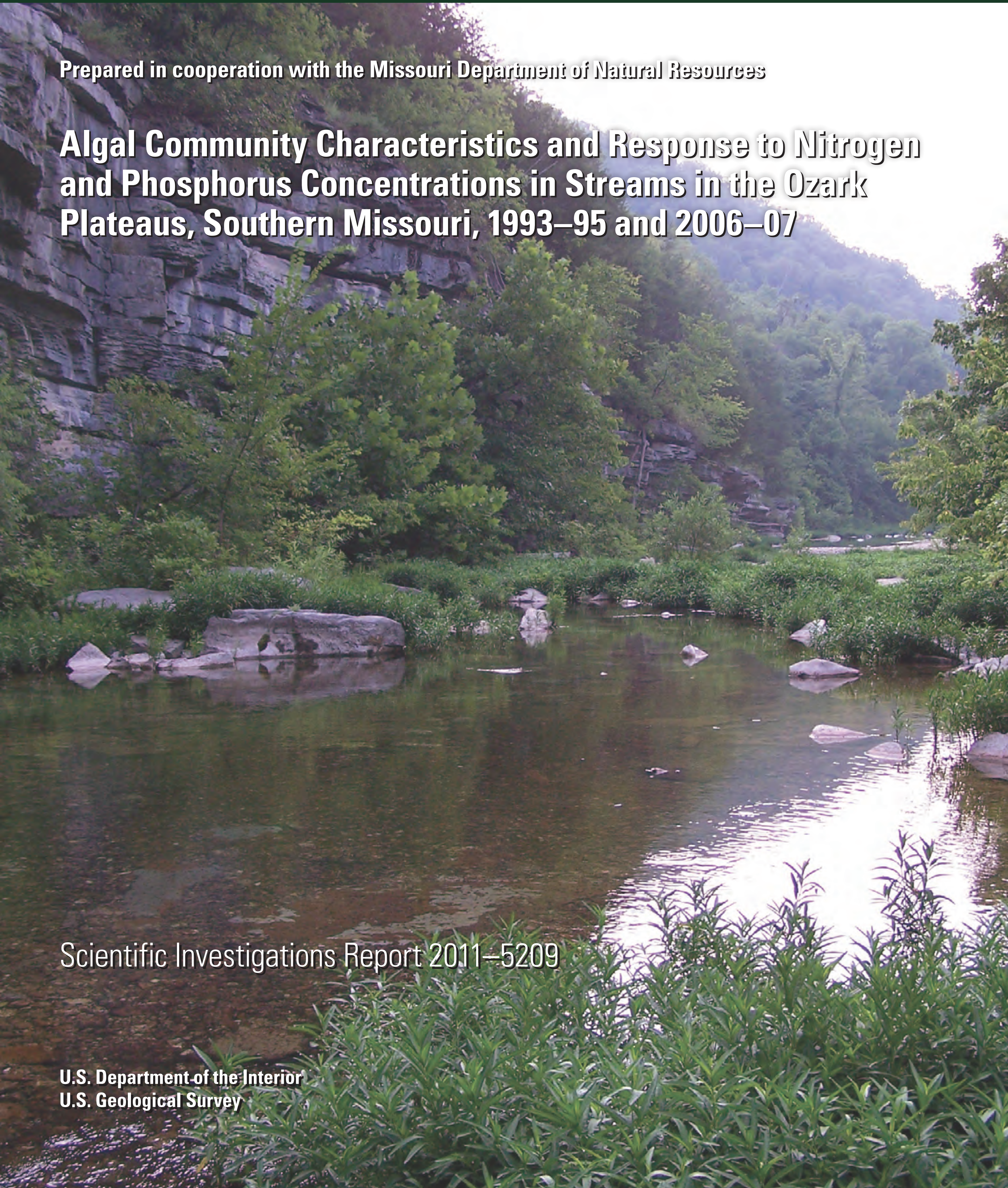

Prepared in cooperation with the Missouri Department of Natural Resources

Algal Community Characteristics and Response to Nitrogen and Phosphorus Concentrations in Streams in the Ozark Plateaus, Southern Missouri, 1993–95 and 2006–07

Scientific Investigations Report 2011–5209

U.S. Department of the Interior
U.S. Geological Survey

Cover photograph. Roasting Ear Creek near Newnata, Arkansas, 2006.

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U.S. Department of the Interior
KEN SALAZAR, Secretary

U.S. Geological Survey
Marcia K. McNutt, Director

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Conversion Factors

SI to Inch/Pound

Multiply	By	To obtain
	Length	
meter (m)	3.281	foot (ft)
meter (m)	1.094	yard (yd)
millimeter (mm)	.03937	inch (in)
micrometer (µm)	.001	millimeter (mm)
	Area	
square kilometer (km^2)	0.3861	square mile (mi^2)
	Volume	
liter (L)	33.82	ounce, fluid (fl. oz)
liter (L)	.2642	gallon (gal)
	Flow Rate	
milligrams per liter (mg/L)	1	parts per million (ppm)
	Mass	
milligram (mg)	0.001	gram (g)

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as follows:

$$°F=(1.8\times°C)+32$$

Concentrations of chemical constituents in water are given either in milligrams per liter (mg/L) or micrograms per liter (µg/L).

Algal Community Characteristics and Response to Nitrogen and Phosphorus Concentrations in Streams in the Ozark Plateaus, Southern Missouri, 1993–95 and 2006–07

By Suzanne R. Femmer

Abstract

Nutrient and algae data were collected in the 1990s and 2000s by the U.S. Geological Survey for the National Water-Quality Assessment program in the Ozark Highlands, southern Missouri. These data were collected at sites of differing drainage area, land use, nutrient concentrations, and physiography. All samples were collected at sites with a riffle/pool structure and cobble/gravel bed material. A total of 60 samples from 45 sites were available for analyses to determine relations between nutrient concentrations and algal community structure in this region. This information can be used by the Missouri Department of Natural Resources to develop the State's nutrient criteria plan. Water samples collected for this study had total nitrogen concentrations ranging from 0.07 to 4.41 milligram per liter (mg/L) with a median of 0.26 mg/L, and total phosphorus concentrations ranging from 0.003 to 0.78 mg/L with a median of 0.007 mg/L. These nutrient concentrations were transformed into nutrient categories consisting of varying percentiles of data. Algal community data were entered into the U.S. Geological Survey's Algae Data Analysis System for the computation of more than 250 metrics. These metrics were correlated with nutrient categories, and four metrics with the strongest relation with the nutrient data were selected. These metrics were Organic Nitrogen Tolerance, Oxygen Tolerance, Bahls Pollution Class, and the Saprobien index with the 25th and 80th percentile nutrient categories. These data indicate that near the 80th percentile (Total Nitrogen = 0.84 mg/L, Total Phosphorus = 0.035 mg/L) the algae communities significantly changed from nitrogen-fixing species dominance to those species more tolerant of eutrophic conditions.

Introduction

Eutrophication, or excess nutrient concentrations, in the Nation's waters have been a concern for many years. An important factor of eutrophication is the anthropogenic inputs of nitrogen and phosphorus. Elevated nitrogen and phosphorus concentrations have resulted in about 40 percent of the Nation's waters having designated use impairments because of nutrients (U.S. Environmental Protection Agency, 1996). In 1997, the U.S. Environmental Protection Agency (USEPA) initiated a Clean Water Action Plan (CWAP) to address the widespread nutrient enrichment situation in the Nation's waters (U.S. Environmental Protection Agency, 1998). Excess algae, especially blue-green (cyanobacteria), can produce harmful toxins that can kill fish, livestock, wildlife, and humans. Frequency of occurrence of these harmful algae has increased in recent years possibly because of increased nutrients from agricultural and other runoff entering the water bodies (Creekmore, 1999). Excess algae also can deplete dissolved oxygen in the water bodies by death and decay causing anoxic conditions, which can have deleterious effects on the biota present (Lembi, 2003).

The Missouri Department of Natural Resources (MDNR) is developing total nitrogen (TN) and total phosphorus (TP) criteria for streams of the State. Because of varied geology, land-use, and ecological regions across the State, the establishment of blanket criteria for the entire State is unlikely. For example, streams in the Ozark Highlands region (Ozarks) typically have smaller nutrient levels than other streams in the State, and even streams within the same Ecological Drainage Unit (EDU; Missouri Resource Assessment Partnership, 2001) may have differing background or baseline TN and TP concentrations. In addition, biologic responses to similar nutrient concentrations are anticipated to differ among and perhaps within various EDUs. An assessment of algal, macroinvertebrate, and fish communities in streams with low-level nutrients found that algal community structure correlated well with increasing nutrient levels (Justus and others, 2009). The use of algal community data to investigate nutrient conditions has been shown to be a successful method in many water-quality monitoring programs such as the U.S. Geological Survey (USGS) National Water-Quality Assessment (NAWQA; Gilliom and others, 1995) and the European Water Framework Directive (European Commission, 2000; Lavoie and others, 2004; Potapova and Charles, 2007; and Porter and others, 2008). Algal community composition attributes have been used as indicators of nutrient conditions and biological integrity (Mills and others, 1993; Pan and others, 1996, 2004; Stevenson and

Bahls, 1999; Hill and others, 2000; Fore and Grafe, 2002; Griffith and others, 2005; Wang and others, 2005). Porter and others (2008) determined that algal indicators of tolerance were positively correlated with nutrient concentrations and suggest that algal metrics might be preferable to using nutrient concentrations for assessing the trophic condition of streams in partially developed catchments.

Because of these findings and the fact that algal data were already available for the Ozarks in the southern Missouri area, the USGS, in cooperation with the Missouri Department of Natural Resources, selected algae for further study of relations between biota and nutrient concentrations in relation to community response.

Benthic algae are the primary producers and a critical component of most stream food webs. These organisms stabilize substrate and provide food and habitat for other aquatic organisms, especially macroinvertebrates. Because benthic algae are a primary food source of macroinvertebrates and some fish; changes in algal communities can affect macroinvertebrate and fish communities (Blinn and Herbst, 2003). Diatoms, a major group of algae, are common in most streams and are good indicators of the environmental integrity of the lotic ecosystem. Diatoms are sensitive to subtle changes or disturbances in the ecosystem that may not be noticeable in other aquatic communities or may only affect the other communities when the changes or disturbances are much larger (Blinn and Herbst, 2003; Dixit and others, 1992; Lowe and Pan, 1996; Stevenson and Pan, 1999). If the algae are being affected, there is probably a disturbance that has occurred or is occurring.

Purpose and Scope

The purpose of this report is to present and document the response of algal communities to different concentrations of nitrogen and phosphorus in streams in the Ozarks. The information in this report can be used to develop nutrient criteria for the State of Missouri to address the USEPA's CWAP. This report presents algal and nutrient data collected by the USGS for the NAWQA program in the Ozarks and describes the response of the algal communities to a gradient of nutrient concentrations.

Description of Study Area

The USGS began the NAWQA program in 1991with the goal to provide a nationally consistent description of the water quality of the Nation's waters. The NAWQA program began a study in 1991 in the Ozarks in northern Arkansas, southeastern Kansas, southern Missouri, and northeastern Oklahoma (fig. 1). The Ozarks are in the Ozark Plateau physiographic province (Fenneman, 1938) and correspond with the Ozark Highlands ecoregion (Omernik, 1987) (fig. 2). Karst features, such as caves, sinkholes, and springs, which are formed by the dissolution of carbonate rocks, are common in the Ozarks (Beveridge and Vineyard, 1990). Land use is primarily pasture and hay fields in the stream valleys with the uplands mostly forested. Poultry, beef, and swine are the primary livestock raised. More information describing the Ozarks can be found in Adamski and others, (1994).

Data Background

Streamflow, water chemical characteristics, biological community, and stream and riparian habitat data were collected for the Ozarks study from 1992 through 1995 at more than 50 sites. In 2005, the NAWQA program's "Effects of nutrient enrichment on stream ecosystems" study (NEET) selected the Ozarks as an area of interest. For the NEET effort, during 2006–07, streamflow, water chemistry, biological community, and stream and riparian habitat data were collected from 30 sites throughout the same region as the earlier NAWQA study in the Ozarks. The 1990s data were collected at sites of various drainage area (18 to 4,318 square kilometers), land use, nutrient concentrations, and physiography. The data from the 2000s were collected at sites on small streams (less than 500 square kilometers), with agricultural and forest land use, which resulted in a gradient of nutrient concentrations (table 1). The nutrient and algal community data from both of these studies were used for this report.

