ECOANALYSTS, INC.

PHYTOPLANKTON COMMUNITY ASSESSMENT AT TUBBS HILL AND UNIVERSITY POINT SAMPLING STATIONS 2007-2017

IDAHO DEPARTMENT OF ENVIRONMENTAL QUALITY

Prepared for
Idaho Department of Environmental Quality
Coeur d'Alene, Idaho

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ACRONYMS AND ABBREVIATIONS

IDEQ:	Idaho Department of Environmental Quality
LMP:	Lake Management Plan
nMDS:	Non-Metric Dimensional Scaling
SIMPER:	Similarity Percentages Analysis
SIMPROF:	similarity profile test
sRP:	Dissolved Ortho-Phosphorus
sTIN:	Nitrite (NO_2) + Nitrate (NO_3) + Ammonia (NH_3)

1. INTRODUCTION

Idaho Department of Environmental Quality (IDEQ) has been monitoring water quality in Coeur d'Alene Lake for many years. IDEQ, in collaboration with the Coeur d'Alene tribe, developed the Lake Management Plan (LMP) in 2009 which has included sampling the water column for phytoplankton communities and other environmental factors (e.g. dissolved metals, nutrients, chlorophyll, etc.). Long-term monitoring was conducted at two primary sampling locations, Tubbs Hill (C1 – Tubbs Hill) and University Point (C4 – University Point) (Figure 1-1). This report will focus on developing trends between phytoplankton communities over time that are associated with the environmental factors at these two stations.

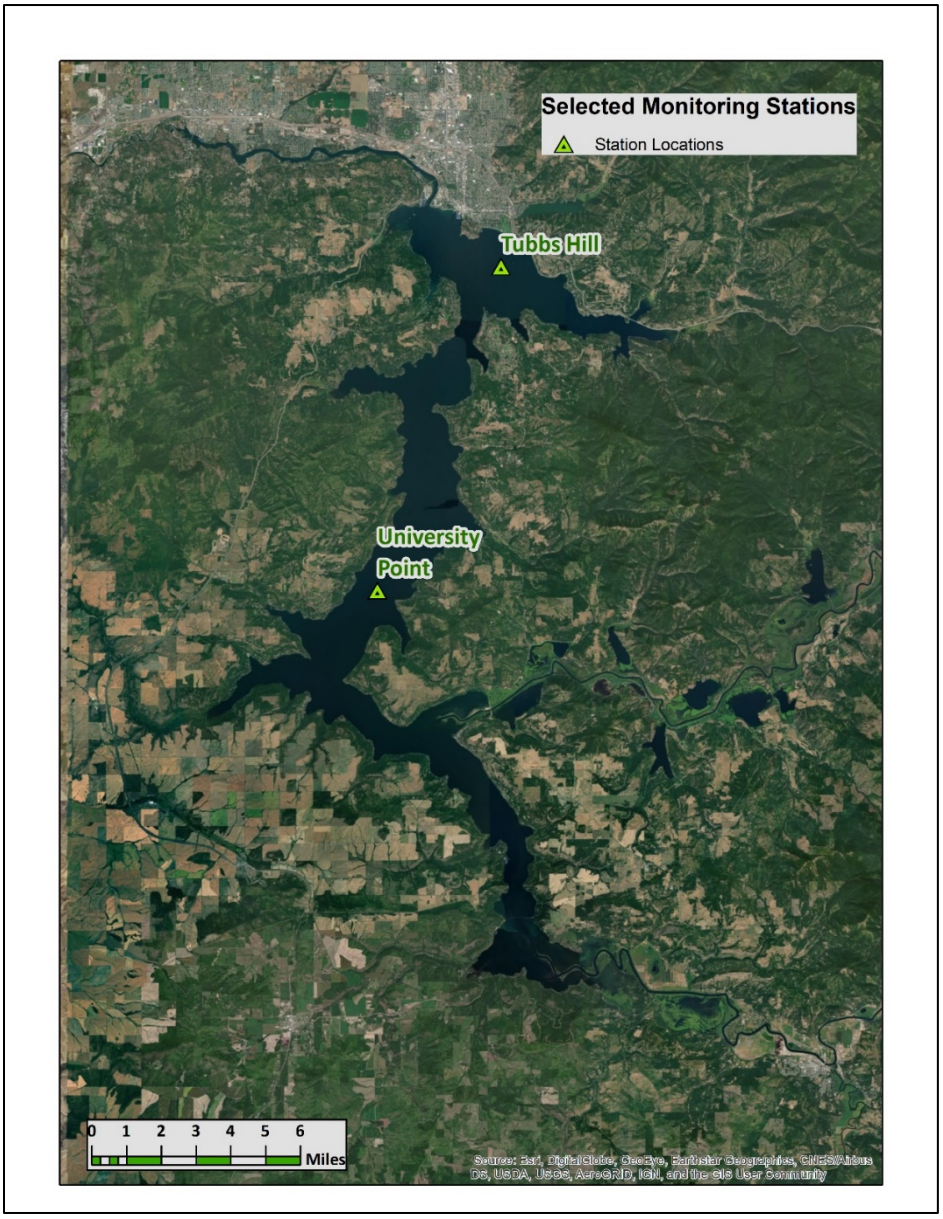

Figure 1-1. Selected Monitoring Station Locations

The seasonal patterns of Coeur d'Alene Lake can be separated by lake functional periods where the water quality conditions of the lake are unique. During a typical year, snow melt runoff will be influencing the lake from around February to June (runoff lake function), while conditions between June and September will show a lake with a stratified warm layer on top (warm stratified lake function). From October to January Coeur d'Alene Lake will be cold and clear (cold clear lake function) (Table 1-1). These seasonal lake function patterns could influence the phytoplankton in the lake and are included in this investigation. Per discussions with IDEQ, June is a transitional month and data collected during this timeframe was included in the analyses for both the runoff and warm stratified periods.

Table 1-1. Lake Function Detail

Lake Function	Time of Year Covered	Abbreviation
Runoff	February - June	RO
Warm Stratified	June – September	WS
Cold Clear	October - January	CC

2. METHODS

2.1 Analytical Data Treatment

Analytical results from water samples collected from 2007 to 2017 were compiled into a single database from many sources provided by IDEQ (Appendix A). The summary tables included in Section 3 reflect analytical detected results only. When the analyte was not detected that sample was not used to calculate averages or ranges. Mean data provided were calculated based on the geometric mean. This mean is calculated by taking the n^{th} root of the product of the detected values. This method of determining a mean is more appropriate for skewed data and balances the influence of outliers.

Analytical data was used to determine correlations with phytoplankton community differences that were indicated by the multivariate analysis. For this analysis, non-detected values were adjusted to a value that represented a minimal detected value throughout the study (Table 2-1). Analytical data were log transformed and normalized prior to conducting the correlation analysis. These treatments are required for conducting this type of analysis so that analytes that naturally have higher concentrations or detection limits don't drive the analysis.

Table 2-1. Non-detected Value for Comparison Correlations

Analyte	Value if Non-Detect
Dissolved Zinc (Diss Zn)	*
Dissolved Cadmium (Diss Cd)	*
Dissolved Lead (Diss Pb)	0.04
Total Phosphorus (TP)	2
Total Nitrogen (TN)	30
Chlorophyll (Chl)	0.5
Nitrite + Nitrate + Ammonia (sTIN)	10
Dissolved Ortho-Phosphorus (sRP)	1
* No non-detected results	

2.2 Phytoplankton Data Treatment

The phytoplankton data collected from 2007 to 2017 was synonymized by expert taxonomists at EcoAnalysts. The synonymization step reviews all identifications to determine if an identification changed following the original identification. This process also ensures that all identifications are at the proper level by aggregating those of a lower level with those of a higher, less-resolved level of identification to avoid artificial inflation of community richness and diversity indices.

2.2.1 Similarity Analysis

The Bray-Curtis coefficient is a measurement that determines similarity between two samples based on variable values. This coefficient is often used to investigate similarity of taxonomy data (each taxon as variables) between biotic samples. Based on the Bray-Curtis results, a resemblance matrix is created that reports the result values for each comparison.

Biological data was pretreated with a square root transformation of biovolume. The Bray-Curtis Similarity Index calculates the relative percent similarity between two different samples based primarily on the relative biovolume of taxa present within each sample.

As defined by Bray and Curtis, the index of similarity is:

$$S_{17} = 100\left(1 - \frac{\sum_i |y_{i1} - y_{i2}|}{\sum_i y_{i1} + \sum_i y_{i2}}\right)$$

Where *Yi* is the count for the ith (of p) species from sample 1, ∑i (….) denotes summation over those species. The results from the Bray-Curtis similarity index are bound between 0 and 1, which is converted to a percentage for comparison purposes. Samples with a result of 1 have the same species composition and samples with a result of 0 do not share any common species.

2.2.2 Hierarchical Clustering

Similarity coefficient values are highly influenced by any transformations that occur during the assessment. Similarity coefficients need to be compared by the rank similarity between stations (i.e. Sample 1 is more similar to Sample 2 than it is to Sample 3) (Clarke, Gorley, Somerfield, & Warwick, 2014). The Bray-Curtis similarity matrix can be displayed using a hierarchical clustering diagram (dendrogram). This diagram is a visual representation of the results of the similarity matrix. The x-axis of this plot represents the individual samples while the y-axis defines the similarity level at which two samples, or a group of samples, can be defined.

To test the significance of the similarity between samples or sample groups in the dendrogram, a similarity profile test (SIMPROF) was performed. The SIMPROF test is a permutation test of the null hypothesis that states there is no difference between the community between two or more samples. SIMPROF uses permutations of species values over the samples to create a set of resemblances among all pairs of samples ranked from smallest to largest which are then ordered and plotted as a dendrogram. The SIMPROF test compares the average absolute departure of the real profile from the mean of the permuted ones. The significance level is determined by the percent of permuted values that are greater than or equal to the observed value (Clarke, Gorley, Somerfield, & Warwick, 2014). Sample groups connected by dashed red lines indicate a fail to reject the null hypothesis and further analyses between samples withing these groups are not appropriate. Sample groups connected by solid black lines indicate that further evaluation of these communities can occur.

2.2.3 Similarity Percentages Analysis

The Similarity Percentages Analysis (SIMPER) in Primer allows for the similarity matrix, in this case based on the Bray-Curtis results, to be broken down into taxa contributions to similarity between (or dissimilarity between) groups. The sample groups can be defined during the initial sampling design (ie. samples collected at C1 Tubbs Hill vs. C4 University Point) or during the analysis (i.e. comparisons based on the groupings based on the hierarchical clustering results). The SIMPER analysis does require at least two samples to perform an in-group assessment and, when this criterion is met, will first indicate what taxa groups are contributing the greatest to the similarity between samples within the group and then it will determine which taxa are contributing the greatest to the dissimilarity between groups. This process will determine the contribution percent for each taxon as well as the cumulation percent of taxa defined in an ordered rank. Also, an important result of this process is the average similarity (or dissimilarity) of each taxon divided by the standard deviation. This result is a good indication of a taxon that contributes relatively consistently to the distinction for all pairs of samples by normalizing the data to the variability of the biovolume of the taxa. The results will be ordered by greatest contributors to lesser. For this report, the top three taxa contributing to either the in-group similarity or between-group dissimilarity have been noted. The full results can be found in Appendix B.

2.2.4 Non-Metric Dimensional Scaling

The similarity results as well as the SIMPROF results can be displayed using a Non-Metric Dimensional Scaling (nMDS) plot that can be utilized further to display correlations with environmental data. This plot "maps" the sample similarity, for this case, in two dimensions (along x and y axes). Each point displayed on the nMDS is a representation of a phytoplankton sample. Samples that have phytoplankton communities that are more similar to each other will be plotted closer on the plot. The similarity matrix created during the Bray-Curtis analysis was ranked by sample similarity. The samples that have the greatest similarity have a rank of 1, the next 2 and onward. This rank is preserved when the matrix is plotted on the nMDS which uses Euclidean distances to determine position. Additionally, the Primer software allows the SIMPROF results to be overlaid on the nMDS plot. This results in oblong circles encompassing groups of samples where the SIMPROF test failed to reject the null hypothesis as discussed in Section 2.2.2.

A concurrent analytical sample was collected with most of the phytoplankton community samples. These analytical samples were analyzed for various environmental factors. Once an nMDS plot has been created that reflects sample similarity based on phytoplankton community as a distance in 2-dimensional space, correlation analyses were performed to attempt to correlate the distance between samples with environmental factors. The first step in the correlation analysis is to correlate the environmental factors with the distance between samples for both the x and y-axes. This correlation analysis results in a correlation coefficient which is bound between -1 and +1. A correlation coefficient of 1 or -1 is a perfect correlation to the axis, while any correlation coefficient over 0.5 was considered "good" and ones over 0.7 were considered "strong". These correlation coefficients are plotted as vectors overlaying the nMDS plot where the relationship to the x and y-axes are combined into one direction indicated by the vector. The point of origin (0, 0) in the vector plot is where all vectors meet. The length of the vector is determined by calculating the hypotenuse length of a triangle where the sides are equal to the individual axis correlation coefficient. The vector lengths range from 0 to just over 1.4 (1.4 would indicate a perfect 1 or -1 correlation with both axes). The longer the vector length, the stronger the correlation the analyte has to the positioning of the sample in the nMDS plot. An environmental factor that shows a "good" (0.5) correlation with both axes of the nMDS plot would have a vector length of 0.7. Analytes that best correlate to the community sample placement for both axes of

the nMDS plot based on the vector length were displayed using a bubble plot. The bubble plots presented illustrate the actual concentration of the selected analyte(s), not transformed data.

3. ANALYTICAL RESULTS

The analytical results section provides a summary of detected values for specific analytes. These summaries are based on water samples collected concurrently to a phytoplankton sample. Annual geometric mean results are provided for each selected analyte in Section 3.1. These results provide a general understanding of the annual trend in concentrations for these analytes. Section 3.2 provides geometric mean results based on the lake function. This data illustrates that analytical concentration in the lake tend to have a seasonal pattern.

3.1 Annual Geometric Mean Results

Geometric mean annual values are provided for selected analytes collected at sampling station Tubbs Hill and University Point to illustrate ranges and general trends (Table 3-1 and Table 3-2). These analytes were selected based on conversations with IDEQ and their assessment of which analytes have the most potential to influence the phytoplankton communities. No samples were collected during the runoff function during 2007. The geometric mean for this year is not reported because this data is not comparable with the other years.

Table 3-1. Tubbs Hill Geometric Mean Values for Selected Analytes

	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017
Dissolved Zinc	50.4	47.2	50.9	46.9	46.1	46.5	41.8	44.0	43.9	40.2
Dissolved Cadmium	0.22	0.22	0.19	0.21	0.20	0.21	0.18	0.18	0.18	0.16
Dissolved Lead	0.21	0.20	0.13	0.38	0.20	0.33	0.20	0.17	0.23	0.24
Total Phosphorus	6.9	6.6	4.6	8.7	5.9	5.7	7.3	8.2	7.2	10.4
Total Nitrogen	120.1	108.9	97.1	86.3	76.3	76.7	86.3	94.7	91.2	116.3
Chlorophyll	4.4	2.5	1.6	1.7	2.3	2.2	2.1	2.2	1.9	1.3
sTIN	43.8	42.8	28.6	41.6	9.8	11.0	24.0	21.0	18.6	33.2
sRP	ND	ND	ND	ND	ND	1.2	1.2	2.5	1.0	1.3
Nitrogen to Phosphorus ratio	17.7	16.1	21.0	9.9	12.8	13.7	11.8	11.6	12.6	11.2
sTIN:sRP ratio	ND	ND	ND	ND	ND	ND	20.0	9.2	15.7	21.1

All values in µg/L except ratios
ND – either not measured or no detected values
sTIN = Nitrite (NO_2) + Nitrate (NO_3) + Ammonia (NH_3)
sRP = Dissolved Ortho-Phosphorus

Table 3-2. University Point Geometric Mean Values for Selected Analytes

	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017
Dissolved Zinc	57.8	49.2	55.5	47.4	53.0	50.2	45.2	49.3	42.2	42.6
Dissolved Cadmium	0.26	0.23	0.21	0.25	0.25	0.24	0.21	0.21	0.19	0.19
Dissolved Lead	0.84	0.28	0.22	0.79	0.73	0.28	0.55	0.31	0.31	0.42
Total Phosphorus	9.4	8.3	5.8	10.1	7.1	5.8	7.0	7.7	9.0	10.2
Total Nitrogen	130.0	123.4	99.4	91.2	83.9	72.6	95.5	111.1	92.6	109.8
Chlorophyll	3.4	2.4	1.8	1.4	2.2	2.4	2.3	2.0	1.6	1.3
sTIN	108.0	69.1	26.9	45.0	37.8	25.3	31.9	48.8	7.8	23.5
sRP	ND	ND	ND	4.1	ND	1.1	3.6	3.1	1.3	1.6
Nitrogen to Phosphorus ratio	ND	14.9	17.3	9.0	11.9	12.6	13.7	14.4	10.2	10.8
sTIN:sRP ratio	ND	ND	ND	25.2	ND	ND	16.7	18.2	4.7	11.1

All values in µg/L except ratios
ND – either not measured or no detected values
sTIN = Nitrite (NO_2) + Nitrate (NO_3) + Ammonia (NH_3)
sRP = Dissolved Ortho-Phosphorus

3.2 Analytical Geometric Mean Results by Lake Function

The following section discusses the geometric mean results for detected analytes over periods of time when the lake is experiencing different lake function patterns (runoff, warm stratified, and cold clear). Some lake function seasons did not have a representative analytical sample (e.g. RO - 2007 or CC - 2012). These are not shown in Figure 3-1 through Figure 3-8. Data presented in this section is only on detected values. When the analyte was not detected during the lake function season there will be a gap in the line and no marker for the data point (see Figure 3-3). The cold clear season was represented by fewer samples and occasionally the geometric mean for this season was only calculated based on one sample.