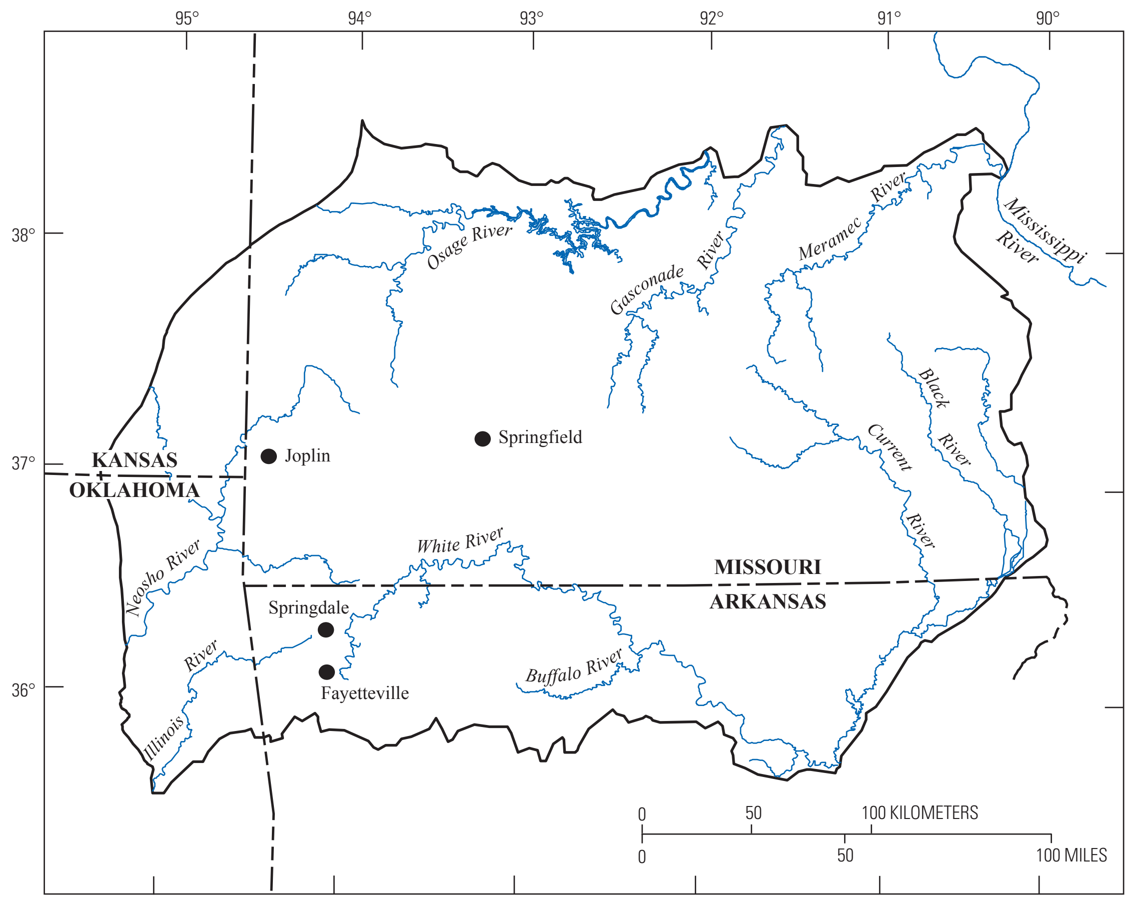
Methods of Study

Nutrient and algae samples used for this study were collected from September 1993 to September 1995 and from July 2006 through November 2007 in the Ozarks (fig. 2). Sampling reaches were established at each site for biological sampling that were a minimum of 150 meters (m) in length and contained a minimum of 2 riffle/pool sequences. Only samples collected during July through November were used for analysis because of life history attributes of the algal community, hydrological conditions, and availability of multiple years of data. This dataset has 60 samples from 45 sites (table 1). All nutrient and algae samples were collected and processed according to protocols developed by the USGS (Moulton and others, 2002, and U.S. Geological Survey, variously dated). All data are stored and available for download at the NAWQA data warehouse (*http://infotrek.er.usgs.gov/apex/f?p=NAWQA:HOME*:0*)*.

Data Collection Protocols

Nutrient Sample Collection and Analysis

At each site, depth- and width-integrated water samples were collected for nutrient analysis [including dissolved nitrate plus nitrite, ammonia, and orthophosphorus, and total Kjeldahl nitrogen (sum of organic and ammonia nitrogen) and



Base from U.S. Geological Survey digital data, 1:100,000, 1983 and 1986
Universe Transverse Mercator projection
Zone 15

Figure 1. The NAWQA Ozark study unit in Arkansas, Kansas, Missouri, and Oklahoma.

phosphorus] from a cross section located in the sampling reach using the equal-width-increment (EWI) method (U.S. Geological Survey, 2006). The EWI method is the collection of samples at evenly spaced distances across the sampling cross section. Samples collected by the EWI method were composited in a 3-liter (L) bottle. Subsamples for the analysis of dissolved (filtered through a 0.45-micrometer pore-size plate or capsule filter) and total nutrients were put in the appropriate containers for preservation and shipping. The nutrient samples were kept on ice and sent to the USGS National Water-Quality Laboratory (NWQL) in Denver, Colorado, for analysis. Samples were analyzed by the laboratory using methods documented in Patton and Truitt (1992, 2000) and Fishman, (1993).

Algal Community Sample Collection and Analysis

Algal community samples were collected from the richest targeted habitat (RTH) present in the sampling reach at each site using sampling protocols documented in Moulton and others (2002). Ozark streams are characterized by riffle/pool sequences and cobble/gravel substrate (Brown and others, 1998; Brussock and Brown, 1991) and the RTH is the riffle (Moulton and others, 2002). All sites sampled for the Ozarks NAWQA were streams of this type. At each site, 5 rocks were randomly selected near 5 randomly selected locations at a minimum of 2 riffles for a total of 25 rocks. Each rock had algae removed from a quantified area, which was composited into one container. Each sample was then preserved with formaldehyde to a 3- to 5-percent solution and shipped to the Academy of Natural Sciences, Philadelphia (ANSP) Phycology Section in Philadelphia, Pennsylvania, for analysis. Each sample was analyzed in the laboratory in accordance with methods documented in Charles and others (2002).

The algae samples collected for the 2006–07 sampling effort were field processed slightly different from those collected for the 1993–95 effort. During 1993–95, a cylinder of a known diameter was held against the selected rock while the algae in the area inside the cylinder was scraped and removed by suction into a polypropylene bottle where all 25 subsamples were composited. Care was given to limit any loss of algae because of a poor seal between the cylinder and the rock. During the 2006–07 sampling effort, a cylinder of a known

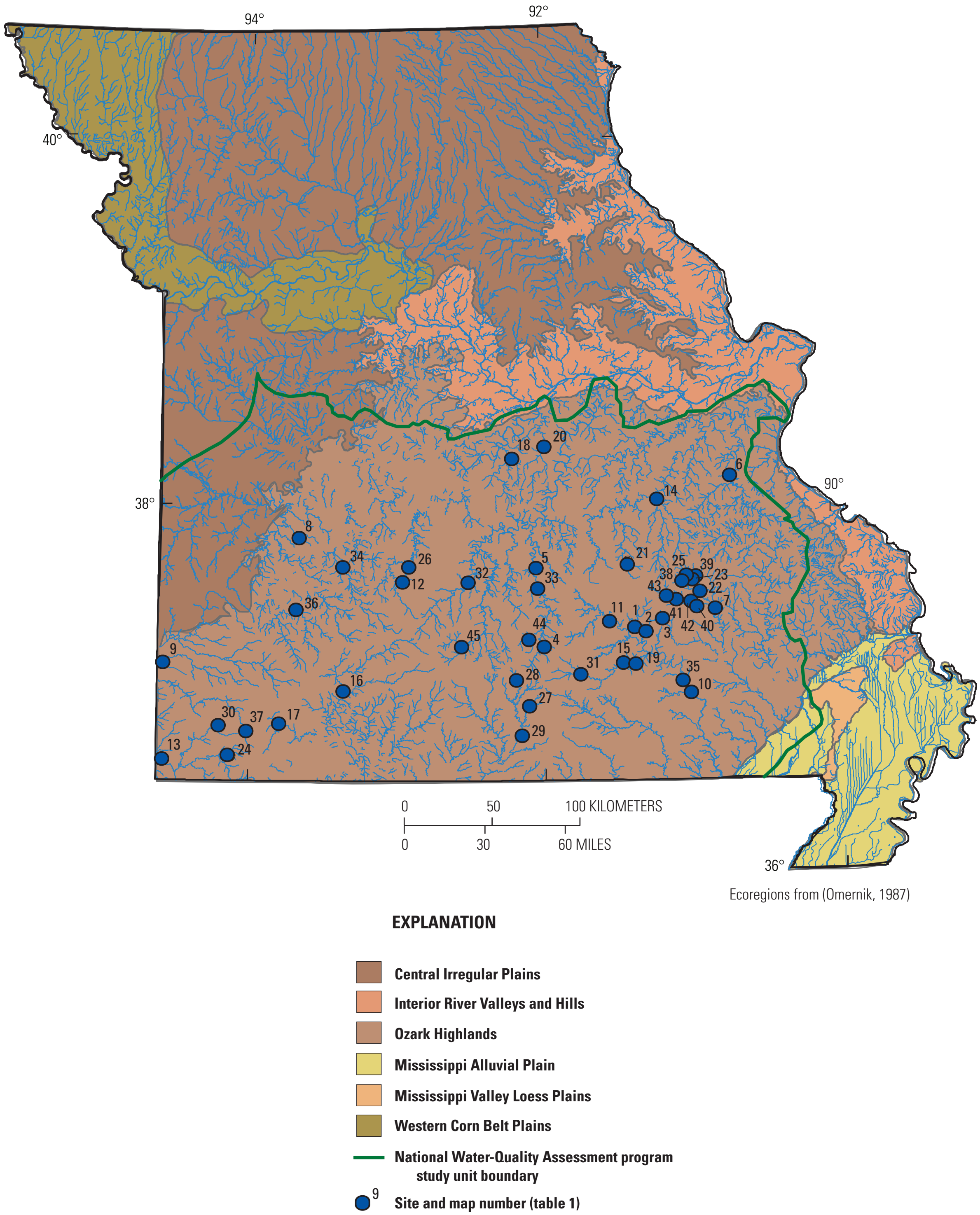


Figure 2. Location of sites where algae samples were collected as part of the National Water-Quality Assessment program.

Table 1. Sites used for this study with ancillary data.

[no., number; km², square kilometer; mg/L, milligram per liter; EDU, ecological drainage unit; .18[c], calculated value; <, less than; Hwy., highway]

Map no.	Site name	U.S. Geological Survey station number	Latitude	Longitude	Drainage area (km²)	Sample date	Total nitrogen (mg/L)	Total phosphorus (mg/L)	EDU	Major basin landuse
1	Barren Fork near Timber, Mo.	07064780	37.346	-91.390	132	8/3/2006	0.07	0.005	Ozark/Black/ Current	Forest
2	Big Creek at Mauser Mill, Mo.	07065040	37.313	-91.316	108	9/22/1994	.18[c]	<.0100	Ozark/Black/ Current	Forest
2	Big Creek at Mauser Mill, Mo.	07065040	37.313	-91.316	108	8/2/2006	.14	.003	Ozark/Black/ Current	Forest
2	Big Creek at Mauser Mill, Mo.	07065040	37.313	-91.316	108	7/18/2007	.12	<.008	Ozark/Black/ Current	Forest
2	Big Creek at Mauser Mill, Mo.	07065040	37.313	-91.316	108	10/30/2007	.15	<.008	Ozark/Black/ Current	Forest
3	Big Creek near Rat, Mo.	07065020	37.389	-91.190	18	9/11/1995	.40[c]	<.010	Ozark/Black/ Current	Forest
4	Big Piney River at Simmons, Mo.	06928730	37.241	-92.009	276	7/31/2006	.25	.024	Ozark/ Gasconade	Mixed
5	Big Piney River near Big Piney, Mo.	06930000	37.666	-92.050	1,427	9/13/1994	.45[c]	.010	Ozark/ Gasconade	Forest
6	Big River near Richwoods, Mo.	07018100	38.159	-90.706	1,904	8/24/1994	.15[c]	<.010	Ozark/Meramec	Mining/ Forest
7	Black River near Lesterville, Mo.	07061400	37.440	-90.832	1,242	9/23/1993	.30[c]	<.010	Ozark/Black/ Current	Forest/ Mining
7	Black River near Lesterville, Mo.	07061400	37.440	-90.832	1,242	8/25/1994	.14[c]	<.010	Ozark/Black/ Current	Forest/ Mining
7	Black River near Lesterville, Mo.	07061400	37.440	-90.832	1,242	8/22/1995	.22[c]	<.010	Ozark/Black/ Current	Forest/ Mining
8	Brush Creek above Collins, Mo.	06919925	37.835	-93.673	143	9/21/1994	.13[c]	<.010	Ozark/Osage	Agriculture
9	Center Creek near Smithfield, Mo.	07186480	37.155	-94.603	761	9/21/1993	3.08	.220	Ozark/Neosho	Mining/ Urban/ Agriculture
9	Center Creek near Smithfield, Mo.	07186480	37.155	-94.603	761	8/29/1995	4.41[c]	.060	Ozark/Neosho	Mining/ Urban/ Agriculture
10	Current River at Van Buren, Mo.	07067000	36.991	-91.014	4,318	8/21/1995	.39[c]	.030	Ozark/Black/ Current	Forest
11	Current River below Akers, Mo.	07064535	37.376	-91.548	761	9/19/1994	.61c	<.010	Ozark/Black/ Current	Forest
12	Dousinbury Creek on Hwy. JJ near Wall Street, Mo.	06923150	37.594	-92.966	106	9/1/1994	.80[c]	.035	Ozark/Osage	Agriculture
12	Dousinbury Creek on Hwy. JJ near Wall Street, Mo.	06923150	37.594	-92.966	106	8/23/1995	.55[c]	.020	Ozark/Osage	Agriculture
13	Elk River near Tiff City, Mo.	07189000	36.631	-94.587	2,258	9/1/1994	.76[c]	.060	Ozark/Neosho	Agriculture
14	Huzzah Creek near Scotia, Mo.	07014300	38.028	-91.213	1,258	8/23/1994	.17[c]	<.010	Ozark/Meramec	Forest
15	Jacks Fork River at Alley Spring, Mo.	07065495	37.144	-91.457	790	9/20/1994	.17[c]	<.010	Ozark/Black/ Current	Forest
16	James River near Boaz, Mo.	07052250	37.006	-93.364	1,202	9/8/1994	3.73[c]	.780	Ozark/White	Urban/ Agriculture
17	Little Flat Creek near McDowell, Mo.	07052790	36.821	-93.794	115	8/3/2006	2.51	.031	Ozark/White	Agriculture
18	Little Tavern Creek near St. Elizabeth, Mo.	06926250	38.268	-92.214	124	8/31/1994	.25[c]	.030	Ozark/Osage	Agriculture

Table 1. Sites used for this study with ancillary data.—Continued

[no., number; km², square kilometer; mg/L, milligram per liter; EDU, ecological drainage unit; .18[c], calculated value; <, less than; Hwy., highway]

Map no.	Site name	U.S. Geological Survey station number	Latitude	Longitude	Drainage area (km²)	Sample date	Total nitrogen (mg/L)	Total phosphorus (mg/L)	EDU	Major basin landuse
19	Mahans Creek above Eminence, Mo.	07065950	37.147	-91.378	140	8/1/2006	0.26	0.009	Ozark/Black/Current	Forest
20	Maries River near Freeburg, Mo.	06926900	38.333	-91.992	482	8/30/1994	.15[c]	.020	Ozark/Osage	Mixed
20	Maries River near Freeburg, Mo.	06926900	38.333	-91.992	482	8/10/2006	.56	.035	Ozark/Osage	Mixed
21	Meramec River above Cook Station, Mo.	07010335	37.688	-91.425	243	7/27/2006	.10	.004	Ozark/Meramec	Forest
21	Meramec River above Cook Station, Mo.	07010335	37.688	-91.425	243	7/19/2007	.14	.006	Ozark/Meramec	Forest
21	Meramec River above Cook Station, Mo.	07010335	37.688	-91.425	243	10/29/2007	.10	.008	Ozark/Meramec	Forest
22	Middle Fork Black River at Black, Mo.	07061163	37.530	-90.935	284	9/14/1995	.21[c]	<.010	Ozark/Black/Current	Mining/Forest
23	Middle Fork Black River at Redmondville, Mo.	07061152	37.616	-90.966	58	9/14/1995	.18[c]	<.010	Ozark/Black/Current	Forest
24	Mikes Creek at Powell, Mo.	07188660	36.626	-94.181	163.6	8/30/1994	.67[c]	<.010	Ozark/Neosho	Forest
25	Neals Creek near Goodland, Mo.	07061161	37.618	-91.026	43	9/19/1995	.18[c]	<.010	Ozark/Black/Current	Mining/Forest
26	Niangua River at Windyville, Mo.	06923250	37.684	-92.924	875	9/7/1994	1.20[c]	.270	Ozark/Osage	Agriculture
27	Noblett Creek near Willow Springs, Mo.	07057420	36.921	-92.096	53	9/7/1994	.21[c]	<.010	Ozark/White	Forest
28	North Fork White River near Cabool, Mo.	07057280	37.054	-92.187	50	7/25/2006	.23	.007	Ozark/White	Forest
29	North Fork White River near Dora, Mo.	07057470	36.760	-92.153	1,046	9/8/1994	.38[c]	<.010	Ozark/White	Forest
30	North Indian Creek near Wanda, Mo.	07188855	36.811	-94.210	113	8/31/1994	3.83[c]	.030	Ozark/Neosho	Agriculture
30	North Indian Creek near Wanda, Mo.	07188855	36.811	-94.210	113	7/27/2006	4.30	.052	Ozark/Neosho	Agriculture
31	North Prong Jacks Fork below Arroll, Mo.	07065160	37.086	-91.750	145	7/20/2006	.22	.007	Ozark/Black/Current	Forest
32	Osage Fork near Russ, Mo.	06927780	37.588	-92.515	909	9/14/1994	.44[c]	.030	Ozark/Gasconade	Agriculture
33	Paddy Creek above Slabtown Spring, Mo.	06929315	37.558	-92.048	79	9/12/1994	.13[c]	<.010	Ozark/Gasconade	Forest
33	Paddy Creek above Slabtown Spring, Mo.	06929315	37.558	-92.048	79	8/24/1995	.13[c]	<.010	Ozark/Gasconade	Forest
34	Pomme de Terre near Polk, Mo.	06921070	37.683	-93.370	715	9/21/1994	.18[c]	.040	Ozark/Osage	Agriculture
35	Rogers Creek near Van Buren, Mo.	07066650	37.049	-91.071	46.4	9/21/1994	.19[c]	.010	Ozark/Black/Current	Forest
36	Sac River near Dadeville, Mo.	06918440	37.443	-93.685	666	9/20/1994	1.31[c]	.050	Ozark/Osage	Agriculture
37	Shoal Creek near Wheaton, Mo.	07186670	36.776	-94.024	112	7/26/2006	2.02	.065	Ozark/Neosho	Agriculture
37	Shoal Creek near Wheaton, Mo.	07186670	36.776	-94.024	112	7/23/2007	3.84	.058	Ozark/Neosho	Agriculture
37	Shoal Creek near Wheaton, Mo.	07186670	36.776	-94.024	112	11/1/2007	3.84	.045	Ozark/Neosho	Agriculture
38	Strother Creek near Oates, Mo.	07061155	37.590	-91.054	23	9/20/1995	.98[c]	.020	Ozark/Black/Current	Mining/Forest

Table 1. Sites used for this study with ancillary data.—Continued

[no., number; km², square kilometer; mg/L, milligram per liter; EDU, ecological drainage unit; .18[c], calculated value; <, less than; Hwy., highway]

Map no.	Site name	U.S. Geological Survey station number	Latitude	Longitude	Drainage area (km²)	Sample date	Total nitrogen (mg/L)	Total phosphorus (mg/L)	EDU	Major basin landuse
39	Strother Creek near Redmondville, Mo.	07061162	37.603	-90.992	94	9/18/1995	0.45[c]	<0.010	Ozark/Black/Current	Mining/Forest
40	West Fork Black River at Centerville, Mo.	07061150	37.445	-90.962	356	9/13/1995	.19[c]	.020	Ozark/Black/Current	Mining/Forest
41	West Fork Black River at West Fork, Mo.	07061135	37.491	-91.099	187	9/12/1995	.40[c]	<.010	Ozark/Black/Current	Mining/Forest
42	West Fork Black River near Centerville, Mo.	07061138	37.478	-91.005	237	9/13/1995	.20[c]	<.010	Ozark/Black/Current	Mining/Forest
43	West Fork Black River near Greeley, Mo.	07061125	37.506	-91.161	107	9/12/1995	.13[c]	<.010	Ozark/Black/Current	Forest
44	West Piney Creek near Bado, Mo.	06928800	37.281	-92.104	72	7/26/2006	.33	.015	Ozark/Gasconade	Mixed
45	Woods Fork near Hartville, Mo.	06927590	37.245	-92.567	118	9/7/1994	.26[c]	.020	Ozark/Gasconade	Agriculture
45	Woods Fork near Hartville, Mo.	06927590	37.245	-92.567	118	7/24/2006	.27	.035	Ozark/Gasconade	Agriculture

diameter was held against the selected rock and the algae in the area outside of the cylinder was brushed and rinsed off. After the rock outside of the cylinder was free of algae, the cylinder was removed and the area of algae that remained was then scraped and rinsed into a polypropylene bottle where all 25 subsamples were composited. Although this change in procedure appears to be minor, there was less likelihood of losing algae from the known area of sampling during processing with the second (2006–07) method than with the first one. There also may have been changes in naming conventions by the analyzing laboratory between the two sampling efforts.

Data Analysis

Total Nitrogen and Total Phosphorus

For this study, the nutrient parameters of total nitrogen (TN) and total phosphorus (TP) were used. For censored data, or data that were reported as less than the laboratory reporting level, one-half of the reporting level [for example, less than (<) 0.2 becomes 0.1] was used. The substitution method (one-half of the reporting level) was used for this study after comparing alternative censor handling scenarios of Kaplan-Meier, substitution, and Regression Order Statistics (ROS) methods (Helsel, 2005) and finding that for this dataset, using the substitution method was appropriate. Where TN concentrations were missing or not directly determined, TN was calculated by adding Kjeldahl nitrogen and nitrate plus nitrite as nitrogen ($KJN + NO_2 + NO_3$). All TN and TP concentrations collected at a site within 1 month before the algae sampling, one to three samples, were averaged for a single value for analysis. Percentile categories were calculated for the nutrient dataset. These percentile categories were used in correlation and similarity calculations, discussed in the next section this report.

Algal Community and Metric Methods of Analysis

Algae were enumerated and identified to the species level (or to the nearest accurate taxonomic level) by the ANSP laboratory. These data were entered into the NAWQA database. Algal data were retrieved from the NAWQA data warehouse in a spreadsheet format. The algal community data were transformed into two forms: relative abundance and calculated metrics.