3.2.1 Dissolved Metals

In general, geometric mean results for dissolved metals (zinc, cadmium, and lead) appear to have seasonal trends in Coeur d'Alene Lake (Figure 3-1 to Figure 3-3). These results indicate that the dissolved metals concentrations are highest during the runoff lake function and lowest during the warm stratified or cold clear lake functions. One exception is for dissolved zinc, which appears to indicate elevated concentrations during the cold clear as well as the runoff lake function. Dissolved metals concentrations appear to be slightly higher at the more southern sampling station, University Point. The dissolved lead result for sample station University Point was elevated during the runoff period of 2008 (see Figure 3-3). One sample collected during this lake function in 2008 may have had particulates when analyzed and the data point is questionable. When this point is removed, the scale of the y-axis allows for better visualizing the other data points (see Figure 3-4).

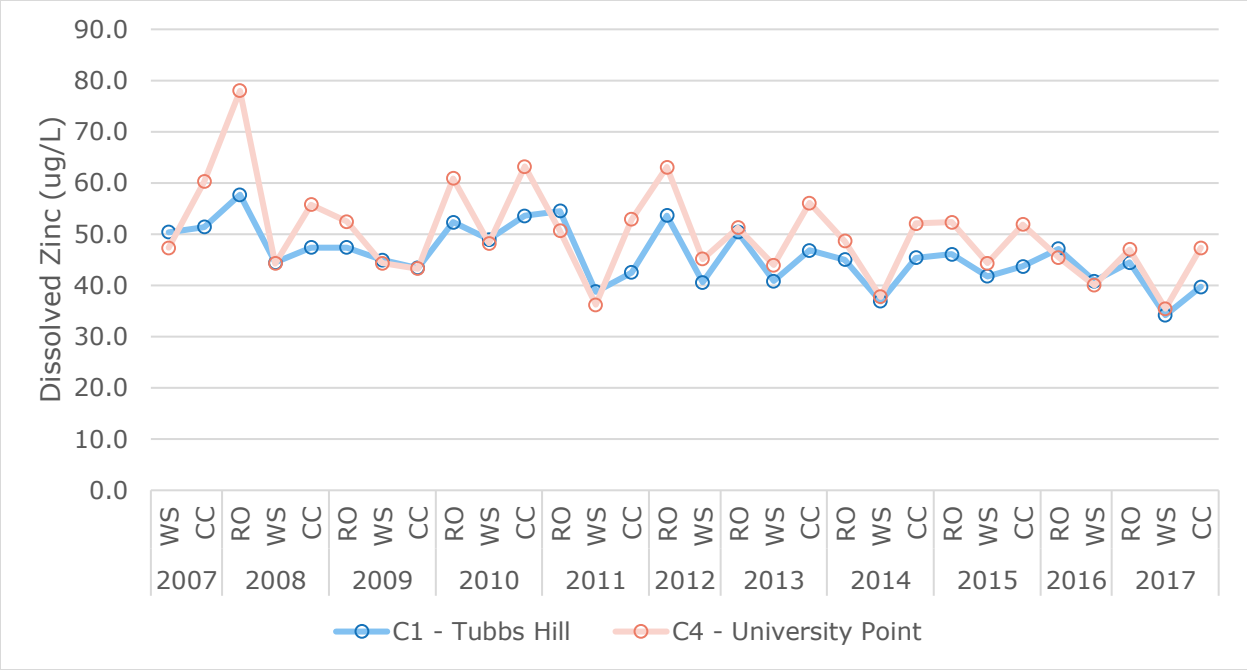

Figure 3-1. Geometric Mean Dissolved Zinc Concentrations by Lake Function and Station

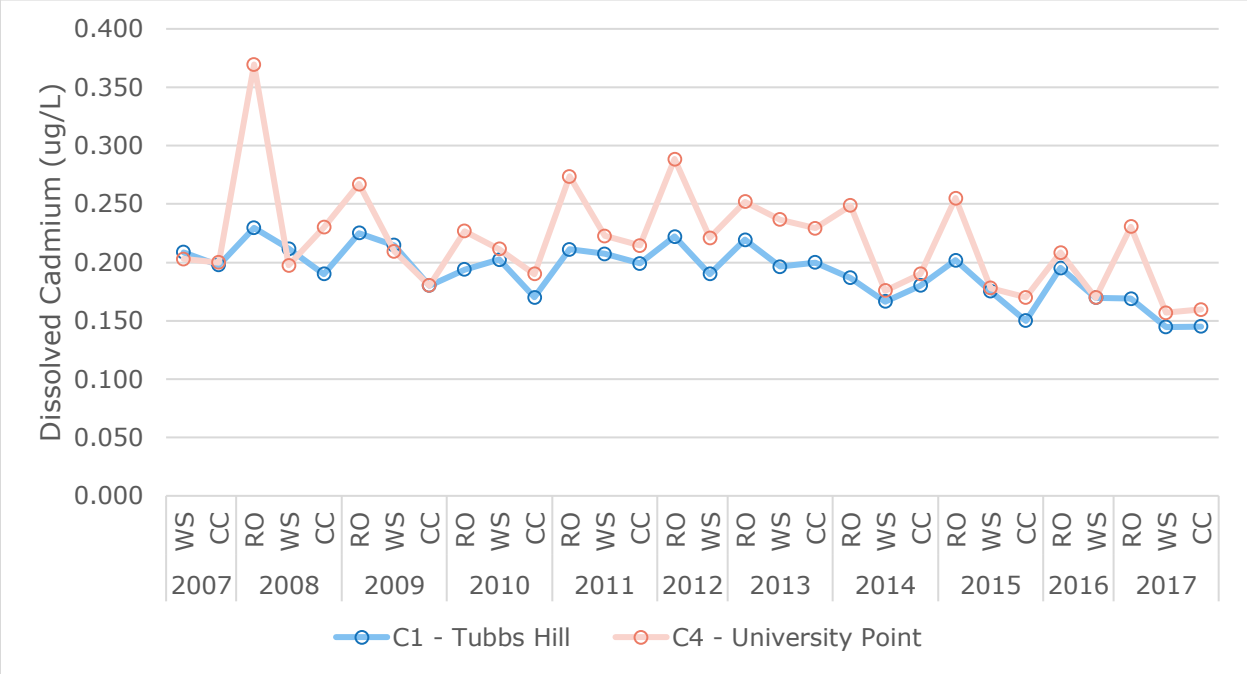

Figure 3-2. Geometric Mean Dissolved Cadmium Concentrations by Lake Function and Station

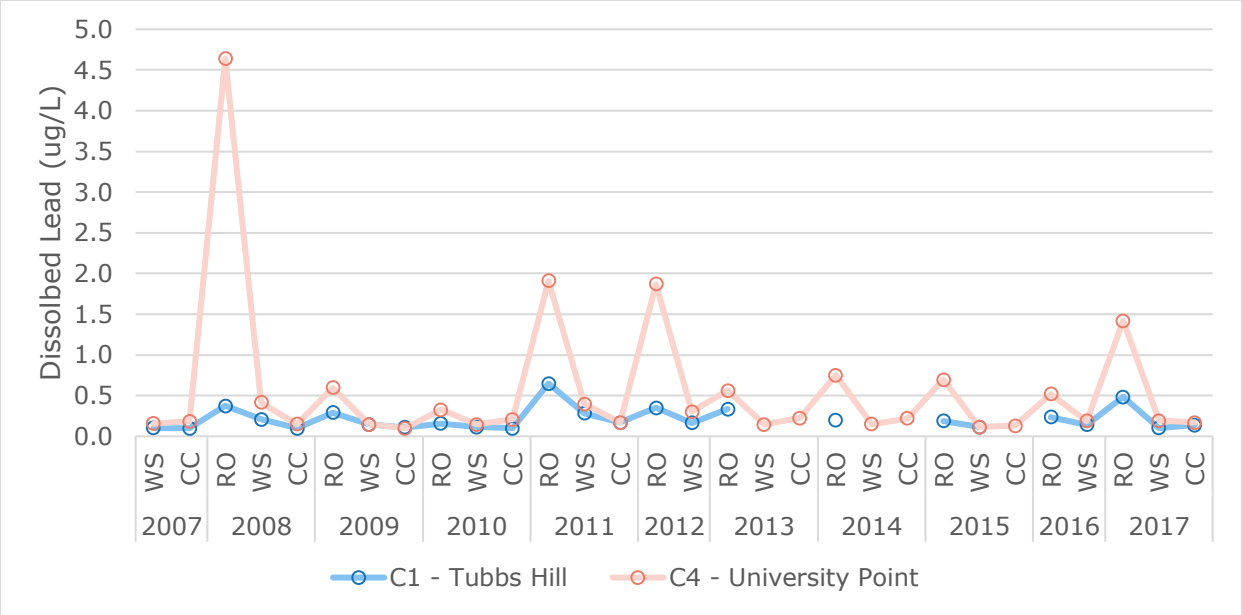

Figure 3-3. Geometric Mean Dissolved Lead Concentrations by Lake Function and Station

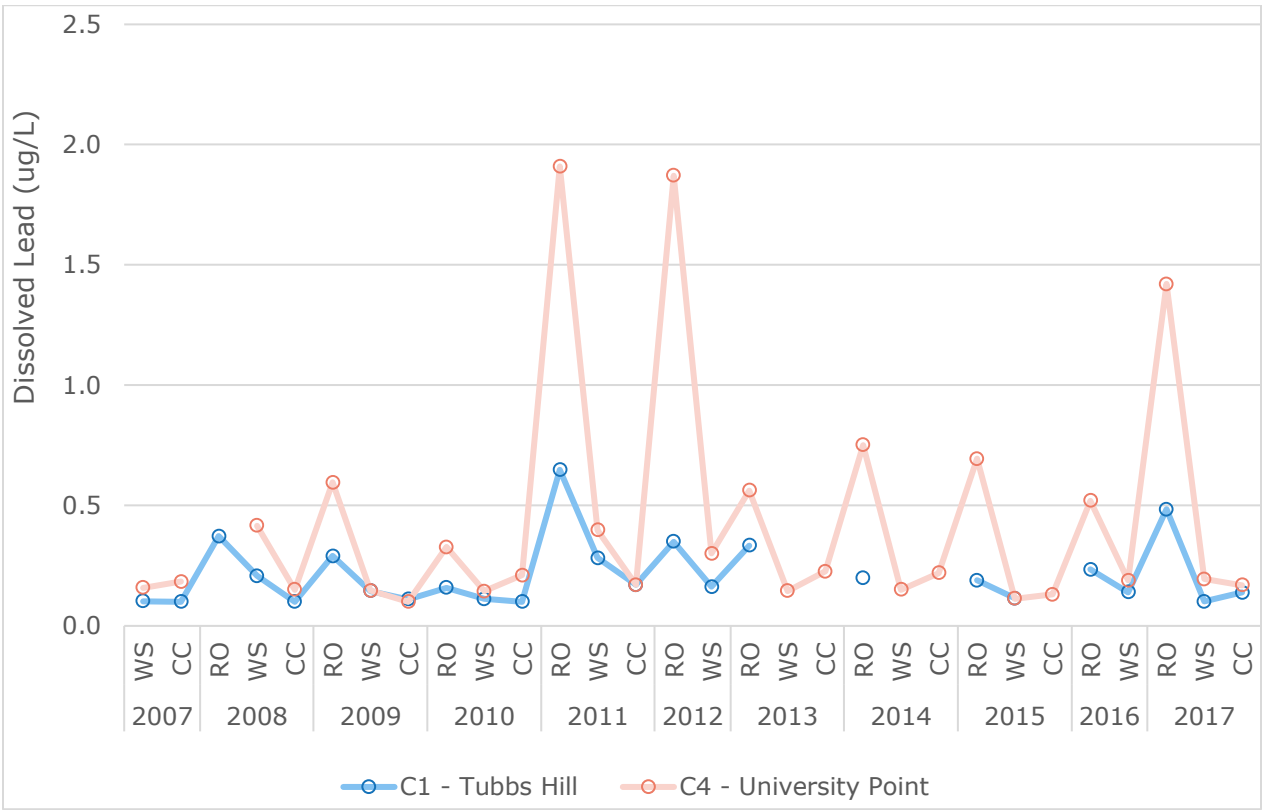

Figure 3-4. Geometric Mean Dissolved Lead Concentration by Lake Function and Station (University Point 2008 Runoff sample removed)

3.2.2 Total Nitrogen, Total Phosphorus and Chlorophyll

Total phosphorus and total nitrogen trends, when viewed with data averaged by lake function and station, indicate a similar seasonal trend as noted for the dissolved metals (see Figure 3-5 to Figure 3-8). Total nitrogen at both sampling stations seem to be following a similar pattern but total phosphorus appears to fluctuate more dramatically at University Point than at the Tubbs Hill sampling station. The ratio of total nitrogen to total phosphorus show one large spike at Tubbs Hill station during the cold clear lake function of 2007 and two spikes during the cold clear lake function of 2014 and 2015 at University Point sampling station. Chlorophyll values indicate two large spikes at both the Tubbs Hill and University Point sampling stations during the runoff and warm stratified lake functions of 2008 and 2009.