Algal Community Analysis

The relative abundance data were analyzed in the PRIMER statistical program (Clarke and Warwick, 2001). Relative abundance is the abundance of a species divided by the total abundance of all the species in the sample combined. The abundance data were square-root transformed with Bray-Curtis resemblance matrix calculations. The resemblance data were then analyzed with nonmetric Multidimensional Scaling (MDS), Analysis of Similarity (ANOSIM), BEST, and SIMPER, which are all components of the PRIMER program. MDS is an ordination technique used to explore similarities and dissimilarities in data and used to present this information in visual form, either 2-dimensional or 3-dimensional. Distances among the samples are calculated, usually with a

Euclidean metric, then these distances are regressed against the original distance matrix, and the predicted ordination distances for each pair of samples are calculated. These distances are then improved by moving the positions of the samples in ordination space by small amounts into the direction in which stress (a measure of the relation between the similarity in species composition and the closeness in ordination space) changes most rapidly. Then the ordination distance matrix is recalculated, the regression performed again, and the stress recalculated. This process is repeated until the optimum value of stress is achieved. A recalculation of 99 times was used for this dataset. On graphs showing MDS results, similar data points are located near each other, whereas those that are more dissimilar are farther apart. ANOSIM calculates differences between groups of community data using a permutation/randomization method on a resemblance matrix. Results are presented as Global R values and significance levels (*p*-values). Global R values are 0 for total dissimilarity and 1 for complete similarity. A 95-percent confidence level was used for these analyses (p=.05 or less). The BEST analysis selects environmental variables (such as total nitrogen or water temperature) or species that best explain the community pattern by maximizing a rank correlation between their respective resemblance matrices. The SIMPER analysis determines the contributions to the average similarity within a group and the dissimilarity between pairs of groups of samples. The SIMPER analysis identifies the species that are contributing to the differences between populations. More information on these statistical programs can be found in Clarke and Warwick (2001).

Algal Metric Analysis

Metrics were calculated from the algal community data using the Algae Data Analysis System (ADAS), a USGS developed software, which calculates more than 250 metrics (Cuffney, T.F., 2010, written commun.). These metrics are based on taxa richness, abundance, dominance, tolerance, and trophic status. The metric data then were analyzed further using PRIMER and SYSTAT13 (SYSTAT13 Software Inc, 2009). In the PRIMER program, the MDS, ANOSIM, and RELATE statistics were used. The RELATE program tests the hypothesis that no relation exists between the multivariate pattern for two sets of samples, in this case, nutrients and algal metrics. In SYSTAT13, Analysis of Variance (ANOVA) and Tukey's pairwise comparisons were used on the metric data. The ANOVA program provides a statistical test of the means of several groups to see if they are all equal. Where ANOVA indicated a significant difference between groups, a Tukey's test was performed to test all pairwise combinations to determine the statistical significance of the difference.

Nitrogen and Phosphorus Concentrations

Concentrations of TN and TP in samples that were collected in conjunction with the algae samples (table 1) were grouped into categories based on percentiles of the dataset. These data were grouped in order to determine inflection points in the relation between nutrients and algal communities. The 10th, 20th, 25th, 30th, 50th (median), 70th, 75th, 80th, and 90th percentiles were calculated for TN and TP concentrations (table 2). Total nitrogen concentrations ranged from 0.07 to 4.41 milligrams per liter (mg/L) with a median of 0.26 mg/L. Total phosphorus concentrations ranged from 0.003 to 0.780 mg/L with a median of 0.007 mg/L (table 2). For further analyses, these data were grouped by percentiles into categories; lower percentiles (10th, 20th, and 25th percentile), middle percentiles (30th, 50th, and 70th) to the upper percentiles (75th , 80th, and 90th), and upper percentiles (greater than 75th, 80th, or 90th). Through further analysis, it was determined that the concentrations in the 25th, 75th, and 80th percentiles had the strongest correlation with the algae data.

Algal Community Characteristics and Responses to Nitrogen and Phosphorus Concentrations

Algal Community Characteristics

The initial MDS analysis of the algal community data resulted in two distinct clusters—samples collected during the 1990s (1993–95) and those collected during the 2000s (2006–07). Sampling techniques that were changed slightly between the two sampling efforts or changes in taxonomic reporting may be the reason the data plot differently. To avoid error introduced by sampling or laboratory differences, the 1993–95 and 2006–07 community abundance data were

Table 2. Selected percentiles of total nitrogen and total phosphorus concentrations.

[mg/L, milligram per liter]

Parameter	Number of samples	Minimum	Maximum	10th	20th	25th	30th	50th	70th	75th	80th	90th
Total nitrogen (mg/L)	60	0.07	4.41	0.13	0.14	0.17	0.18	0.26	0.55	0.62	0.84	3.14
Total phosphorus (mg/L)	60	.003	.780	.005	.005	.005	.005	.007	.030	.030	.035	.052

analyzed independently. When this dataset was analyzed by each sampling effort, the two had similar results in that there was a significant difference in community structure between the sites with smaller nutrient concentrations and the sites with larger nutrient concentrations. Analyzing the two groups separately in relation to nutrient categories (less than 25th, 25–50th, 50–75th, greater than 75th) resulted in a significant difference (p=0.00) between the less than 25th and the greater than 75th categories. Although there were significant differences found with the community data, the problems noted made this method suboptimal for further analyses.

Algal Metric Characteristics

More than 250 algal metrics were calculated from the algal community data by the ADAS program. Definition and descriptions of these metrics can be found in Porter (2008). Using these metrics, no distinctions between sampling periods (1990s and 2000s) were determined. These metrics were based on type of species (for example, by functional feeding group, tolerances to different variables) and were calculated by percent of sample population instead of only on species taxonomy as in the community analysis. The dataset used for metric analyses included all of the algae samples together. Using ANOSIM, the four metrics with the greatest correlation to the nutrient categories were selected. All four metrics are tolerance metrics using percent richness of diatoms. These metrics are: Organic Nitrogen Tolerance (ON), Oxygen Tolerance (OT), Bahls Pollution Class (PC), and the Saprobien index (SP). The ON metric classifies diatoms with tolerance for low inorganic nitrogen, high tolerance for inorganic nitrogen, facultative organic nitrogen, or obligate organic nitrogen. The OT metric classifies diatoms as requiring high dissolved oxygen, fairly high dissolved oxygen, moderately high dissolved oxygen, tolerant of low dissolved oxygen, and tolerant of very low dissolved oxygen. The PC metric classifies diatoms as most tolerant, less tolerant, and sensitive. The SP metric classifies diatoms as oligosaprobous [those that need high oxygen saturation (greater than 85 percent) and very slight dissolved organic matter] , b-mesosaprobous (those that live in 70–85 percent oxygen saturation and low levels of dissolved organic matter), a-mesosaprobous (those that live in 25–70 percent oxygen saturation and moderate levels of dissolved organic matter), a-meso/polysaprobous (those that tolerate 10–25 percent dissolved oxygen saturation and elevated levels of dissolved organic matter), or polysaprobous [those that tolerate low dissolved oxygen (less than 10 percent saturation) and high levels of dissolved organic matter].

Algal Metrics Response to Nitrogen and Phosphorus Concentrations

MDS analyses of the algal metrics indicate a change in community structure as TN and TP levels increase. Many combinations of percentile categories were analyzed for this effort, by quartiles and by every 10th percentile. ANOSIM analyses indicate that the most substantial differences between communities, as indicated by higher Global R values, were between the 25th and 80th percentile categories for both TN and TP (table 3). There also were significant differences between the 25th/75th and 25th/90th percentile categories of TN or TP values (table 3). There were no significant differences in results in using either the 10th, 20th, or 25th percentiles in relation to the upper percentiles; therefore, the 25th percentile was used as the lower percentile category and assumed to represent the baseline or “natural” condition throughout the rest of the analyses. The baseline or “natural” condition in these streams tend toward algal species that are nitrogen fixing and sensitive to high concentrations of nutrients, or organic materials, or low dissolved oxygen, or a combination of all three. Those species that are known to be tolerant of high(er) concentrations of nutrient and organic materials, and low dissolved oxygen were found in communities that had nutrient concentrations that fell into the greater than 75th percentile category (especially the greater than 80th percentile category).

MDS plots of TN and TP categories with the four selected algal metrics consistently indicated strong distinction between algal communities at both the 75th and 80th percentile category (figs. 3–7) and not as strong at the 90th percentile category in relation to the 25th percentile category

Table 3. Analysis of similarity (ANOSIM) Global R values of selected total nitrogen and total phosphorus percentiles compared to selected algal metrics.

[all p-values less than 0.05]

Metric	Total nitrogen 25th compared to 75th percentile	Total nitrogen 25th compared to 80th percentile	Total nitrogen 25th compared to 90th percentile	Total phosphorus 25th compared to 75th percentile	Total phosphorus 25th compared to 80th percentile	Total phosphorus 25th compared to 90th percentile
Organic Nitrogen Tolerance	0.173	0.183	0.132	0.278	0.294	0.262
Oxygen Tolerance	.127	.283	.134	.285	.291	.236
Bahls Pollution Class	.127	.282	.144	.313	.378	.317
Saprobien index	.132	.215	.042	.260	.271	.239

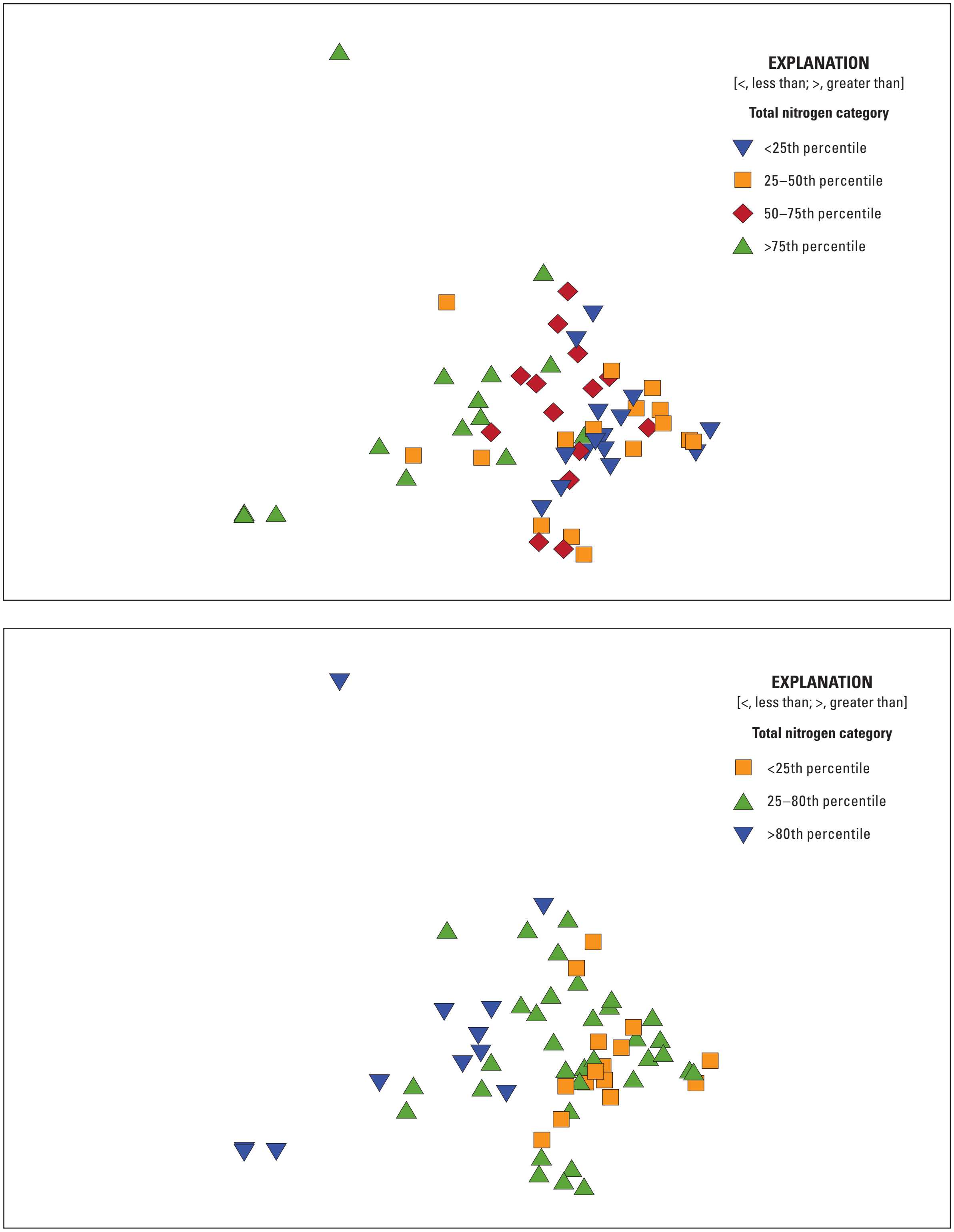
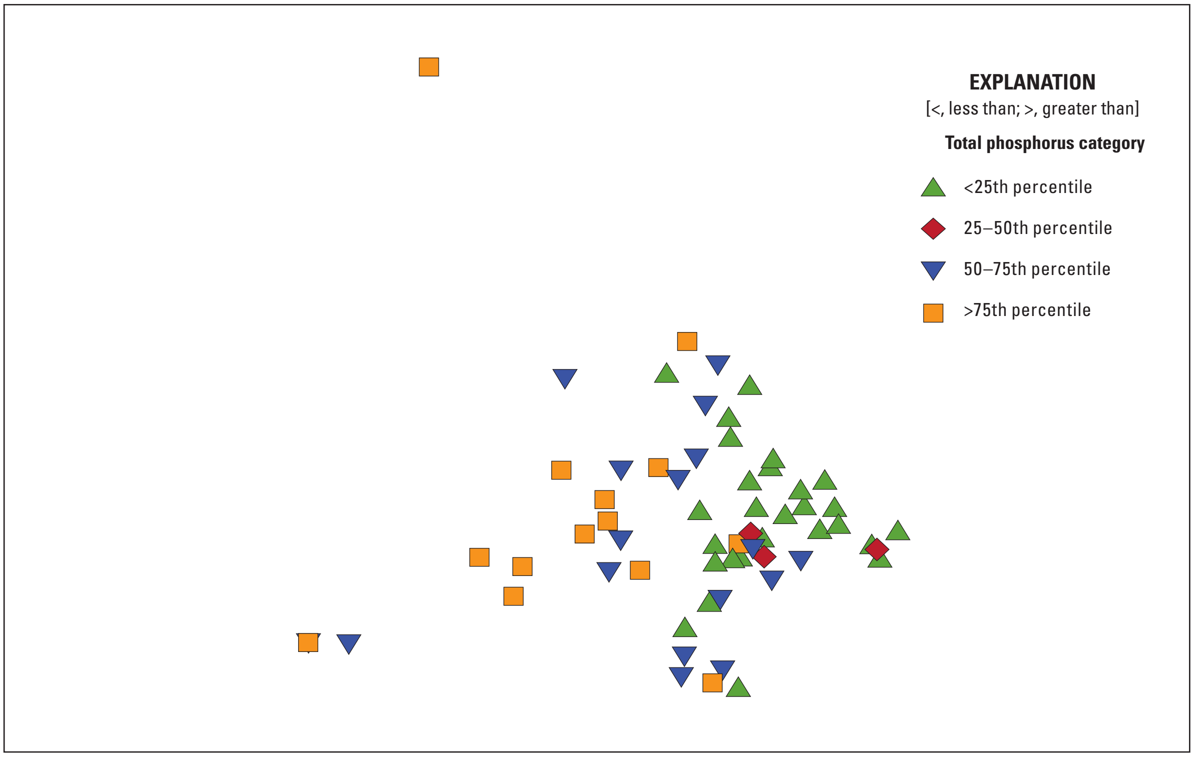


Figure 3. Multidimensional scaling (MDS) plot of Organic Nitrogen Tolerance metric (percent abundance) and total nitrogen categories.



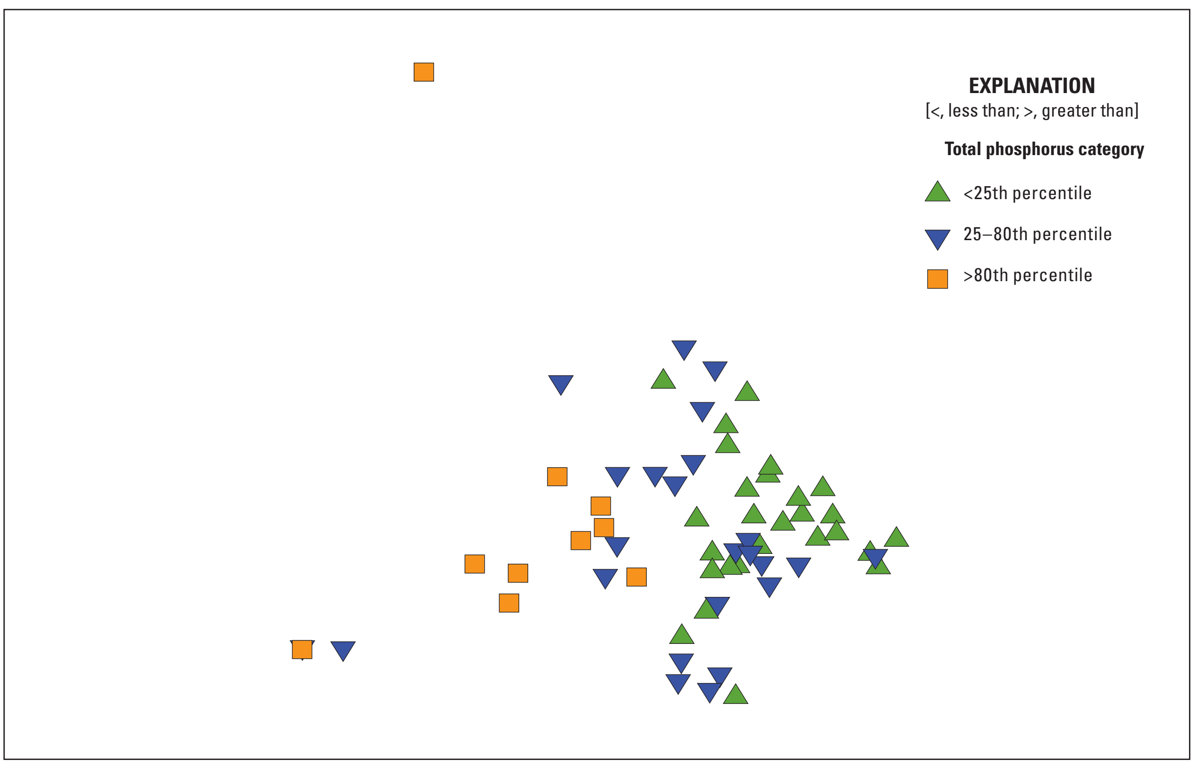
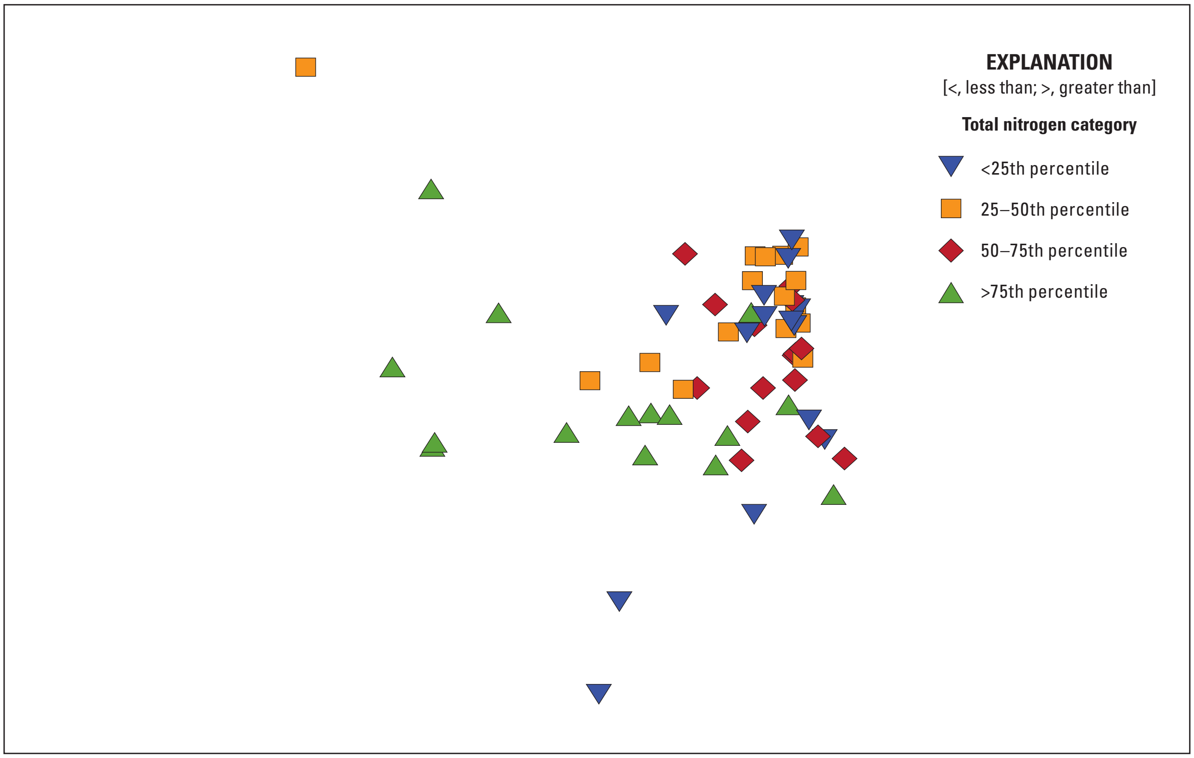


Figure 4. Multidimensional scaling (MDS) plot of Organic Nitrogen Tolerance metric (percent abundance) and total phosphorus categories.



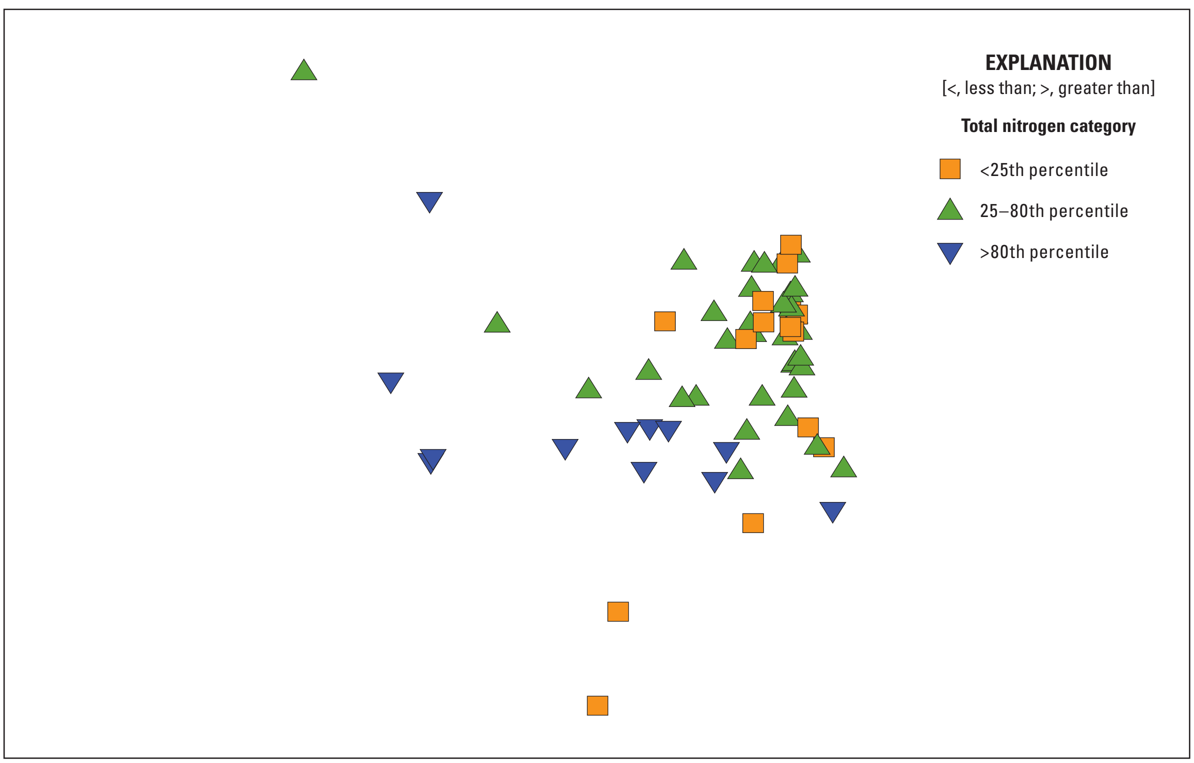
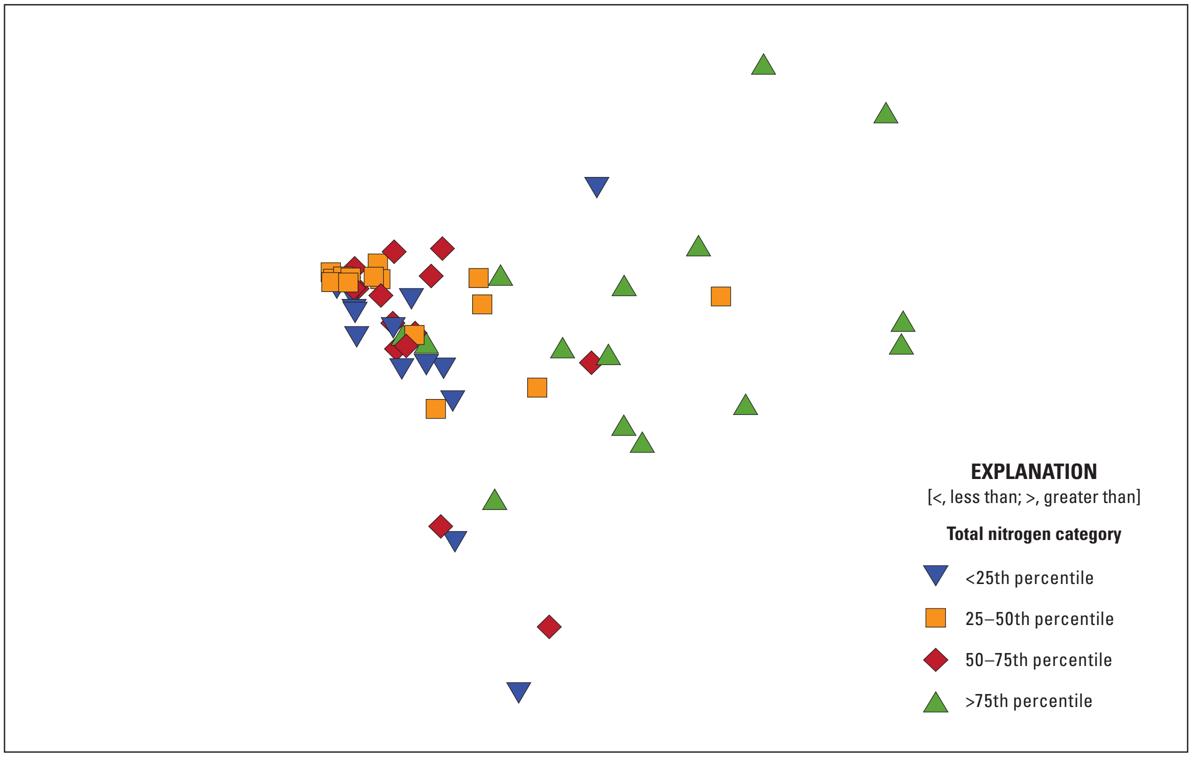