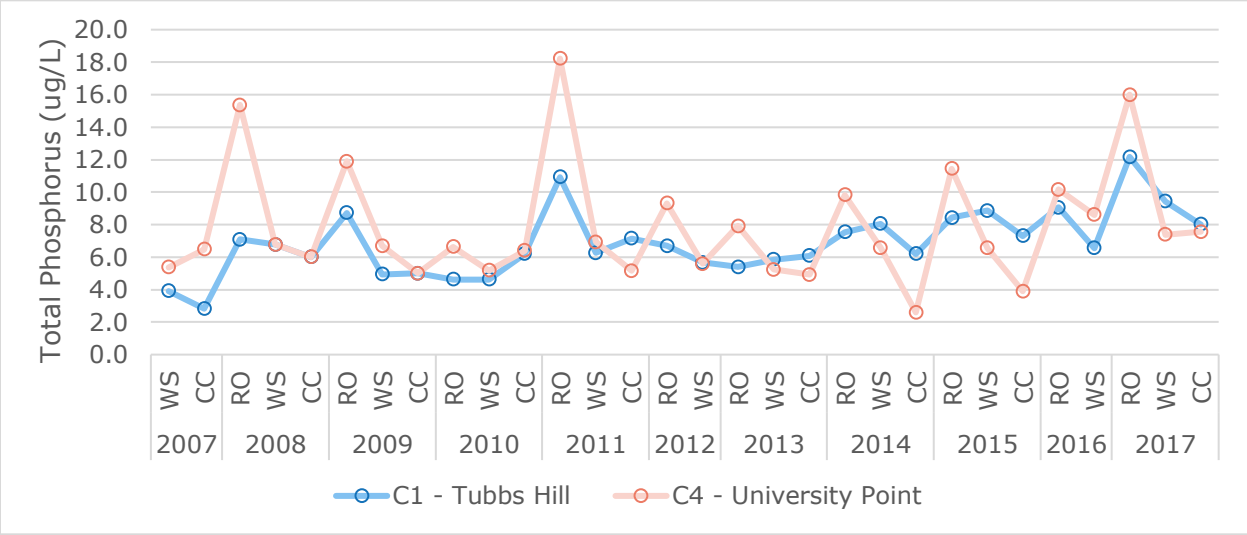

Figure 3-5. Geometric Mean Total Phosphorus Concentrations by Lake Function and Station

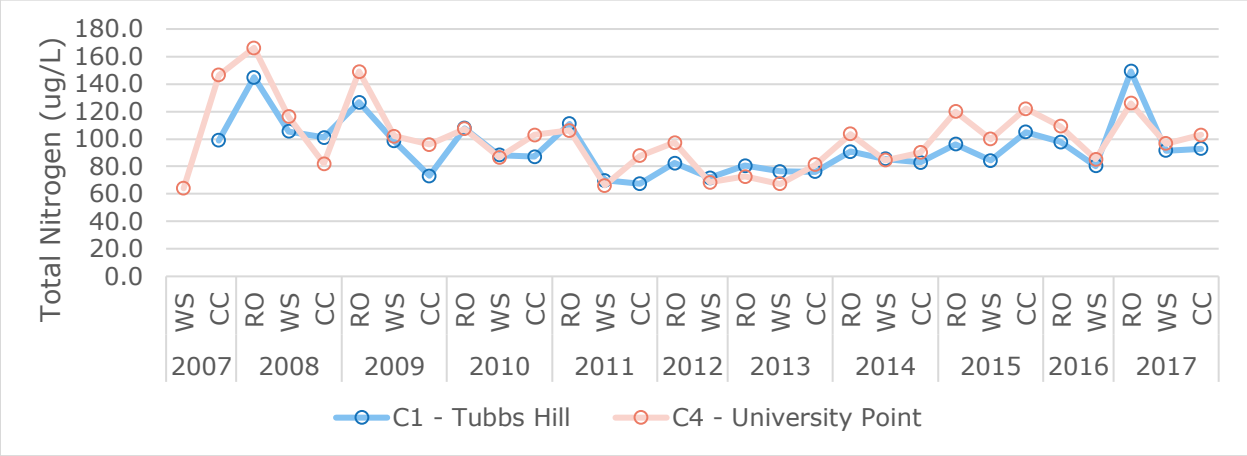

Figure 3-6. Geometric Mean Total Nitrogen Concentrations by Lake Function and Station

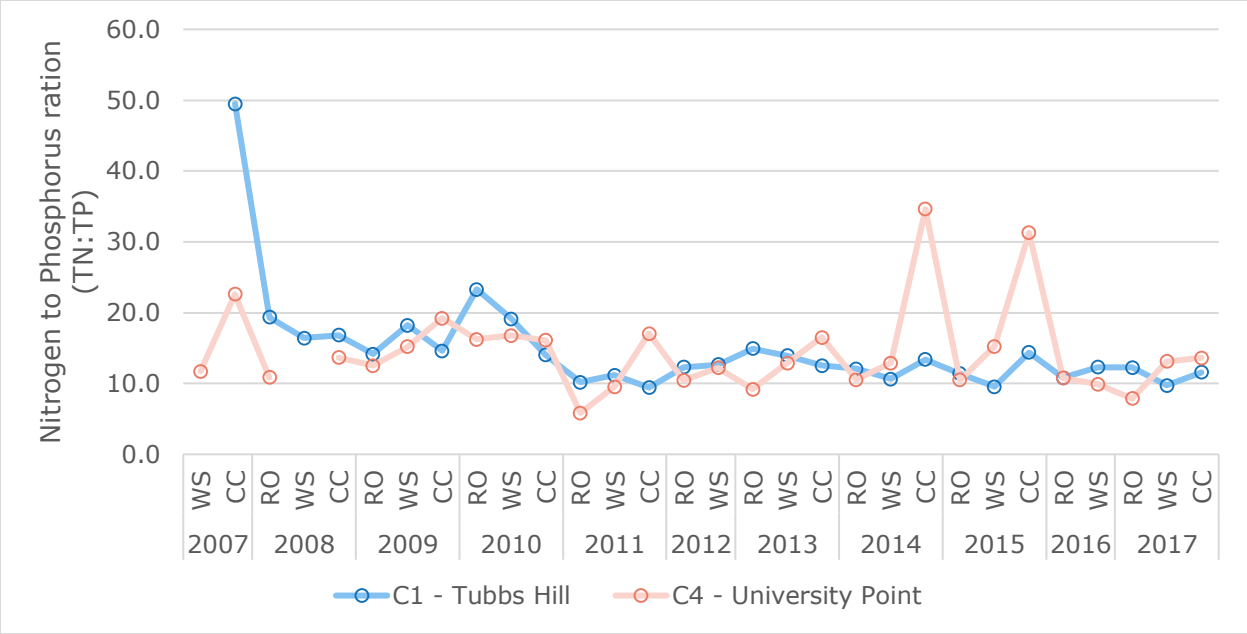

Figure 3-7. Geometric Mean Total Nitrogen to Total Phosphorus Ratio by Lake Function and Station

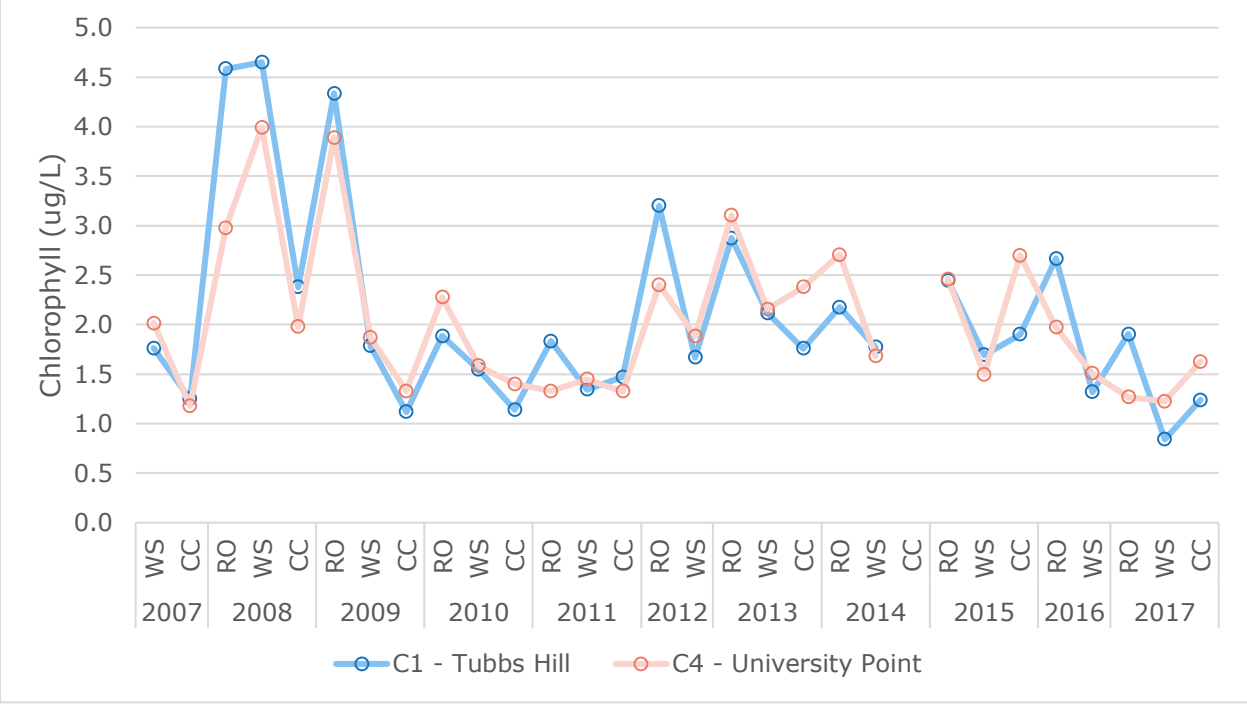

Figure 3-8. Geometric Mean Chlorophyll Concentration by Lake Function and Station

4. PHYTOPLANKTON COMMUNITY RESULTS

The phytoplankton summaries discussed in Section 4 provide results based on phytoplankton data after the synonymization process. During the course of this monitoring program 82 unique taxa of phytoplankton taxa have been identified in the samples collected from the Tubbs Hill location during the runoff lake functional period. The same sampling efforts at the University Point location resulted in 72 uniquely identified phytoplankton. *Asterionella formosa* was the major contributor of average biovolume at both sampling station. The average biovolume from the top 20 taxa during this period are shown in Figure 4-1 for the Tubbs Hill Station and Figure 4-2 for the University Point station during the runoff period.

During the warm stratified function period of this monitoring program, 78 unique taxa were identified at the Tubbs Hill station while 76 unique taxa were identified at the University Point station. *Asterionella formosa* was the major contributor of average biovolume at both sampling station. The top 20 taxa with the highest average biovolume are provided in Figure 4-3 for Tubbs Hill and Figure 4-4 for University Point.

During the cold clear function period 62 unique taxa were identified for Tubbs Hill as well as 65 unique taxa identified for University Point. *Microcystis spp*. was the taxon that contributed the highest average biovolume for samples collected at Tubbs Hill while *Tabellaria flocculosa* and *Cryptomonas spp.* were the taxa that contributed the highest average biovolume to the samples collected at University Point. The top 20 taxa with the highest average biovolume are provided in Figure 4-5 for Tubbs Hill station and Figure 4-6 for University Point station.

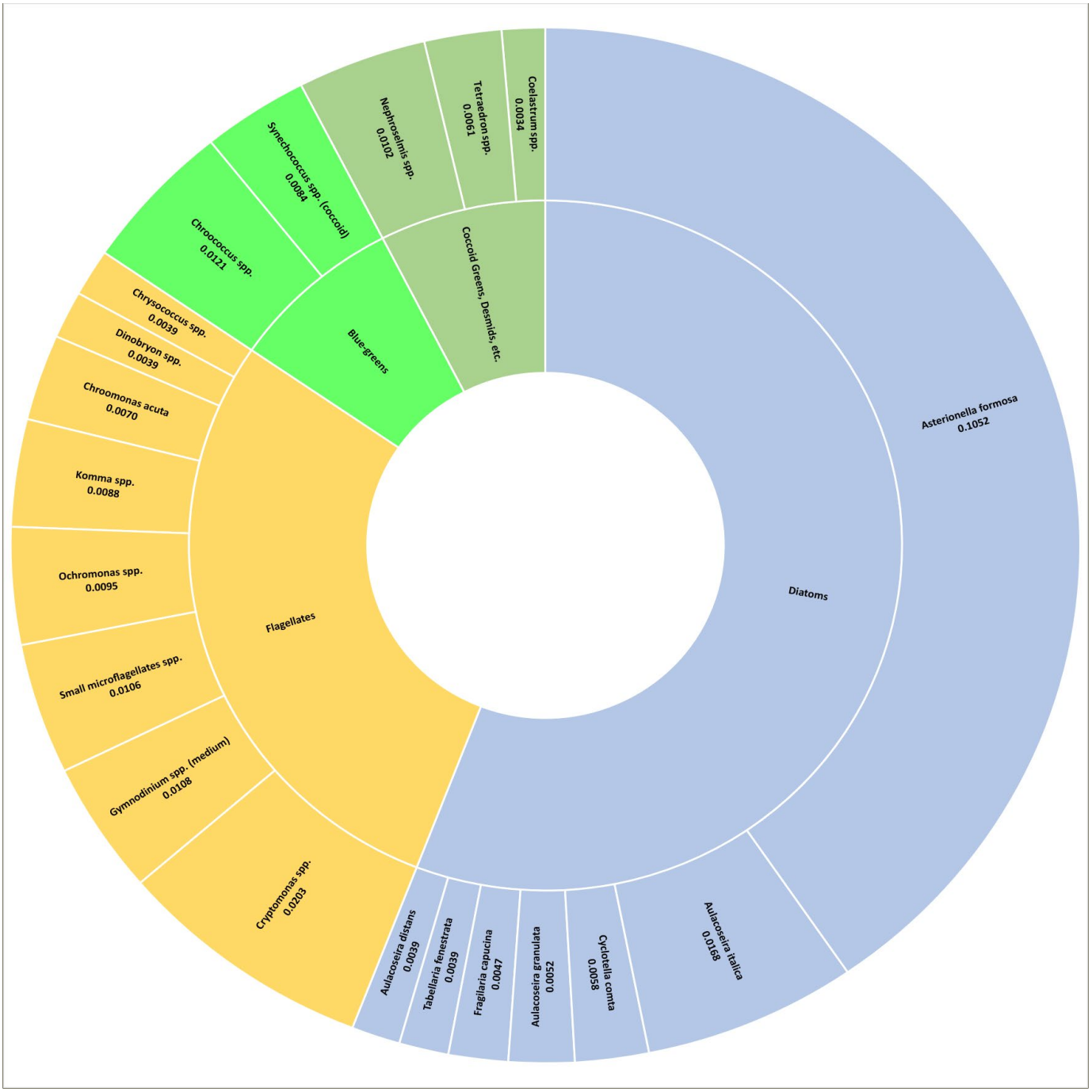

Figure 4-1. Tubbs Hill Runoff top 20 taxa by average biovolume mm^3/L

Figure 4-2. University Point Runoff top 20 taxa by average biovolume mm^3/L

Figure 4-3. Tubbs Hill Warm Stratified top 20 taxa by average biovolume mm^3/L

Figure 4-4. University Point Warm Stratified top 20 taxa by average biovolume mm^3/L

Figure 4-5. Tubbs Hill Cold Clear top 20 taxa by average biovolume mm^3/L

Figure 4-6. University Point Cold Clear top 20 taxa by average biovolume mm^3/L

5. PHYTOPLANKTON COMMUNITY ANALYSIS

Phytoplankton communities at two sampling locations (Tubbs Hill and University Point) in Coeur d'Alene Lake were analyzed using multivariate statistical techniques as described in Section 2. These techniques were used to investigate phytoplankton community differences between the two sampling locations during the three lake functions. The first step to the analysis was to determine if there were any samples collected at a sampling station during a specific lake function that indicated a significantly different phytoplankton population. In order to determine this a Bray-Curtis similarity analysis was performed on samples collected at each station during each lake function season. Following the Bray-Curtis similarity analysis a SIMPROF test was conducted to determine when sample groupings were significantly different based on the phytoplankton communities.

Following these steps, selected environmental factors were analyzed for correlations of these dissimilarities. The results from the Bray-Curtis analyses were used to graph sample dissimilarities on an nMDS plot. Correlation analysis was performed according to the methods defined in Section 2.2.4 to determine how well the placement of samples in the nMDS plot correlated with paired analytical data. Environmental data was log transformed and normalized prior to vector correlation analysis. A correlation coefficient of 1 or -1 indicates a perfect correlation to the axis, while any individual correlation coefficient over 0.5 was considered "good" and ones over 0.7 were considered "strong".

Following the correlation analyses, further investigation was conducted to determine which taxa were contributing to the similarity within defined sample groups as well as which taxa were contributing to the dissimilarity between these groupings. Most of the phytoplankton samples collected at each station during the three lake functional periods had phytoplankton communities that were not significantly different from each other. The sample groups that contains these are referred to as the "Major Group" in this report. Sample groupings indicating significant differences were then compared to the Major Group at the sampling station during the corresponding lake function.

The dissimilarity between a Bray-Curtis defined group and the Major Group based on the top three taxa indicate an increase or decrease in average relative biovolume for these taxa. A radial plot is provided for each lake function at each sample station that shows the increase or decrease in average relative biovolume of the taxa that are contributing to the dissimilarity between the comparison groups. The increase or decrease of the average relative biovolume is determined by finding the difference the sample group and the Major group. Positive values are an indication that the dissimilarity is being driven by an increase in biovolume of this taxon in the comparative group from the Major Group. Negative values are an indication that the dissimilarity between these groups are being driven by a reduction of biovolumes in the comparative group from the Major Group.

5.1 Runoff Lake Function

5.1.1 Tubbs Hill

The results from the Bray-Curtis analysis and subsequent SIMPROF test for samples collected from Tubbs Hill during the runoff lake function are presented in Figure 5-1. The SIMPROF results indicate four distinct groups of samples. The majority of the phytoplankton community results during the runoff period are not significantly different from each other and are contained in Group D (Major Group). Group A (June 2008), Group B (March – May 2008) and Group C (April and May 2009) indicate the phytoplankton communities are significantly different than those communities found in Group D.