Figure 5. Multidimensional scaling (MDS) plot of Oxygen Tolerance metric (percent abundance) and total nitrogen categories.



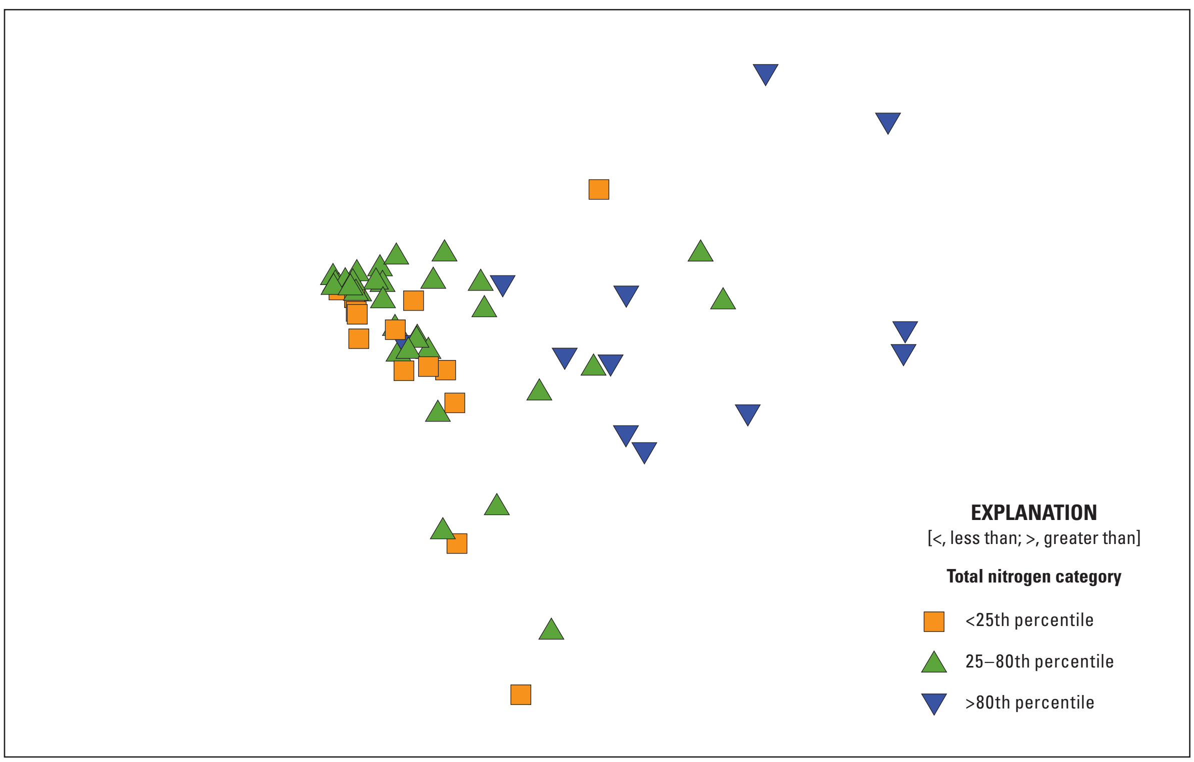


Figure 6. Multidimensional scaling (MDS) plot of Bahls Pollution Class metric (percent abundance) and total nitrogen categories.

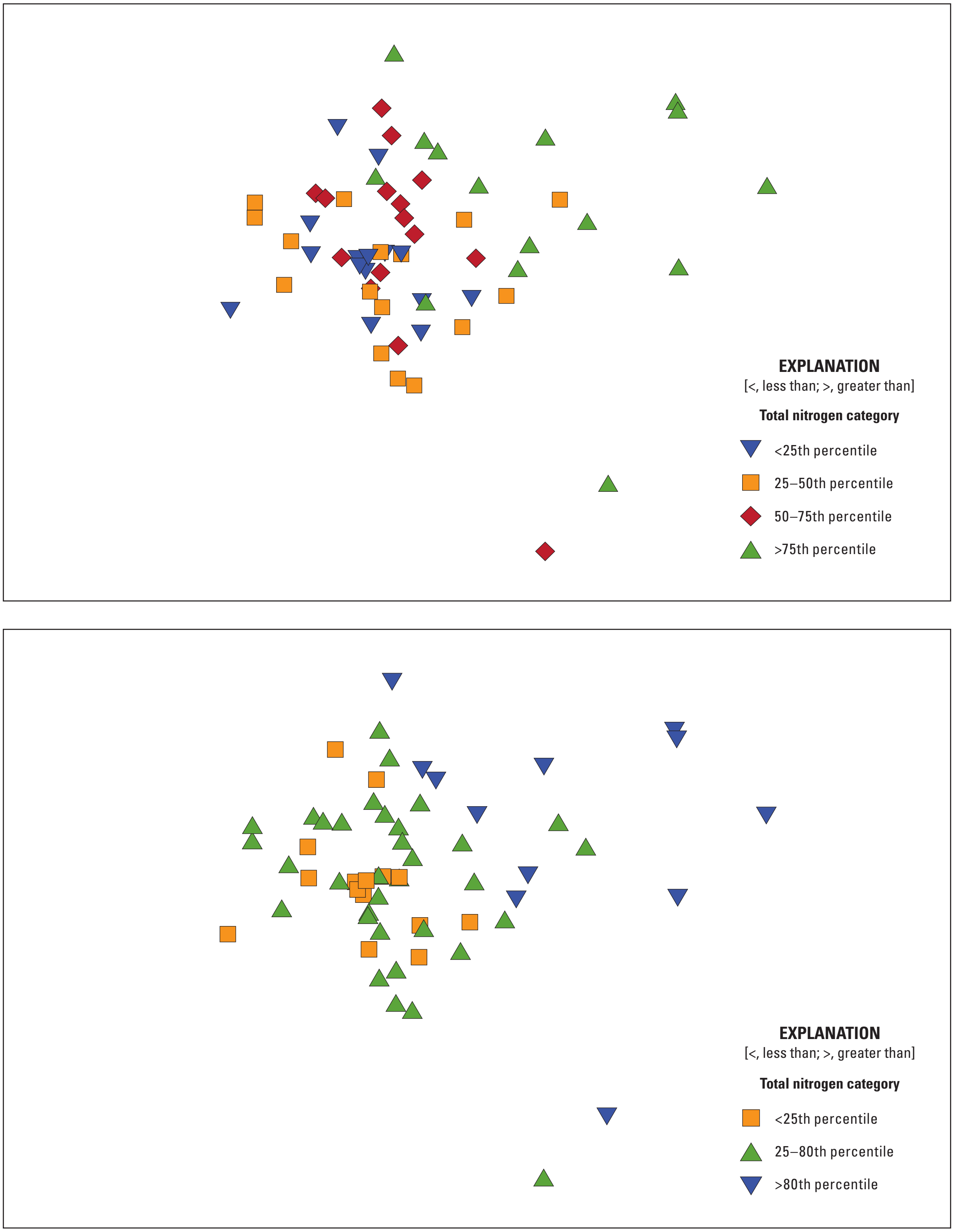


Figure 7. Multidimensional scaling (MDS) plot of Saprobien Index metric (percent abundance) and total nitrogen categories.

(table 3). The figures 3–7 illustrate this distinction by showing that the sites with larger nutrient concentrations plot to the left and the sites with the smallest nutrient concentrations plot together on the right side, separated by the sites that have moderate nutrient concentrations. The fact that there were only a few samples in the 90th percentile category may explain the decreased relation. The total phosphorus MDS plots are shown for just the ON metric because the trend continued throughout the other metrics. If the plot only contains the less than 25th percentile and greater than 80th percentile (fig. 8), the separation between the low and elevated nutrient levels are more discernable.

Using ANOVA and Tukey's test to establish the significance of these differences (table 4), the most sensitive/natural species tend to exhibit the most significant differences in algal populations (figs. 9–12). When observing relations between TN percentile categories and algal metrics, the consistent break between sensitive/natural algae species and moderately tolerant algae species is around the 25th percentile. The 80th percentile presents the most substantial difference for the communities with major changes in structure. In general, the low nutrient, sensitive algal species such as *Calothrix* sp., *Cymbella affinis, Cymbella delicatula, Achnanthidium minutissimum,* and *Achnanthidium deflexum* (Spaulding and others, 2010) were found in larger relative abundances at sites that fell into the lower 25th percentile category of nutrient concentrations (fig. 13; TN=0.17 mg/L or less, TP=0.005 mg/L or less, table 2) than at sites in the greater than 80th percentile category. Algal species that often are associated with eutrophic waters, such as *Navicula minima, Pleurocapsa minor*, and *Homoeothrix janthina* (Spaulding and others, 2010), were found in larger relative abundance in the 80th percentile category of nutrient concentrations (fig. 13; TN= 0.84 mg/L, TP= 0.035 mg/L, table 2) than at sites in the 25th percentile category. These are nutrient levels in which community change occurs.

The relations found in the TN data also were found in the TP data with respect to the algal metrics. The 80th percentile was the most significant for differences in community structure (per metrics). The MDS plots and boxplots (figs. 3–12) both show the same patterns of statistically significant differences in the algal community structure between streams with smaller nutrient concentrations (those streams with concentrations in the less than 25th percentile) and larger nutrient concentrations (those streams with concentrations in the greater than 75th and 80th percentiles). These patterns are consistent regardless of stream size, land use, and time periods.