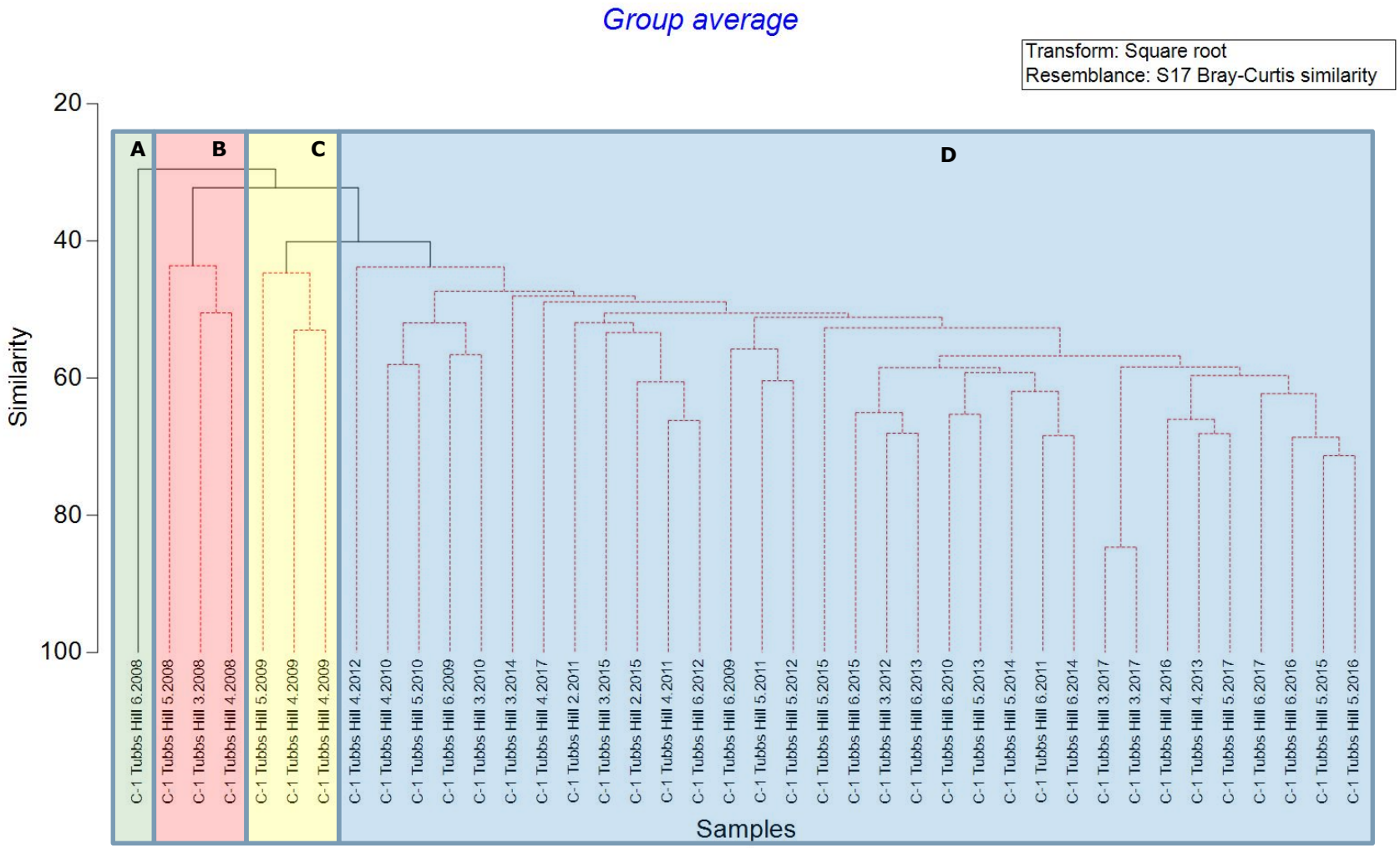

Figure 5-1. Tubbs Hill Runoff Sample Group Designations Based on Similarity Grouping

Results of the correlation analysis for samples collected during the runoff lake function period at Tubbs Hill are provided in Figure 5-2. These results indicated no analytes have a "good" correlation with the positioning of the samples in the nMDS plot. Chlorophyll demonstrated the best correlation with the two axes (vector 0.50). Individual axis correlations as well as the combined vector length for each analyte are provided in Table 5-1. Figure 5-3 illustrates the concentrations of chlorophyll for each sample. The Bray-Curtis sampling groupings are also provided to indicate the samples which demonstrated significant differences during the phytoplankton community analysis.

Table 5-1. Tubbs Hill Runoff correlation results for individual nMDS axes and combined vertex length

	Diss. Zn	Diss. Cd	Diss. Pb	TP	TN	Chl.	sTIN	sRP	TN:TP	sTIN: sRP
MDS1 (x)	0.13	0.33	0.27	0.02	0.03	0.36	0.19	-0.04	0.01	0.22
MDS2 (y)	0.10	0.28	0.03	-0.30	-0.21	0.35	0.00	-0.35	0.14	0.11
Vector	0.16	0.43	0.27	0.30	0.21	0.50	0.19	0.35	0.14	0.25

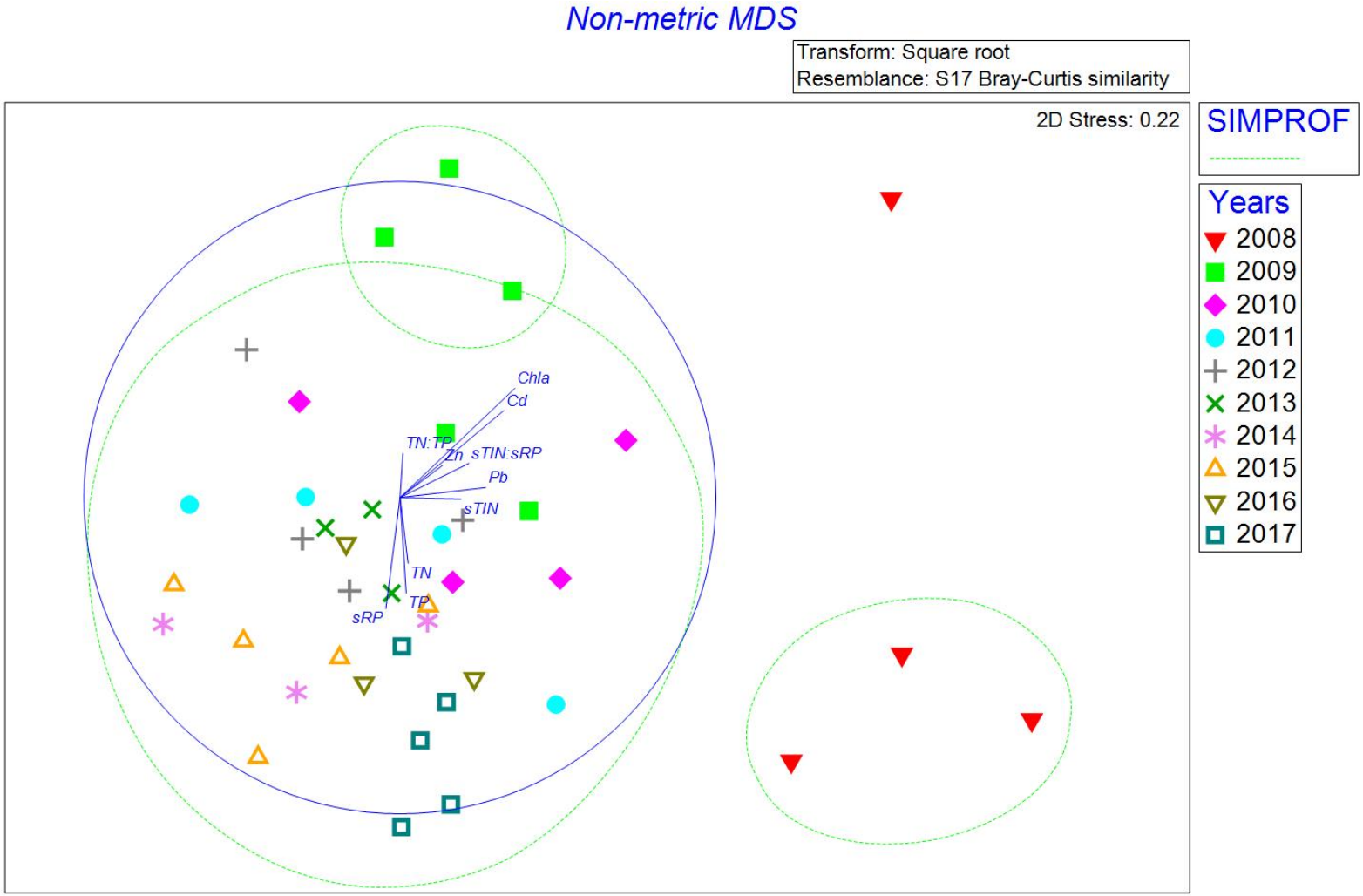

Figure 5-2. Tubbs Hill Runoff Vector Correlation Results

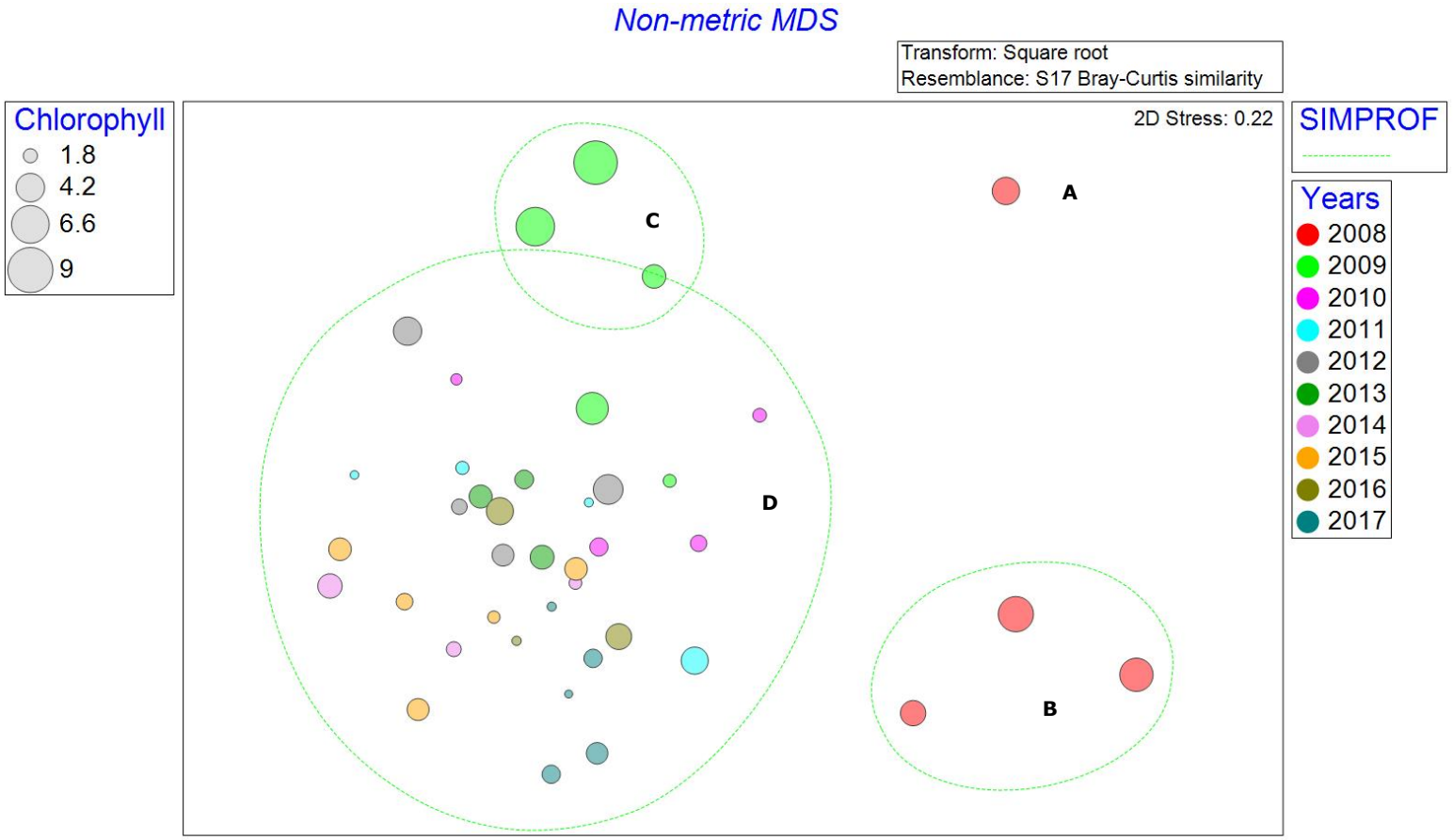

Figure 5-3. Tubbs Hill Runoff Sample Bubble Plot Indicating Concentration of Selected Analytes. (Alpha codes indicate SIMPER Groupings)

The SIMPER results for in-group similarity for the samples collected during the runoff lake function period at Tubbs Hill are provided in Table 5-2. Group A only contained one sample so in-group similarity could not be assessed. For sample Groups B, C, and D, *Asterionella formosa* was the taxon that contributed the greatest to the similarity within each group while *Cryptomonas spp.* was also a major contributor.

Table 5-2. SIMPER Results Tubbs Hill Runoff In-group Similarity

Group (% Similarity)	Taxa	Phytoplankton Type	Average Relative Biovolume	Similarity/ Standard Deviation	Contribution %	Cumulative %
Group A[1]						
Group B (45.9%)	*Asterionella formosa*	Diatom	0.29	4.56	24.9	24.9
	Cryptomonas spp.	Flagellate	0.18	10.05	14.2	39.1
	Oscillatoria sp2	Blue-green	0.15	2.14	10.6	49.7
Group C (47.5%)	*Asterionella formosa*	Diatom	0.46	5.68	30.5	30.5
	Aulacoseira italica	Diatom	0.32	2.12	16.9	47.4
	Cryptomonas spp.	Flagellate	0.20	9.88	11.4	58.8
Group D *Major Group* (51.7%)	*Asterionella formosa*	Diatom	0.29	2.35	20.4	20.4
	Cryptomonas spp	Flagellate	0.13	1.92	9.2	29.5
	Small microflagellates spp.	Flagellate	0.10	3.14	8.5	38.0

[1] Group contains one sample. SIMPER in-group similarity not calculatable.

Selected results from the SIMPER comparison for between-group dissimilarity conducted on samples collected during the runoff lake function at station Tubbs Hill are provided in Table 5-3. This table was truncated to provide only the results of the comparisons between each Bray-Curtis defined sample grouping to the sample group that contained the majority of the samples. A complete table of results is provided in Appendix B.

The taxon contributing to the majority of the dissimilarity (42.7%) between sample Group A and D was a blue-green alga (*Microcystis spp.*) which indicated an increase of *Microcystis spp.* in samples from Group A. The comparison between Group B and D also indicated a blue-green alga (*Oscillatoria sp2.*) contributed the greatest to the dissimilarity (5.3%) between these groups where there was an increase of *Oscillatoria sp2.* in sample Group B. The comparison between sample Group C and D indicate a

diatom (*Aulacoseira italica*) was the taxon contributing the greatest to the dissimilarity (8.8%) where there was an increase of *Aulacoseira italica* in samples collected in sample Group C.

Table 5-3. SIMPER Results Tubbs Hill Runoff Between-group Dissimilarity (Selected Results)

Group Comparison (% Dissimilarity)	Taxa	Phytoplankton Type	Group 1 Average Relative Biovolume	Group 2 Average Relative Biovolume	Dissimilarity/ Standard Deviation	Contribution %	Cumulative %
Group A (1) to Group D (2) (70.5%)	*Microcystis spp.*	Blue-green	1.52	0.01	9.24	42.7	42.7
	Fragilaria crotonensis	Diatom	0.12	0.01	3.58	3.1	45.8
	Asterionella formosa	Diatom	0.26	0.29	0.79	2.8	48.6
Group B (1) to Group D (2) (67.3%)	*Oscillatoria sp2*	Blue-green	0.15	0.00	2.68	5.3	5.3
	Aulacoseira granulata	Diatom	0.14	0.02	1.29	5.0	10.3
	Asterionella formosa	Diatom	0.29	0.29	0.87	3.9	14.2
Group C (1) to Group D (2) (59.9%)	*Aulacoseira italica*	Diatom	0.32	0.08	1.53	8.8	8.8
	Asterionella formosa	Diatom	0.46	0.29	1.58	7.4	16.2
	Tabellaria flocculosa	Diatom	0.18	0.01	0.72	6.0	22.2

A radial plot is provided (Figure 5-4) which illustrates the difference in biovolume of each of the top three taxa that are contributing to the dissimilarity between the sample groups (A, B, and C) and the Major Group (D). The comparison between sample Group A and the Major Group shows the average relative biovolume of *Microcystis spp.* is much greater in sample Group A than in the Major Group. The difference in average relative biovolume of *Asterionella formosa* was -0.03 indicating that this taxon was found to have less average relative biovolume in samples for Group A than the Major Group.

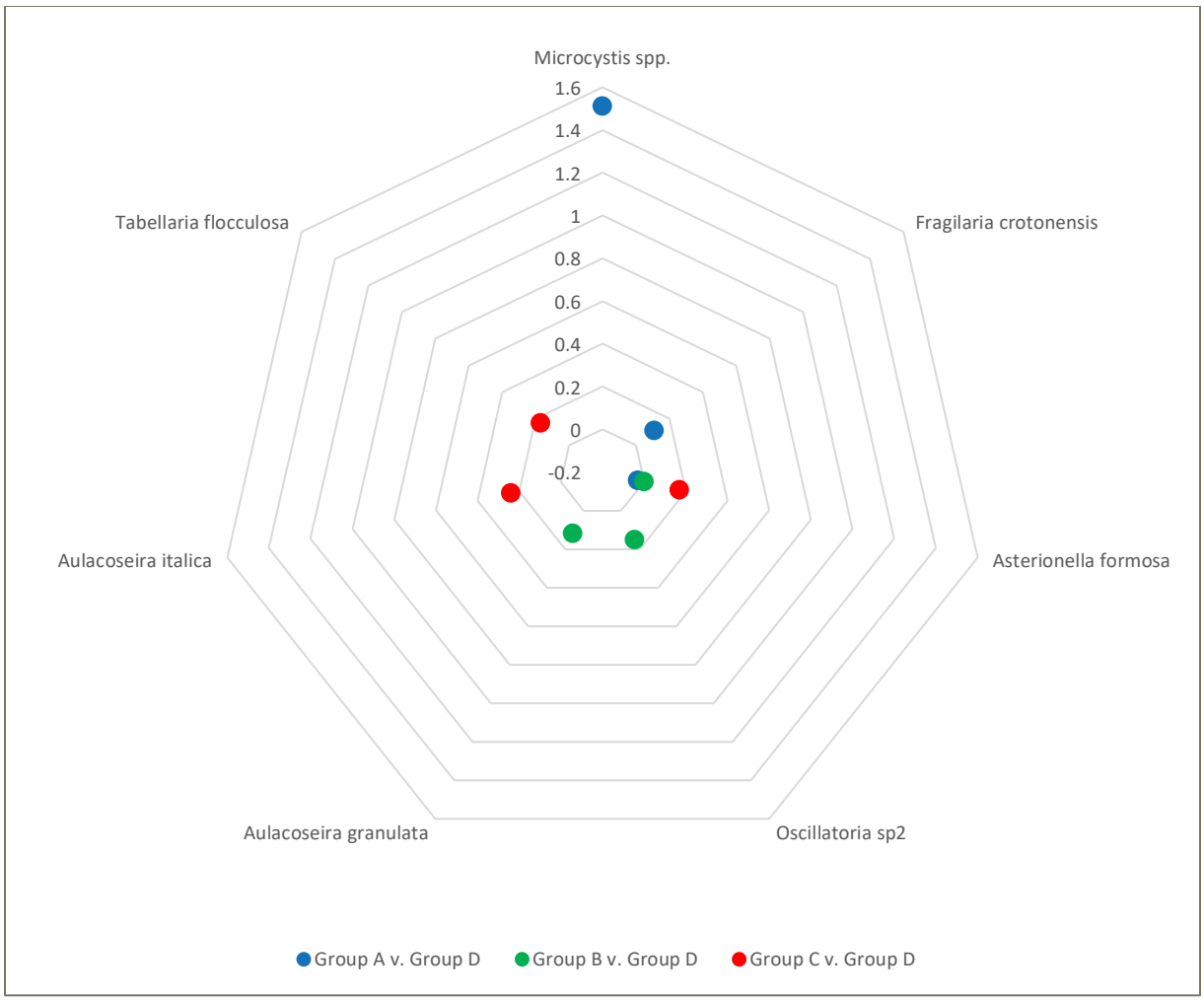

Figure 5-4. Average relative biovolume (mm^3/L) difference for taxa contributing to dissimilarity (Tubbs Hill Runoff)

5.1.2 University Point

The results from the Bray-Curtis analysis and subsequent SIMPROF test for samples collected from University Point during the runoff lake function are presented in Figure 5-5. The SIMPROF results indicate six distinct groups of samples. Samples contained in Group A were the least similar to the majority of the samples and were all collected during the spring of 2008. Samples from Group B and C were collected during the 2011 and 2012 sampling efforts. Samples from Groups D and E were most similar to those in Group F (Major Group).

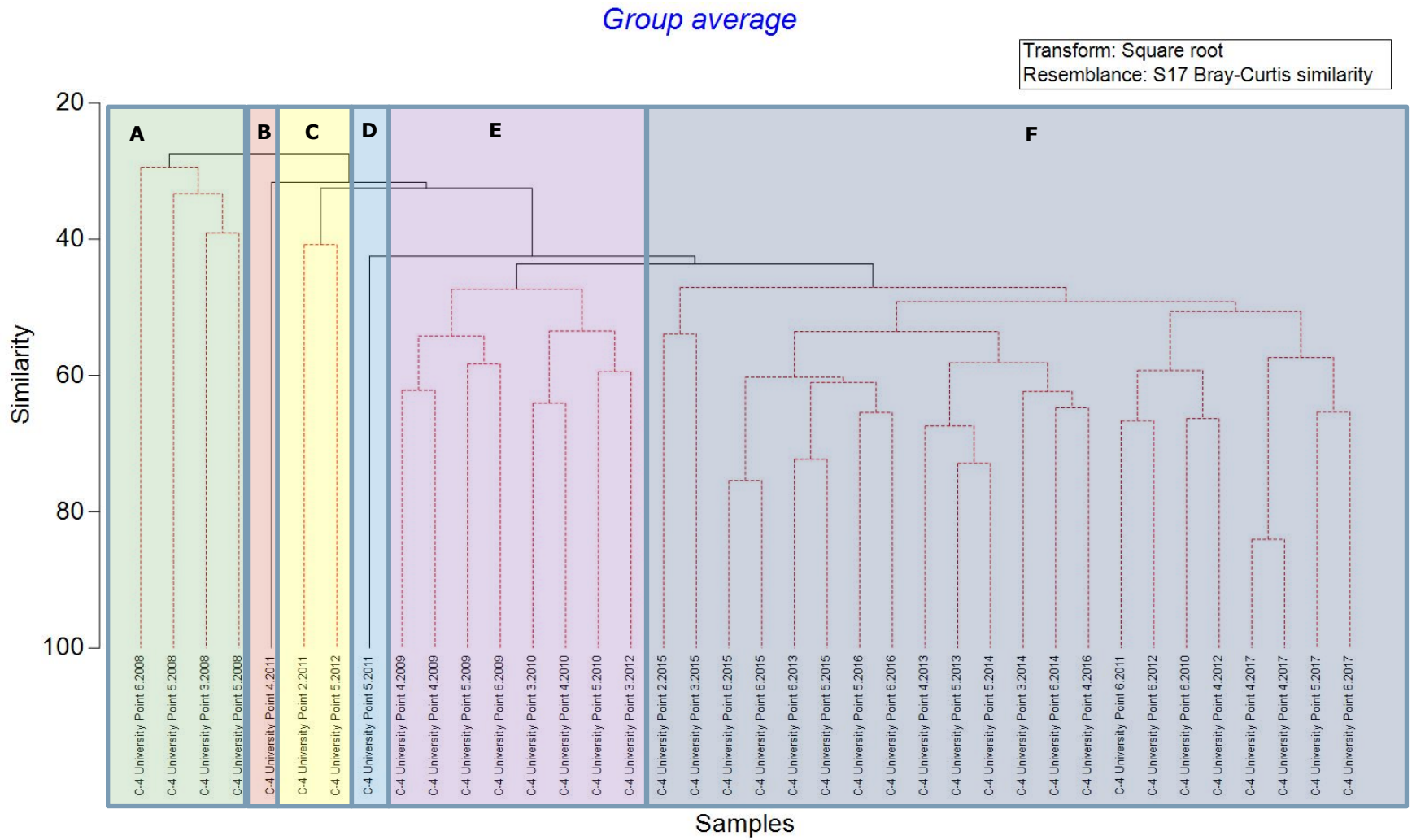

Figure 5-5. University Point Runoff Sample Group Designations Based on Similarity Grouping

Results of the correlation analysis for samples collected during the runoff lake function period at University Point are provided in Figure 5-6. These results indicated Chlorophyll has a "good" correlation (vector 0.68) with the positioning of the samples in the nMDS plot. Individual axis correlations for each analyte are provided in Table 5-4. Figure 5-7 illustrates the concentrations of chlorophyll measured for each sample. The Bray-Curtis sampling groupings are also provided to indicate the samples which demonstrated significant differences during the phytoplankton community analysis.

Table 5-4. University Point Runoff correlation results for individual nMDS axes and combined vertex length

	Diss. Zn	Diss. Cd	Diss. Pb	TP	TN	Chl.	sTIN	sRP	TN:TP	sTIN: sRP
MDS1 (x)	-0.19	-0.16	-0.04	0.05	-0.32	-0.60	-0.13	0.41	-0.33	-0.39
MDS2 (y)	0.14	0.33	0.45	0.30	0.06	-0.33	0.17	0.27	-0.29	0.03
Vector	0.24	0.37	0.45	0.30	0.33	0.68	0.21	0.49	0.44	0.39

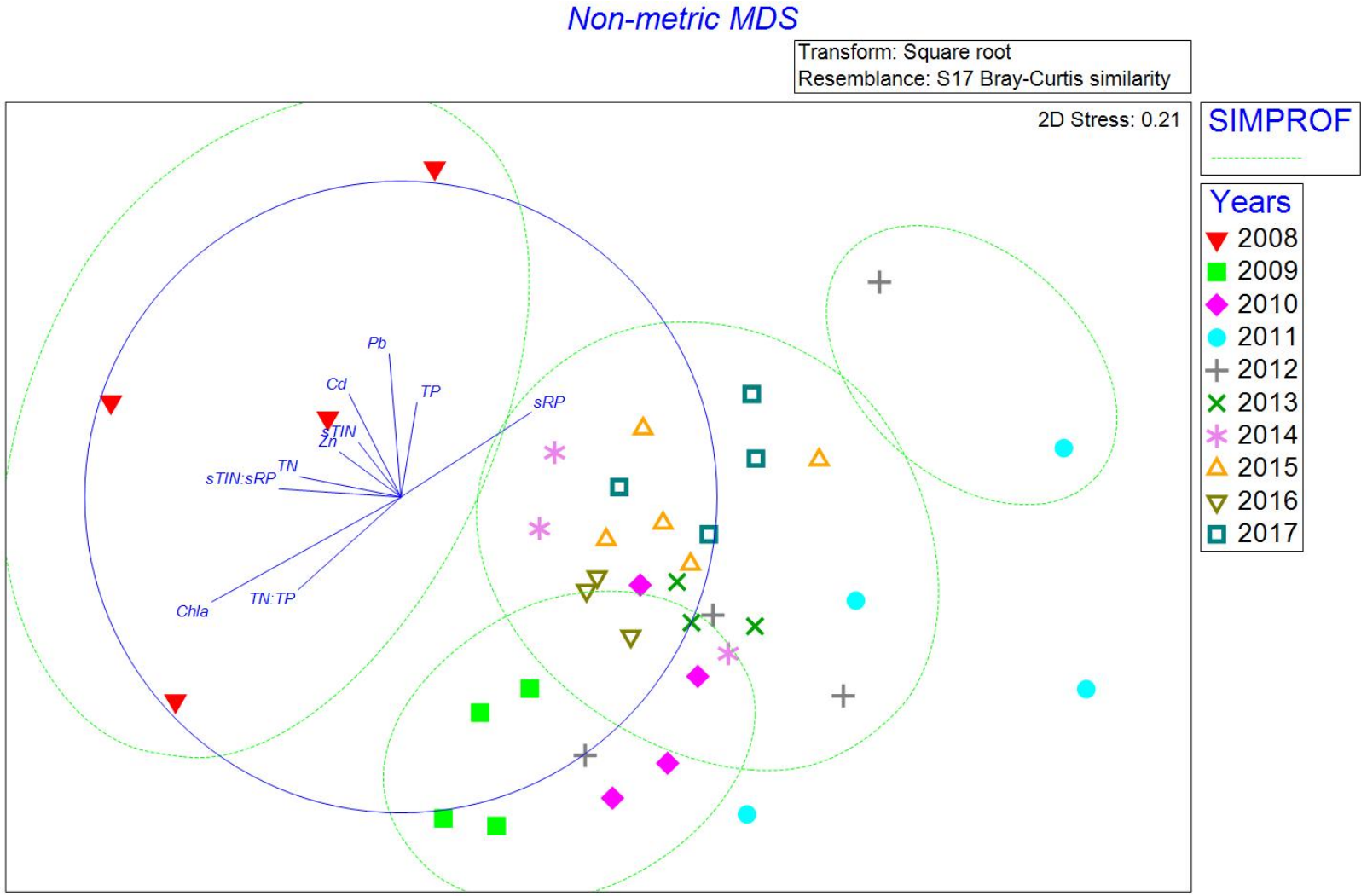

Figure 5-6. University Point Station Runoff Vector Correlation Results

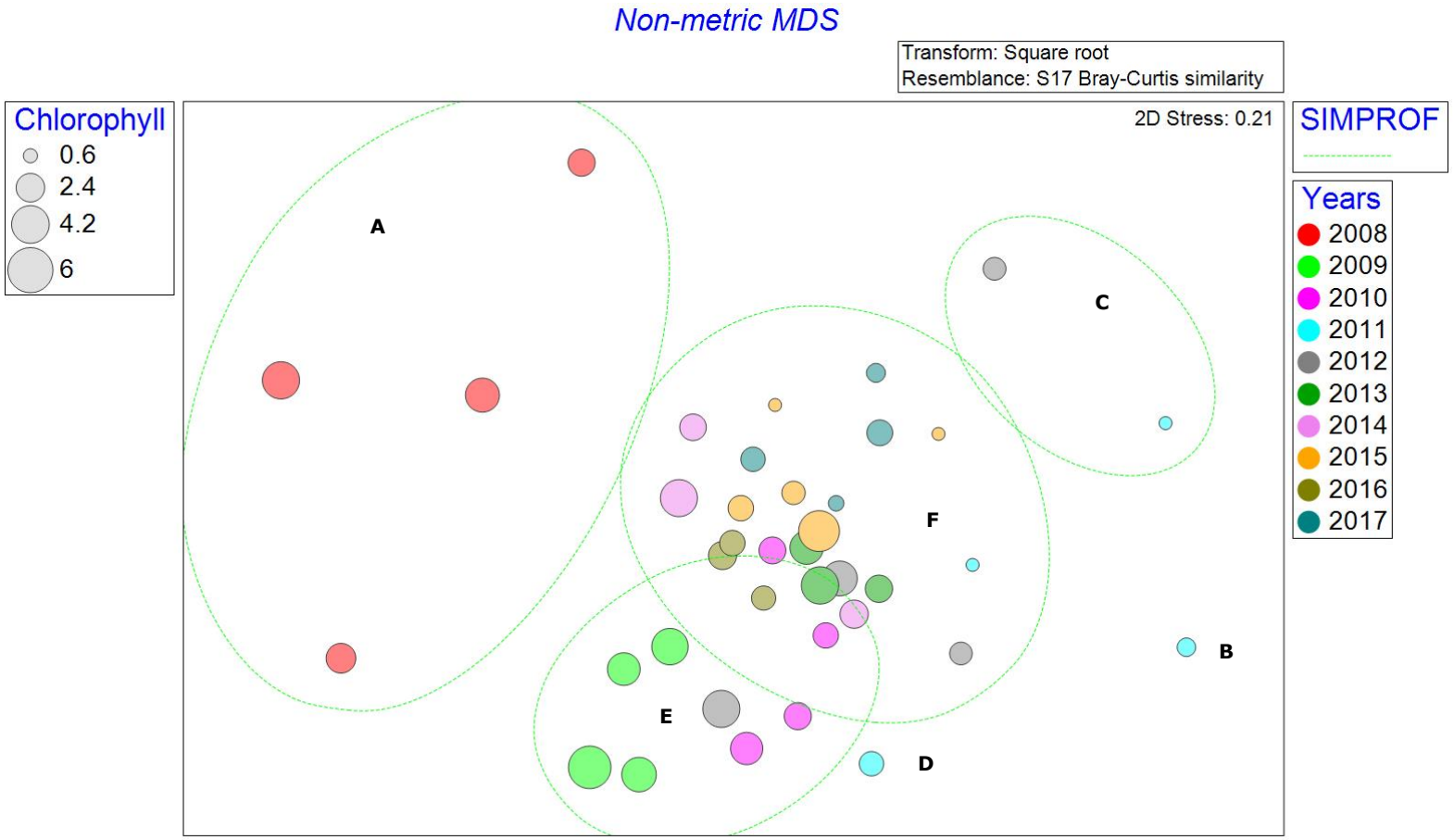

Figure 5-7. University Point Runoff Sample Bubble Plot Indicating Concentration of Selected Analytes. (Alpha codes indicate SIMPER Groupings)

The SIMPER results for in-group similarity for the samples collected during the runoff lake function period at University Point are provided in Table 5-5. Sample Group B and D only contained one sample so in-group similarity could not be assessed.