Quantitative algae samples are collected from a variety of rocks that are randomly selected throughout the riffles of each site. This is the scraping method used in the 1993–95 samples. Photograph courtesy of Jim Petersen, U.S. Geological Survey, 2002.

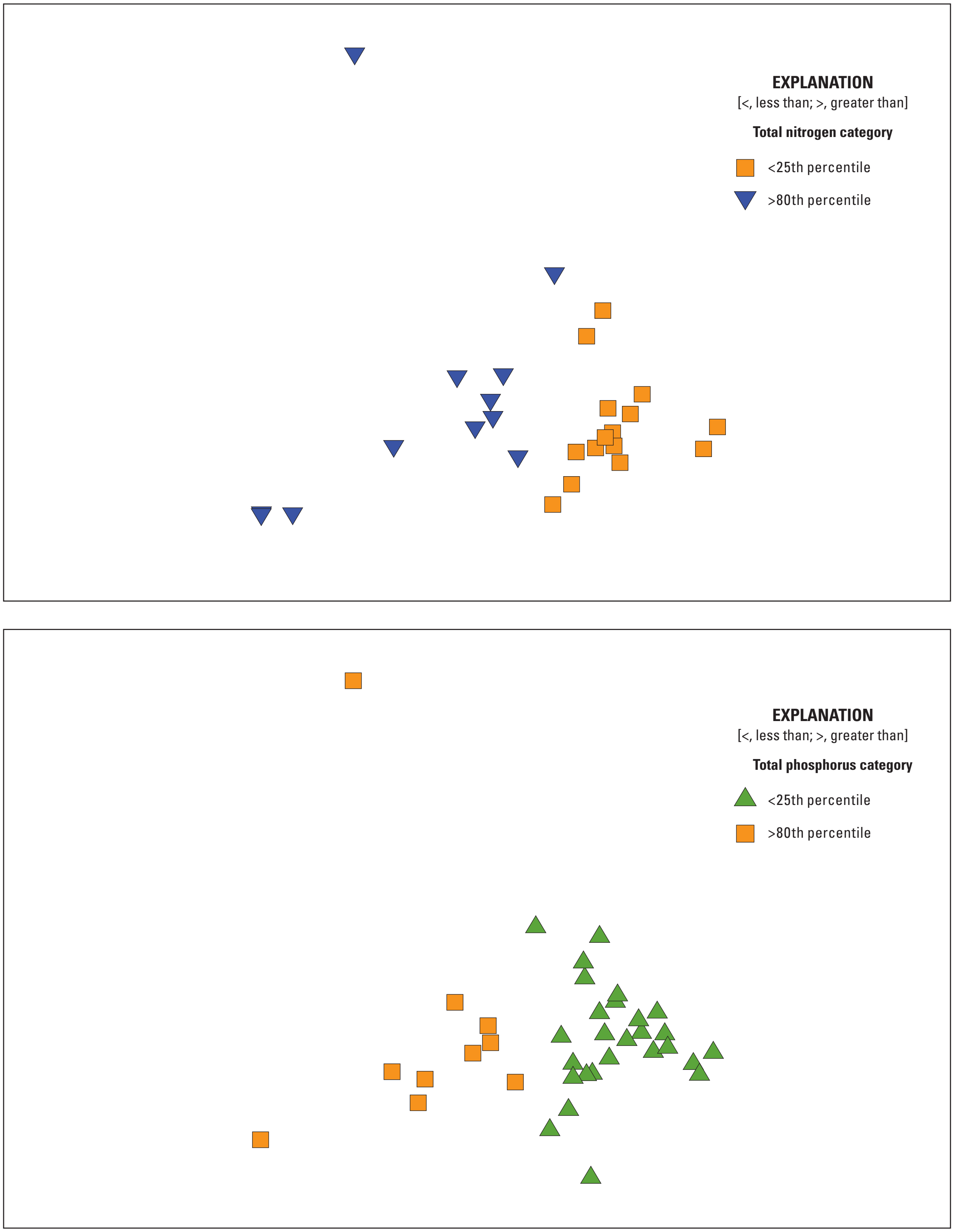


Figure 8. Multidimensional scaling (MDS) plot of Organic Nitrogen Tolerance metric and the upper and lower percentile categories of total nitrogen and total phosphorus.

Table 4. Comparison of differences (*p* values) between most natural condition (less than 25th percentile) and the most changed condition (greater than 80th percentile) for selected metrics.

[*p* values less than 0.05 are statistically significant]

Metric	Total nitrogen	Total phosphorus
Organic Nitrogen Tolerance		
Nitrogen autotroph, low inorganic nitrogen	0	0
Nitrogen autotroph, high inorganic nitrogen	.156	.111
Nitrogen heterotroph, facultative organic nitrogen	0	0
Nitrogen heterotroph, obligate organic nitrogen	.096	.052
Unknown or unclassified for nitrogen	.806	.965
Nitrogen heterotroph, facultative and obligate	0	0
Oxygen Tolerance		
Diatoms requiring high dissolved oxygen	0	0
Diatoms requiring fairly high dissolved oxygen	.134	.273
Diatoms requiring moderately high dissolved oxygen	.039	0
Diatoms tolerant of low dissolved oxygen	.000	0
Diatoms tolerant of very low dissolved oxygen	.421	.105
Diatoms with an unknown oxygen tolerance	.793	.970
Bahls Pollution Class		
Most tolerant diatoms	0	0
Less tolerant diatoms	0	0
Sensitive diatoms	0	0
Unknown or unclassified	.416	.184
Saprobien index		
Oligosaprobous	0.001	0
b-mesosaprobous	.002	.108
a-mesosaprobous	.002	0
a-meso/polysaprobous	0	0
polysaprobous	.192	.117
Unknown or unclassified	.888	.584

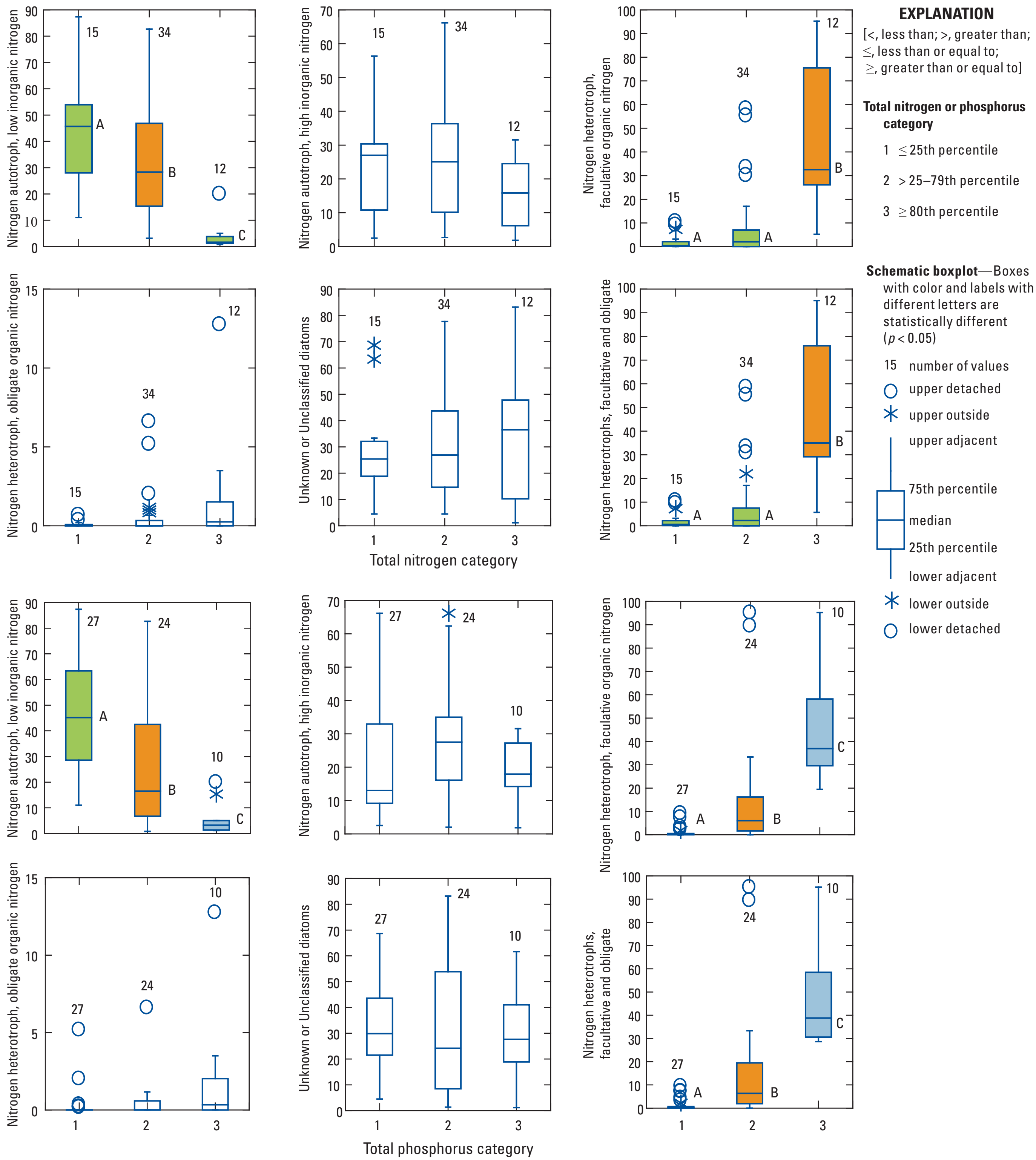


Figure 9. Organic Nitrogen Tolerance metric (percent of population) compared to total nitrogen and total phosphorus categories.

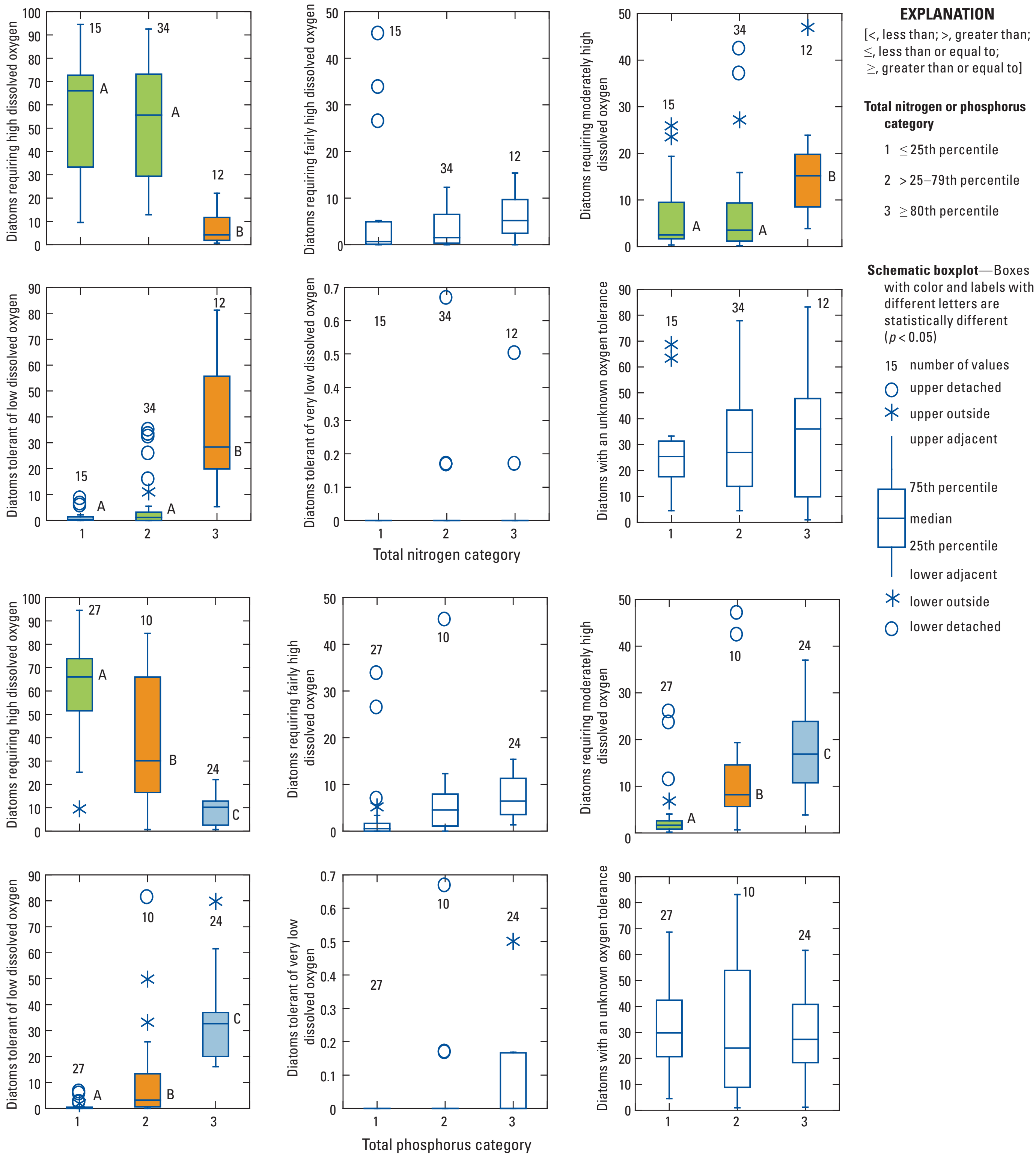


Figure 10. Oxygen Tolerance metric (percent of population) compared to total nitrogen and total phosphorus categories.