Cryptomonas spp. was the taxon that contributed the greatest to the similarity (17.4%) within sample Group A while the taxon identified as Small microflagellates spp. contributed the greatest (35.7%) to the similarity of samples within sample Group C. *Asterionella formosa* was the taxon that contributed the greatest similarity to both sample Group E (20.1%) and Group F (9.8%).

Table 5-5. SIMPER Results University Point Runoff In-group Similarity

Group (% Similarity)	Taxa	Phytoplankton Type	Average Relative Biovolume	Similarity/ Standard Deviation	Contribution %	Cumulative %
Group A (32.3%)	*Cryptomonas spp.*	Flagellates	0.19	3.04	17.4	17.4
	Asterionella formosa	Diatom	0.20	2.86	15.4	32.8
	Small microflagellates spp.	Flagellate	0.14	2.63	15.1	47.9
Group B[1]						
Group C (40.8%)	Small microflagellates spp.	Flagellate	0.15	*	35.7	35.7
	Synechococcus spp. (coccoid)	Blue-green	0.14	*	35.1	70.9
Group D[1]						
Group E (51.2%)	*Asterionella formosa*	Diatom	0.30	4.44	20.1	20.1
	Aulacoseira italica	Diatom	0.29	2.20	14.6	34.7
	Cryptomonas spp.	Flagellate	0.16	1.60	9.6	44.2
Group F *Major Group* (52.0%)	*Asterionella formosa*	Diatom	0.18	1.19	9.8	9.8
	Small Microflagellates spp.	Flagellate	0.13	5.07	9.5	19.4
	Cryptomonas spp.	Flagellate	0.14	2.17	9.4	28.7

[1] Group contains one sample. SIMPER in-group similarity not calculatable.
* Group contains two samples. Not able to calculate standard deviation.

Table 5-6 provides the selected results from the SIMPER comparison for between-group dissimilarity conducted on samples collected during the runoff lake function at the University Point sample station. The majority of the samples collected at University Point during the runoff lake function are found in sample Group F. The selected SIMPER results contain comparisons made between each sample group to sample Group F. A complete table of results is provided in Appendix B.

Although the SIMPER results indicate that sample Group D, E, and F are significantly different, they are most similar with each other. The major contributions of dissimilarity between these groups is based on the relative biovolume of the diatom, *Aulacoseira italica*. Sample Group D contains one sample collected in May of 2011, while sample Group E contains samples predominantly collected from 2009 and 2010. Sample Group F contains samples collected from 2010 to 2017. These results indicate a temporal shift in the *Aulacoseira italica* biovolume measurements decreasing in the more recent samples. A decrease in *Aulacoseira italica* could indicate either an impact to this taxon, which caused a reduction in biovolume from 2010 to 2017, or a bloom during the 2009 and 2010 sampling seasons.

The taxon contributing to most of the dissimilarity (7.7%) between sample Group A and F was a blue-green alga (*Anabaena spp.*) which indicated an increase of *Anabaena spp.* in sample Group A. The comparison between Group B and F and Group C and F indicate a diatom (*Asterionella formosa*) was the major contributor to the dissimilarity between these groups. These results indicate an decrease of *Asterionella formosa* in sample Group B and Group C when compared to Group F.

Table 5-6. SIMPER Results University Point Runoff Between-group Dissimilarity (Selected Results)

Group Comparison (% Dissimilarity)	Taxa	Phytoplankton Type	Group 1 Average Relative Biovolume	Group 2 Average Relative Biovolume	Dissimilarity / Standard Deviation	Contribution %	Cumulative %
Group A (1) to Group F (2) (71.5%)	*Anabaena spp.*	Blue-green	0.36	0.00	0.58	7.7	7.7
	Microcystis spp.	Blue-green	0.23	0.00	0.57	7.5	15.2
	Aulacoseira granulata	Diatom	0.16	0.04	0.84	5.0	20.2
Group B (1) to Group F (2) (65.1%)	*Asterionella formosa*	Diatom	0.00	0.18	1.55	8.8	8.8
	Cryptomoas spp.	Flagellate	0.00	0.14	2.54	6.8	15.5
	Small Microflagellates spp.	Flagellate	0.02	0.13	3.45	5.2	20.7
Group C (1) to Group F (2) (65.5%)	*Asterionella formosa*	Diatom	0.00	0.18	1.55	8.8	8.8
	Chroococcus spp.	Blue-green	0.00	0.10	0.93	4.5	13.3
	Gymnodinium spp.	Flagellate	0.00	0.09	1.46	4.4	17.7
Group D (1) to Group F (2) (57.4%)	*Aulacoseira italica*	Diatom	0.24	0.04	2.62	7.1	7.1
	Cyclotella comta	Diatom	0.23	0.05	2.29	6.3	13.4
	Cryptomonas spp.	Flagellate	0.00	0.14	2.54	4.8	18.2
Group E (1) to Group F (2) (56.3%)	*Aulacoseira italica*	Diatom	0.29	0.04	1.76	9.6	9.6
	Asterionella formosa	Diatom	0.30	0.18	1.02	6.4	16.0
	Cyclotella comta	Diatom	0.11	0.05	1.07	4.4	20.3

A radial plot is provided (Figure 5-8) which illustrates the difference in biovolume of each of the top three taxa that are contributing to the dissimilarity between the sample groups (A through E) and the Major Group (F). The comparison between sample Group A and the Major Group F shows the average relative biovolume of *Anabaena spp.* is greater in sample Group A. All of the top three taxa driving the dissimilarity between Group C and the Major Group are shown to have less average relative biomass.

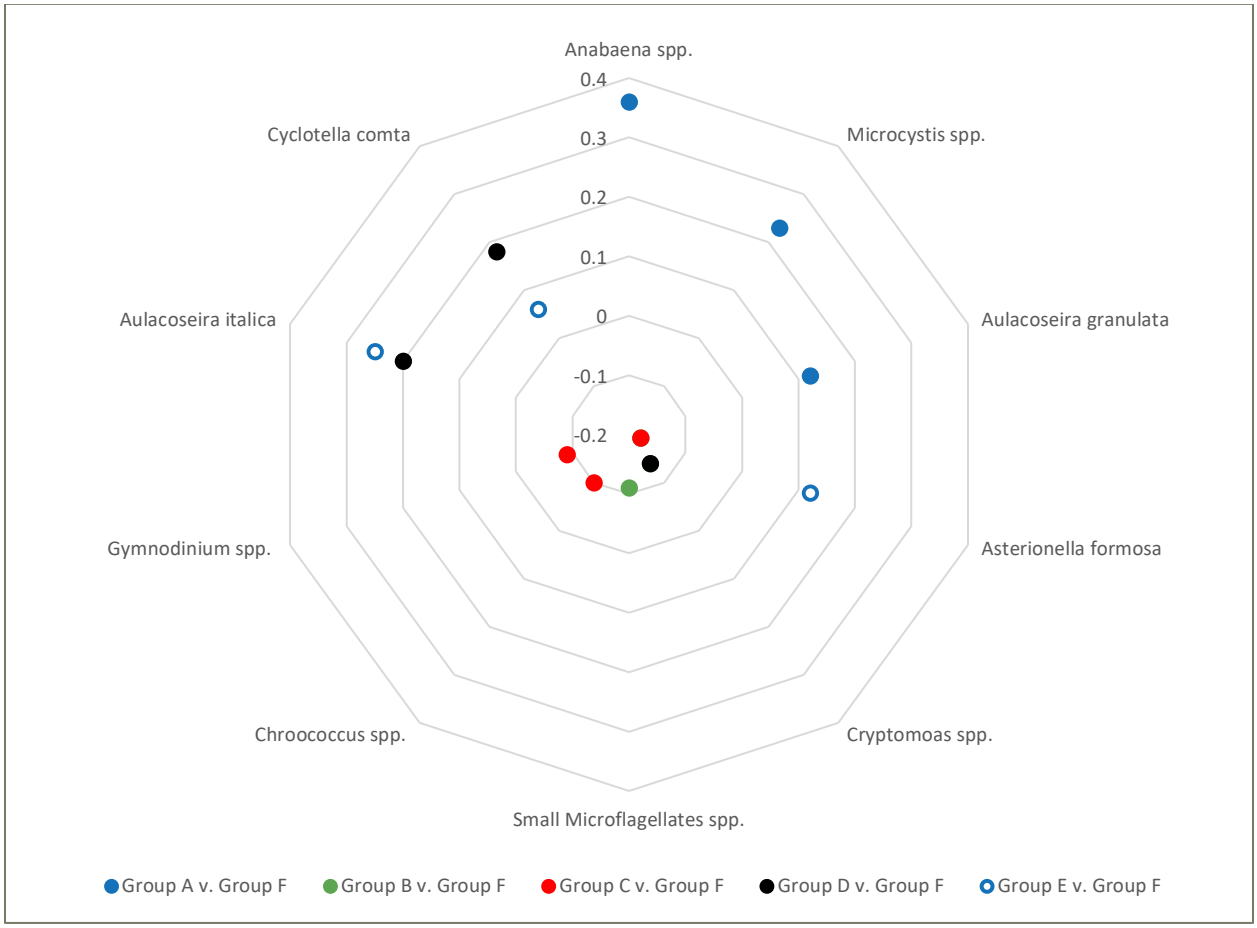

Figure 5-8. Average relative biovolume (mm^3/L) difference for taxa contributing to dissimilarity (University Point Runoff)

5.2 Warm Stratified Lake Function

5.2.1 Tubbs Hills

The results from the Bray-Curtis analysis and subsequent SIMPROF test for samples collected from Tubbs Hill during the warm stratified lake function period are presented in Figure 5-9. The SIMPROF results indicate eight distinct groups of samples. The majority of the phytoplankton community results during the warm stratified period are not significantly different from each other and are contained in Group H (Major Group). These samples represent multiple field efforts and were collected from 2009 to 2017. Sample Group A, B, and C were all collected during the 2007 sampling effort. Samples in Group D were collected during the 2008. Although sample Group E/F (collected in 2009 and 2017) and Group G (collected in 2016 and 2017) were more similar to the Major Group samples, this analysis did indicate that there were significant differences between these groups.

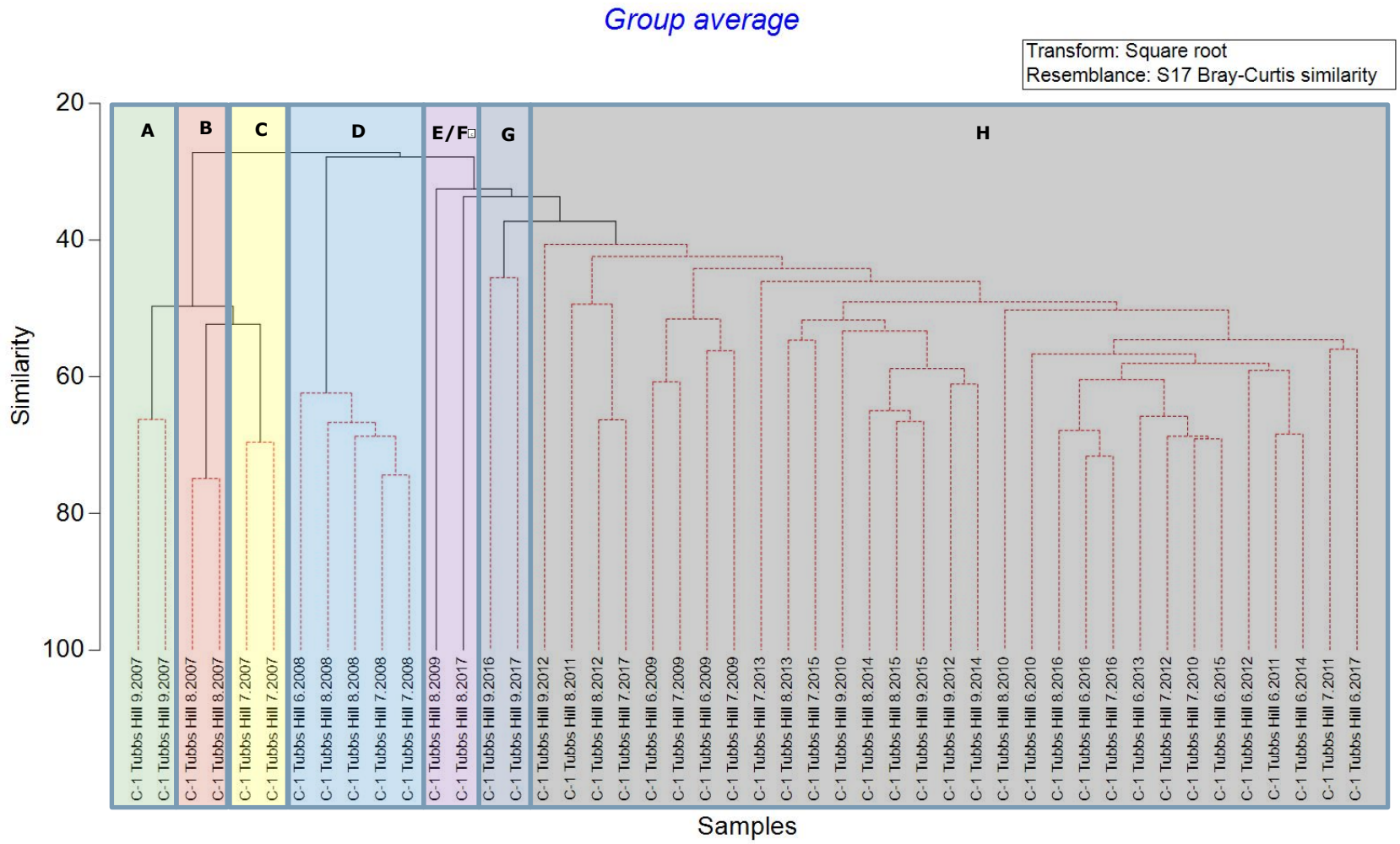

Figure 5-9. Tubbs Hill Warm Stratified Sample Group Designations Based on Similarity Grouping

Results of the correlation analysis for samples collected during the warm stratified lake function period at Tubbs Hill are provided in Figure 5-10. These results indicate three analytes have the best correlations with the positioning of the samples in the nMDS plot (dissolved cadmium, total nitrogen, and chlorophyll). Individual axis correlations for each analyte are provided in Table 5-7. Figure 5-11 illustrates the concentrations of the three analytes for each sample. The Bray-Curtis sampling groupings are also provided to indicate the samples which demonstrated significant differences during the phytoplankton community analysis.