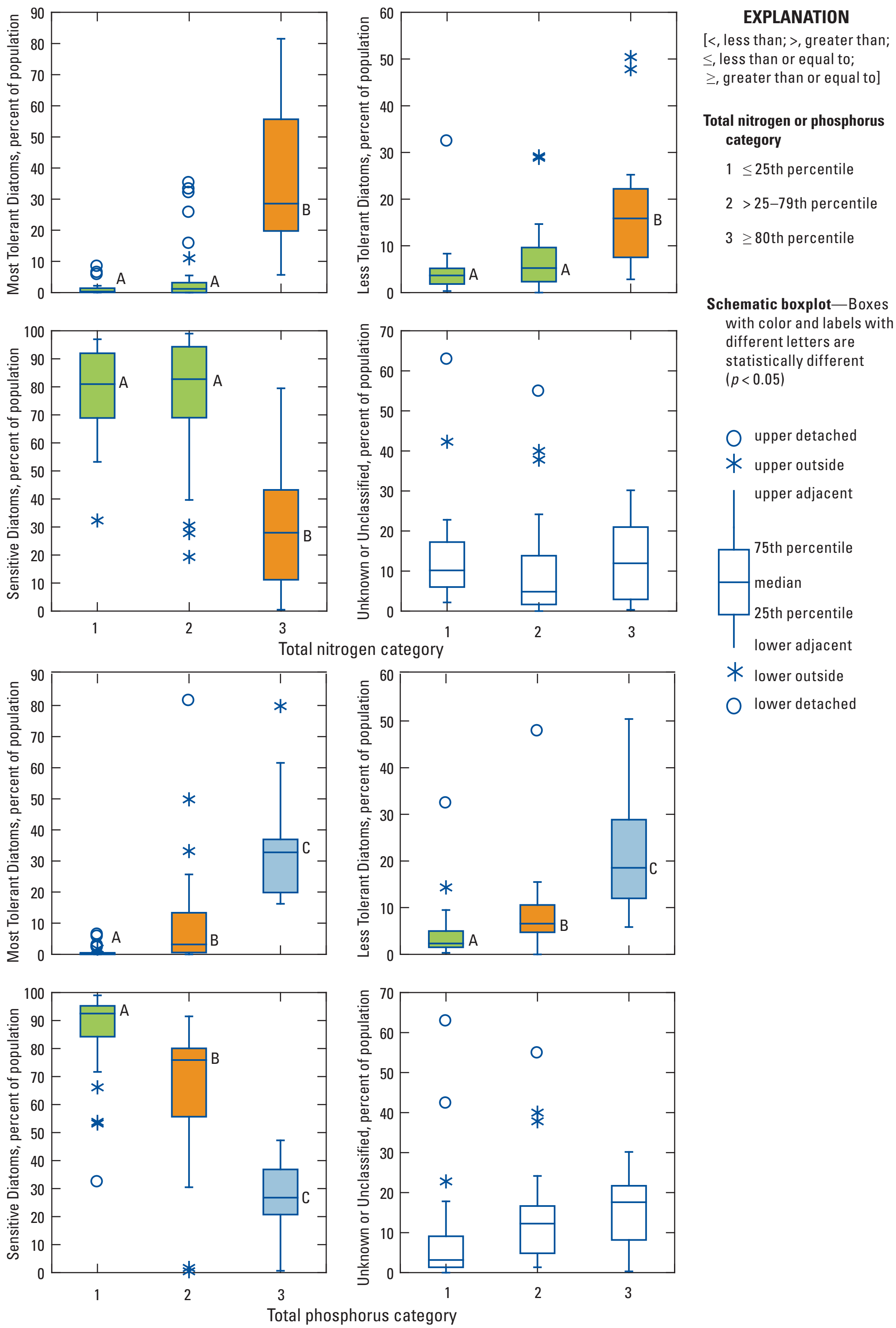


Figure 11. Bahls Pollution Class metric (percent of population) compared to total nitrogen and total phosphorus categories.

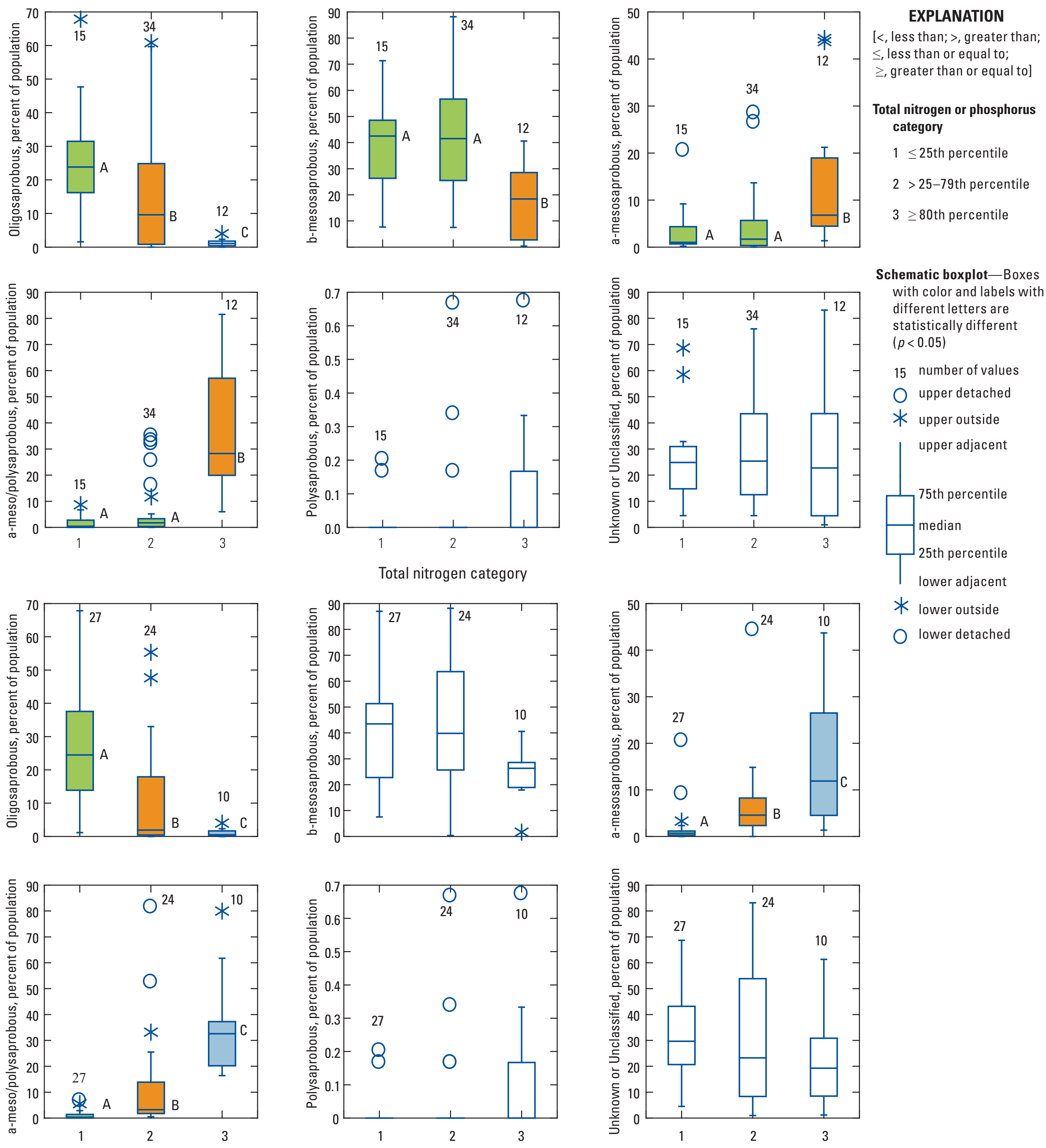


Figure 12. Saprobien index metric (percent of population) compared to total nitrogen and total phosphorus categories.

Figure 13. Multidimensional scaling (MDS) plots illustrating differences in (*A*) nutrient sensitive (for example, *Cymbella delicatula* Kutzing) and (*B*) nutrient tolerant (for example, *Navicula minima* Grunow) species at sites with smaller nutrient concentrations (1) and those sites with larger nutrient concentrations (3).

Summary and Conclusions

In 1997, the U.S. Environmental Protection Agency initiated a Clean Water Action Plan to address the widespread nutrient enrichment situation in the Nation's waters. To address the nutrient enrichment issue, the Missouri Department of Natural Resources is developing total nitrogen (TN) and total phosphorus (TP) criteria for streams of the State. In cooperation with the Missouri Department of Natural Resources, the U.S. Geological Survey selected algae for further study of the relations between biota and nutrient concentrations in the Ozarks of southern Missouri.

Data collected during 1993–95 and 2006–07 for the U.S. Geological Survey National Water-Quality Assessment program included nutrient and algal community data. These data were collected in the Ozark Highlands in southern Missouri at sites of differing drainage areas, land use, nutrient concentrations, and physiography. All sites were riffle/pool structure with the dominant substrate being cobble/gravel. A total of 60 samples from 45 sites were available for analysis. These sites had nutrient concentrations that covered a gradient of values. Total nitrogen concentrations ranged from 0.07 to 4.41 milligrams per liter (mg/L) with a median of 0.26 mg/L. Total phosphorus concentrations ranged from 0.003 to 0.780 mg/L with a median of 0.007 mg/L.

The nutrient concentration data were transformed into nutrient categories consisting of varying percentiles of data. Analyses of these data using several statistical programs with a variety of analyses, such as MDS, ANOSIM, and ANOVA, show significant changes in the algal community structure in relation to nutrient concentrations. Four metrics that have the strongest relation were selected: Organic Nitrogen Tolerance, Oxygen Tolerance, Bahls Pollution Class, and the Saprobien index. These changes in community structure primarily are characterized by a combination of nitrogen-fixing and organic/nutrient tolerant species and their changes in percent contribution to the total algal population. Nitrogen fixing or nutrient sensitive species such as, *Calothrix* sp., *Cymbella delicatula*, and *Achnanthidium minutissimum* were found at larger relative abundances at sites that fell into the less than 25th percentile nutrient concentration category than at sites in the greater than 80th percentile nutrient concentration category. Algal species that typically are found in eutrophic conditions and are more tolerant to higher organics and nutrient levels, such as, *Navicula minima*, *Pleurocapsa minor*, and *Homoeothrix janthina*, were found in larger relative abundances at the sites that were in the greater than 80th percentile nutrient concentration categories and at lesser relative abundances at sites that were in the less than 25th percentile nutrient concentration categories. These four metrics, nutrient data, and species composition data indicate that there is a significant change in algae community structure in the Ozark region when total nitrogen and phosphorus concentrations reach the 80th percentile of this dataset (0.84 mg/L, total nitrogen and 0.035 mg/L, total phosphorus).

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