Table 5-7. Tubbs Hill Warm Stratified correlation results for individual nMDS axes and combined vertex length

	Diss. Zn	Diss. Cd	Diss. Pb	TP	TN	Chl.	sTIN	sRP	TN:TP	sTIN: sRP
MDS1 (x)	0.51	0.52	0.28	-0.19	-0.57	0.46	0.13	-0.18	-0.32	0.14
MDS2 (y)	-0.03	-0.33	-0.37	-0.15	-0.36	-0.45	-0.25	-0.02	-0.18	-0.25
Vector	0.51	0.62	0.46	0.24	0.67	0.64	0.28	0.18	0.37	0.29

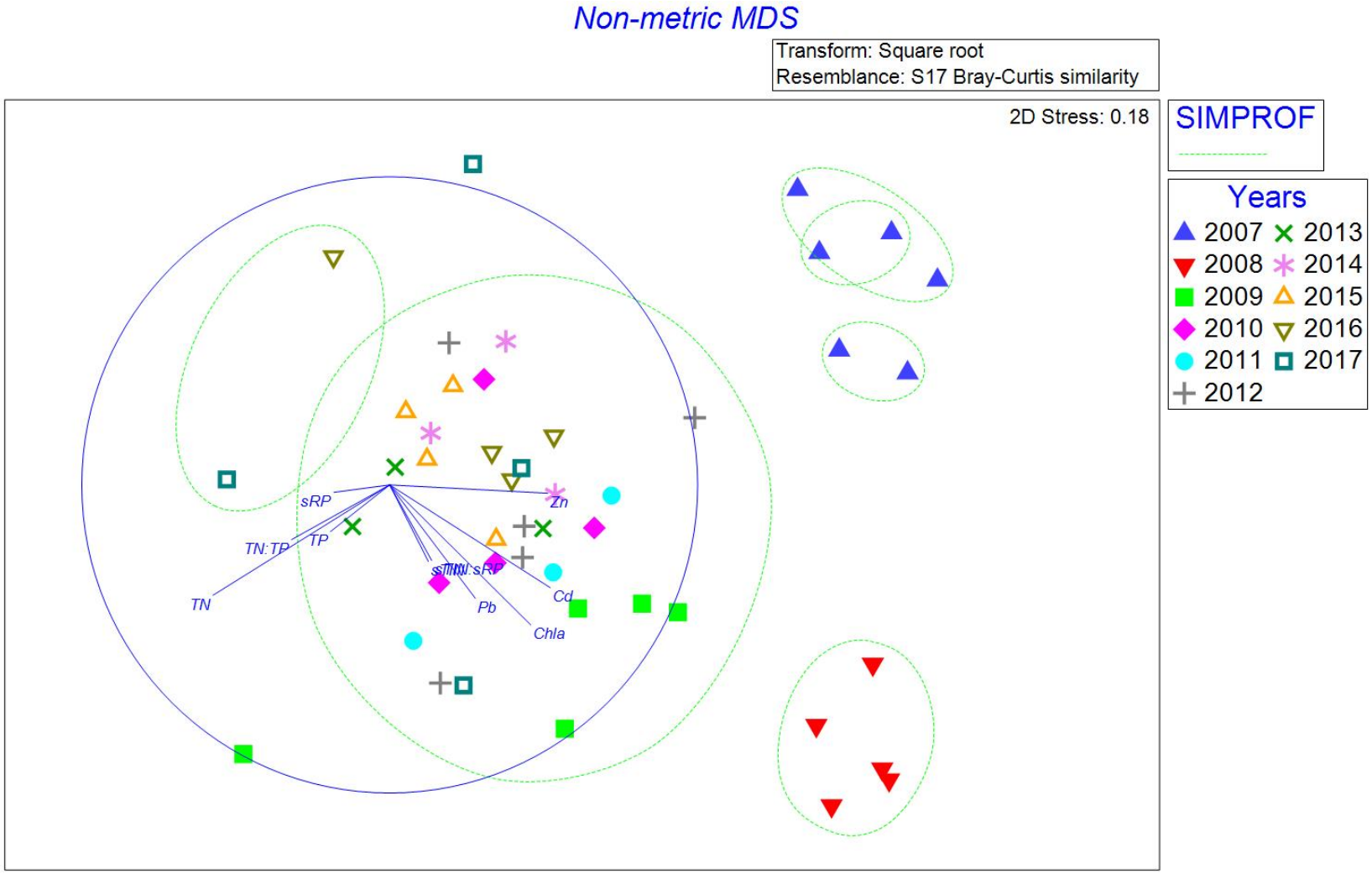

Figure 5-10. Tubbs Hill Station Warm Stratified Vector Correlation Results

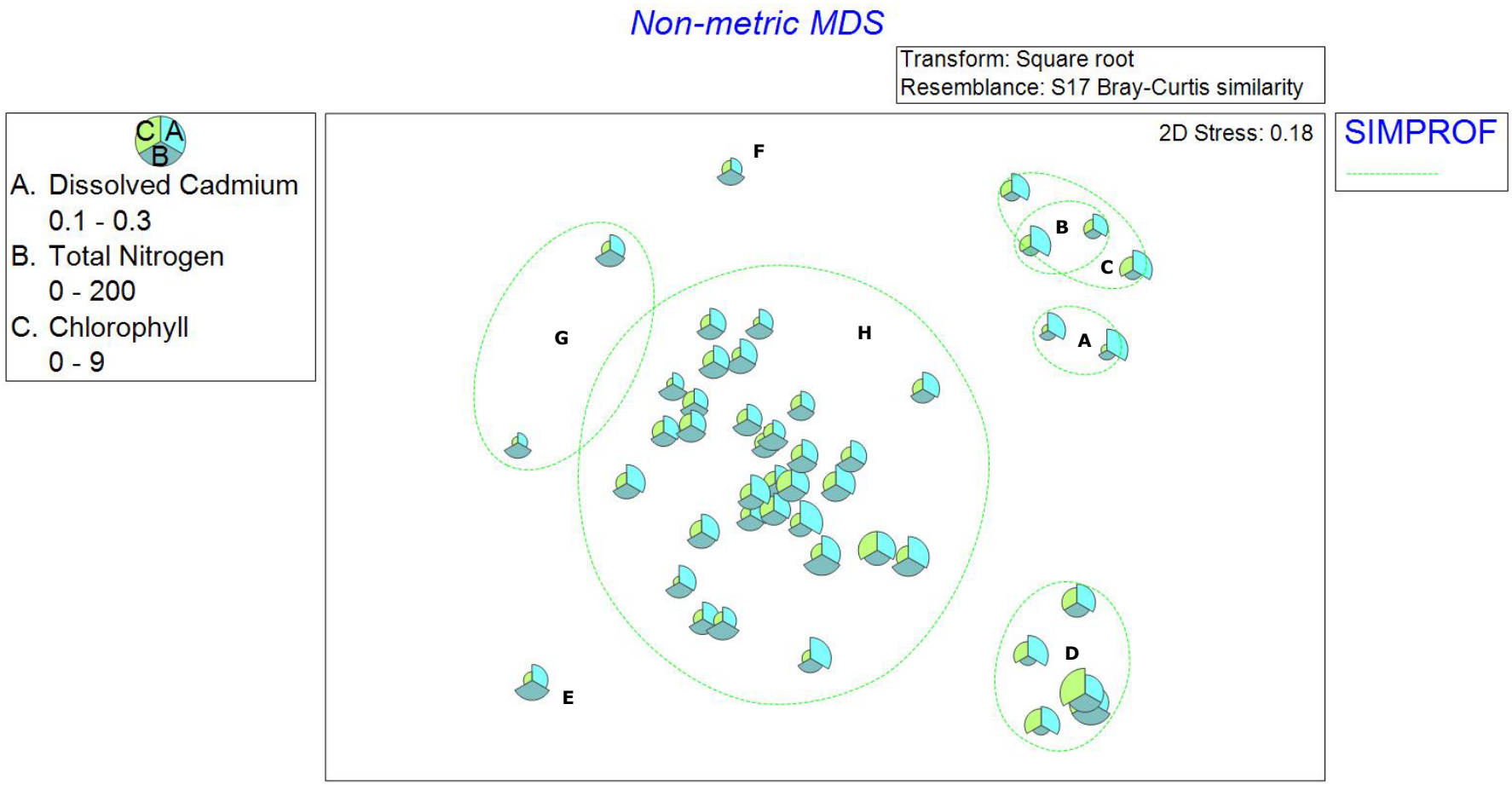

Figure 5-11. Tubbs Hill Warm Stratified Sample Bubble Plot Indicating Concentration of Selected Analytes. (Alpha codes indicate SIMPER Groupings)

The SIMPER results for in-group similarity for the samples collected during the warm stratified lake function period at Tubbs Hill are provided in Table 5-8. Sample Group E and F only contained one sample therefore in-group similarity could not be assessed.

SIMPER results indicate that *Tabellaria fenestrata* was the taxon that contributed the greatest to the similarity to both sample Group A (25.2%) and Group D (41.3%). Willea spp. was identified as the taxon driving most of the similarity of samples in Group B (14.6%) while *Cryptomonas spp*., *Chroococcus spp*., and *Asterionella formosa* were the taxa driving the similarity in Group C (15.8%), Group G (14.3%), and Group H (16.3%), respectively.

Table 5-8. SIMPER Results Tubbs Hill Warm Stratified In-group Similarity

Group (% Similarity)	Taxa	phytoplankton Type	Average Relative Biovolume	Similarity / Standard Deviation	Contribution %	Cumulative %
Group A (66.3%)	*Tabellaria fenestrata*	Diatom	0.41	*	25.2	25.2
	Asterionella formosa	Diatom	0.21	*	13.1	38.3
	Cryptomonas spp.	Flagellate	0.17	*	9.5	47.9
Group B (74.9%)	*Willea spp.*	Chlorophyte	0.28	*	14.6	14.6
	Crytomonas spp.	Flagellate	0.24	*	11.3	25.9
	Planktosphaeria spp.	Chlorophyte	0.21	*	10.6	36.5
Group C (69.6%)	*Cryptomonas spp.*	Flagellate	0.39	*	15.8	15.8
	Asterionella formosa	Diatom	0.23	*	12.6	28.4
	Oocystis spp.	Chlorophyte	0.14	*	10.0	38.3
Group D (66.2%)	*Tabellaria fenestrata*	Diatom	0.91	8.1	41.3	41.3
	Asterionella formosa	Diatom	0.45	4.4	18.2	59.5
	Cryptomonas spp.	Flagellate	0.21	2.4	9.1	68.6
Group E[1]						
Group F[1]						
Group G (45.5%)	*Chroococcus spp.*	Blue-green	0.12	*	14.3	14.3
	Ochromonas spp.	Flagellate	0.08	*	11.1	25.4
	Synechococcus spp. (rod)	Blue-green	0.06	*	9.6	34.9
Group H *Major Group* (48.4%)	*Asterionella formosa*	Diatom	0.19	1.8	16.3	16.3
	Cryptomonas spp.	Flagellate	0.12	1.8	11.0	27.3
	Small Microflagellates spp.	Flagellate	0.09	5.1	10.1	37.3

[1] Group contains one sample. SIMPER in-group similarity not calculatable.
* Group contains two samples. Not able to calculate standard deviation.

Selected results from the SIMPER comparison for between-group dissimilarity conducted on samples collected during the warm stratified lake function at station Tubbs Hill are provided in Table 5-9. This table was truncated to provide only the results that compared each sample grouping to Group H, which contained the majority of the samples. A complete table of results is provided in Appendix B.

The taxon contributing to the majority of the dissimilarity (14.7%) between sample Group A and H was a diatom (*Tabellaria fenestrata.*) which indicated an increase of *Tabellaria fenestrata*. in samples from Group A. The comparison between Group B and H indicated a chlorophyte (*Willea spp.*) contributed the greatest to the dissimilarity (9.3%) between these groups where there was an increase of *Willea spp.* in sample Group B. The SIMPER results indicate that *Cryptomonas spp*. is the taxon that is contributing the most dissimilarity between Group C and H. while *Microcystis spp*., *Asterionella formosa*, *Amphidinium spp*., and *Asterionella formosa* were the taxa contributing to the dissimilarity between Group D, E, F and G, respectively to Group H.

Table 5-9. SIMPER Results Tubbs Hill Warm Stratified Between-group Dissimilarity (Selected Results)

Group Comparison (% Dissimilarity)	Taxa	Phytoplankton Type	Group 1 Average Relative Biovolume	Group 2 Average Relative Biovolume	Dissimilarity / Standard Deviation	Contribution %	Cumulative %
Group A (1) to Group H (2) (72.1%)	*Tabellaria fenestrata*	Diatom	0.41	0.00	7.8	14.7	14.7
	Planktosphaeria spp.	Chlorophyte	0.33	0.04	1.1	10.9	25.6
	Staurodesmus spp.	Chlorophyte	0.10	0.00	5.9	3.6	29.2
Group B (1) to Group H (2) (73.2%)	*Willea spp.*	Chlorophyte	0.28	0.00	8.3	9.3	9.3
	Tabellaria fenestrata	Diatom	0.21	0.00	8.1	7.0	16.2
	Planktosphaeria spp.	Chlorophyte	0.21	0.04	3.1	6.2	22.5
Group C (1) to Group H (2) (68.7%)	*Cryptomonas spp.*	Flagellate	0.39	0.12	1.6	10.8	10.8
	Rhodomonas spp.	Flagellate	0.15	0.00	4.7	5.9	16.7
	Gymnodinium spp.	Dinoflagellate	0.15	0.00	4.8	5.9	22.6
Group D (1) to Group H (2) (70.9%)	*Microcystis spp.*	Blue-green	0.91	0.01	3.2	29.4	29.4
	Asterionella formosa	Diatom	0.45	0.19	1.2	8.9	38.3
	Cryptomonas spp.	Flagellate	0.21	0.12	1.4	3.5	41.8
Group E (1) to Group H (2) (67.4%)	*Asterionella formosa*	Diatom	0.05	0.19	2.0	9.0	9.0
	Cryptomonas spp.	Flagellate	0.00	0.12	2.1	7.6	16.5
	Chroococcus spp.	Blue-green	0.00	0.08	1.3	4.9	21.4
Group F (1) to Group H (2) (66.3%)	*Amphidinium spp.*	Dinoflagellate	0.36	0.00	7.4	15.0	15.0
	Euglena spp.	Euglenoid	0.35	0.02	4.2	14.2	29.2
	Planktosphaeria spp.	Chlorophyte	0.33	0.04	3.2	12.6	41.8
Group G (1) to Group H (2) (62.8%)	*Asterionella formosa*	Diatom	0.00	0.19	2.1	10.4	10.4
	Cryptomonas spp.	Flagellate	0.00	0.12	2.0	6.7	17.1
	Euglena spp.	Euglenoid	0.12	0.02	1.0	6.0	23.1

A radial plot is provided (Figure 5-12) which illustrates the difference of biovolume of each of the top three taxa that are contributing to the dissimilarity between the sample groups (A through G) and the Major Group (H). The comparison between sample Group D and the Major Group H shows the average relative biovolume of *Microcystis spp.* to be greater in sample Group D.

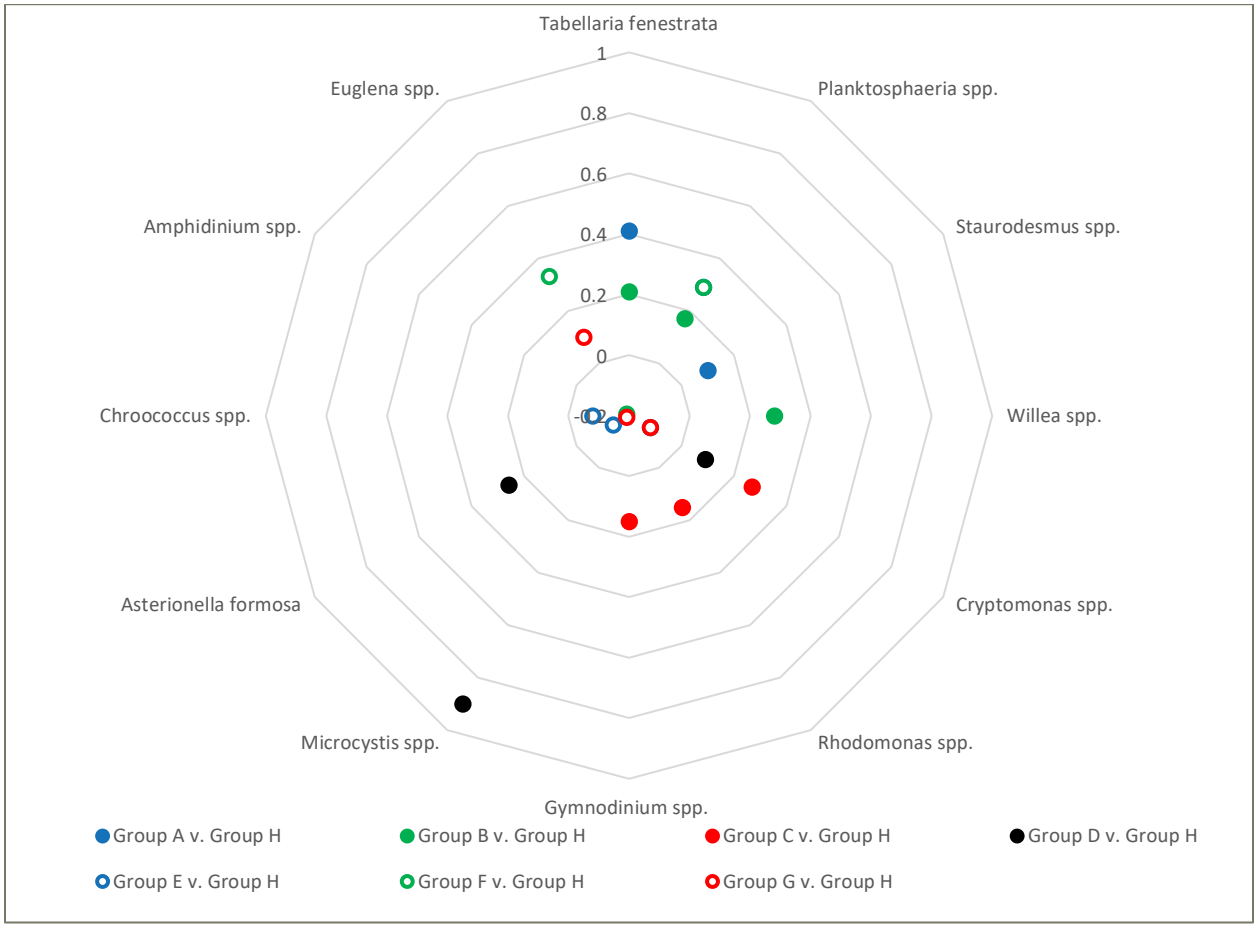

Figure 5-12. Average relative biovolume (mm^3/L) difference for taxa contributing to dissimilarity (Tubbs Hill Warm Stratified)

5.2.2 University Point

The results from the Bray-Curtis analysis and subsequent SIMPROF test for samples collected from University Point during the warm stratified lake function period are presented in Figure 5-13. The SIMPROF results indicate seven distinct groups of samples. The majority of the phytoplankton community results during the warm stratified period are not significantly different from each other and are contained in Group G (Major Group). These sample represent multiple sampling efforts and were collected from 2009 to 2017. The sample from Group A was collected in 2017 while samples collected from Group B, C, and D were all collected in 2007. Samples from Group E were collected in 2008 and Group F represents a sample from 2011 and one from 2012.

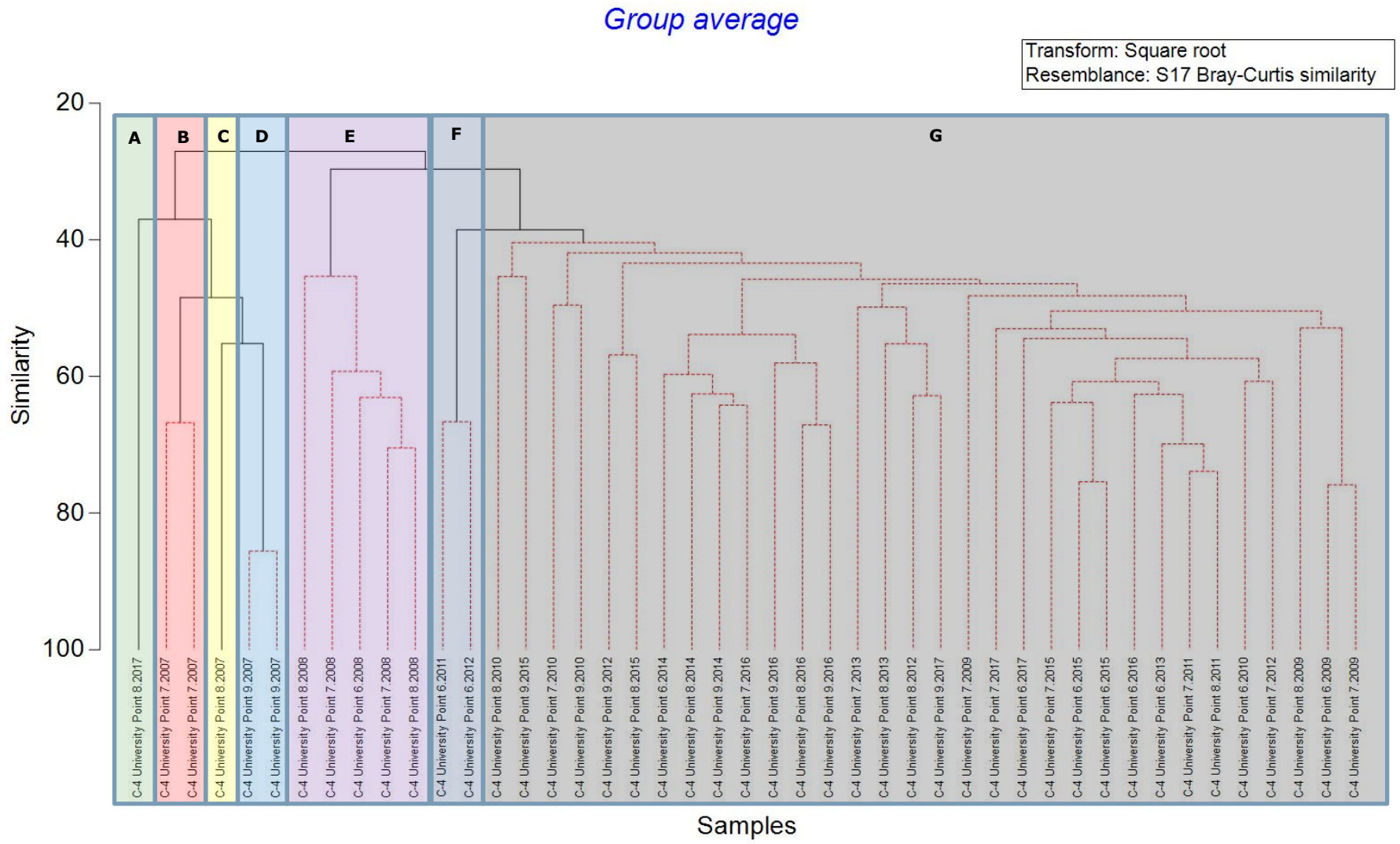

Figure 5-13. University Point Warm Stratified Sample Group Designations Based on Similarity Grouping

Results of the correlation analysis for samples collected during the warm stratified lake function period at University Point are provided in Figure 5-14. These results indicate chlorophyll has the best correlation (0.59) with the positioning of the samples in the nMDS plot. Individual axis correlations for each analyte are provided in Table 5-10. Figure 5-15 illustrates the concentrations of chlorophyll for each sample. The Bray-Curtis sampling groupings are also provided to indicate the samples which demonstrated significant differences during the phytoplankton community analysis.

Table 5-10. University Point Warm Stratified correlation results for individual nMDS axes and combined vertex length

	Diss. Zn	Diss. Cd	Diss. Pb	TP	TN	Chl.	sTIN	sRP	TN:TP	sTIN: sRP
MDS1 (x)	-0.17	0.07	-0.08	-0.06	0.28	-0.27	0.02	0.16	0.29	-0.05
MDS2 (y)	-0.28	-0.32	-0.48	-0.13	-0.30	-0.53	-0.18	0.09	-0.18	-0.20
Vector	0.33	0.33	0.49	0.14	0.41	0.59	0.18	0.18	0.34	0.21

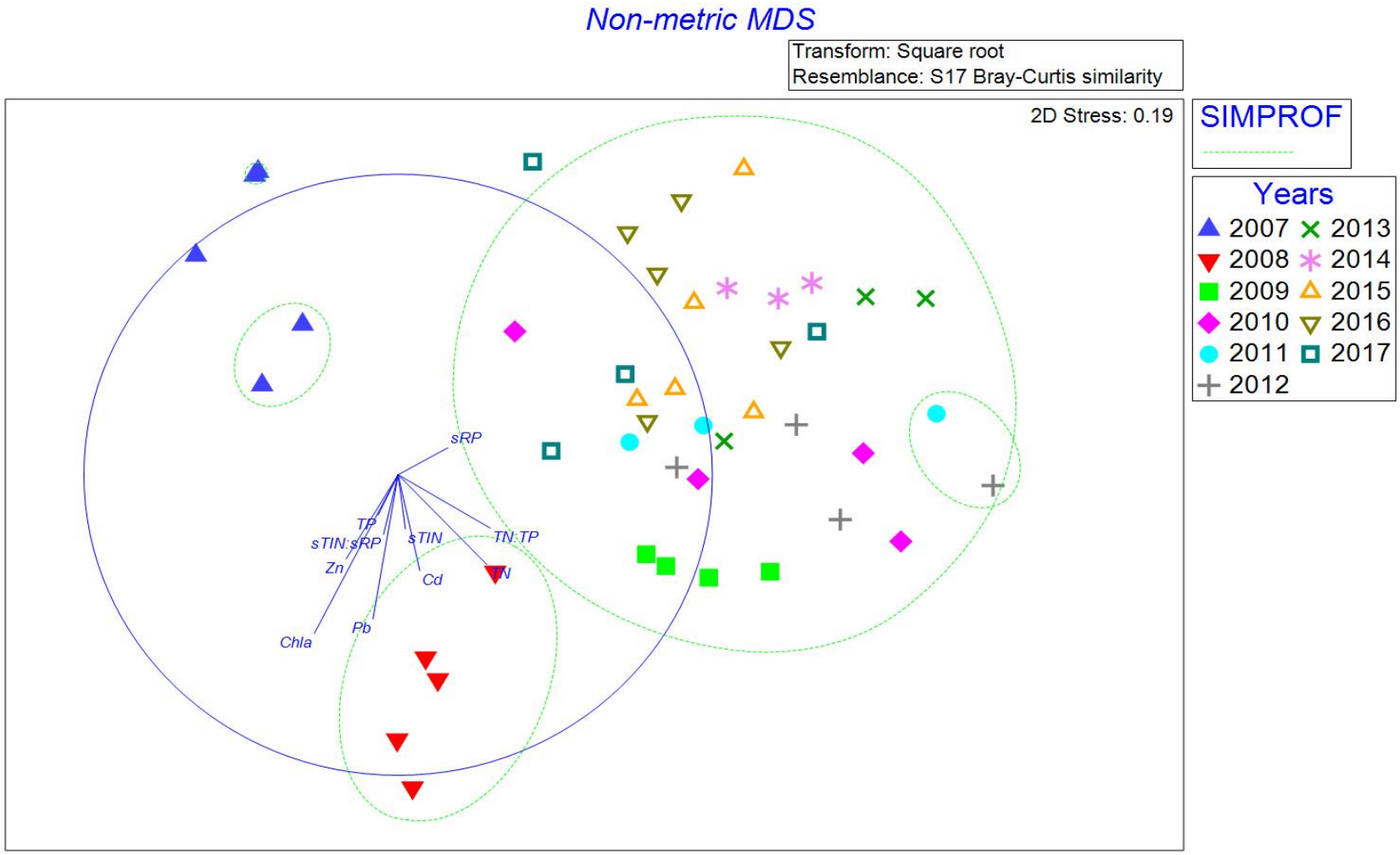

Figure 5-14. University Point Station Warm Stratified Vector Correlation Results

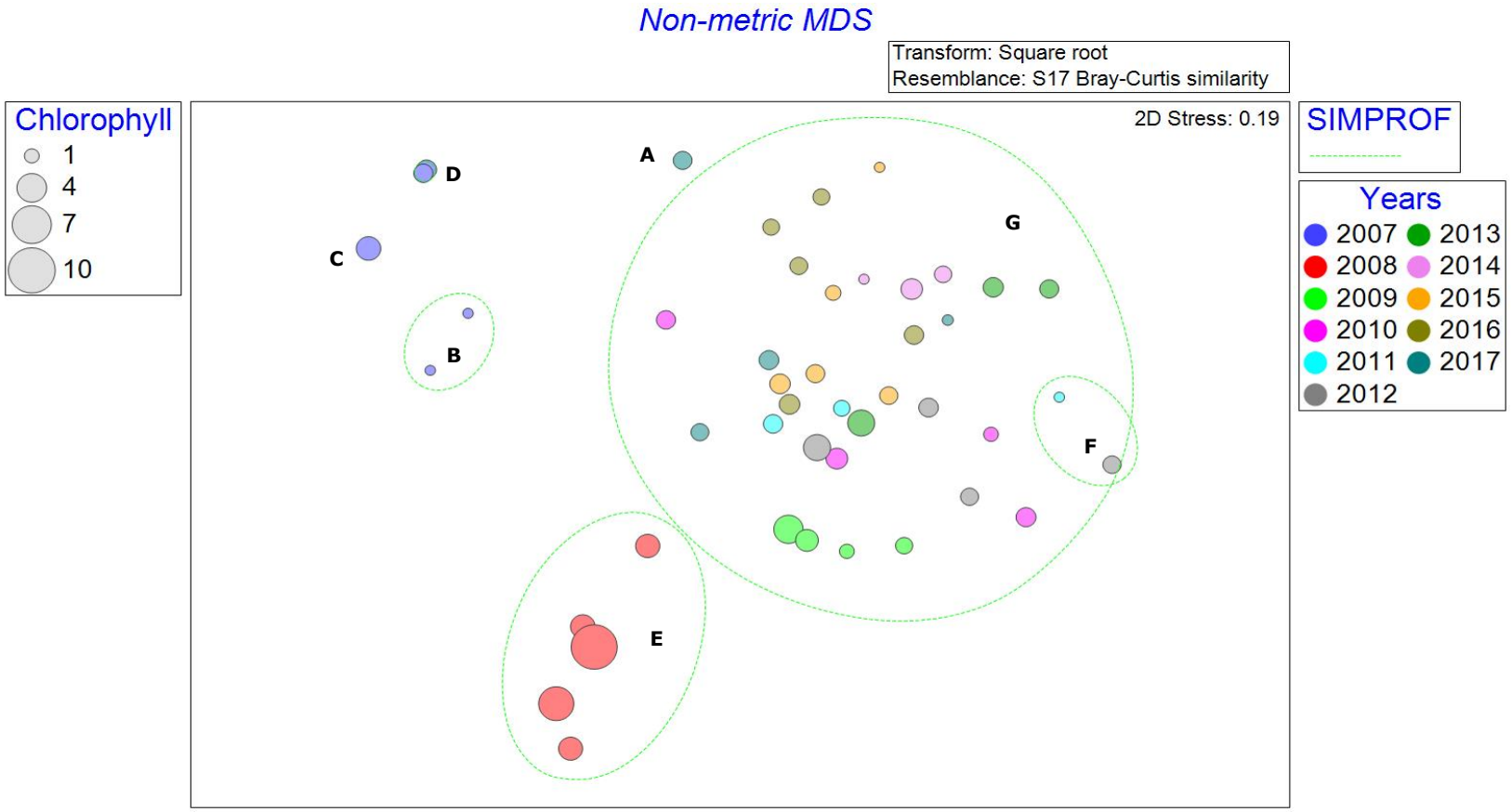

Figure 5-15. University Point Warm Stratified Sample Bubble Plot Indicating Concentration of Selected Analytes (Alpha codes indicate SIMPER Groupings)

The SIMPER results for in-group similarity for the samples collected during the warm stratified lake function period at University Point are provided in Table 5-11. Sample Group A and C only contained one sample therefore in-group similarity could not be assessed.

SIMPER results indicate that *Asterionella formosa* was the taxon that contributed the greatest to the similarity to both sample Group B (18.7%) and Group G (11.5%). *Planktosphaeria spp.* was identified as the taxon driving most of the similarity of samples in Group D (20.7%) while *Microcystis spp*. and *Aulacoseira granulata* were the taxa driving the similarity in Group E (27.6%), and Group F (14.5%), respectively.

Table 5-11. SIMPER Results University Point Warm Stratified In-group Similarity

Group (% Similarity)	Taxa	Phytoplankton Type	Average Relative Biovolume	Similarity/ Standard Deviation	Contribution %	Cumulative %
Group A[1]						
Group B (66.8%)	*Asterionella formosa*	Diatom	0.38	*	18.7	18.7
	Cryptomonas spp.	Flagellate	0.32	*	17.8	36.5
	Planktosphaeria spp.	Chlorophyte	0.20	*	10.6	47.1
Group C[1]						
Group D (85.6%)	*Planktosphaeria spp.*	Chlorophyte	0.66	*	20.7	20.7
	Tabellaria fenestrata	Diatom	0.57	*	20.2	40.8
	Asterionella formosa	Diatom	0.18	*	6.8	47.6
Group E (55.6%)	*Microcystis spp.*	Blue-green	0.72	1.1	27.6	27.6
	Cryptomonas spp.	Flagellate	0.22	7.4	15.3	42.9
	Asterionella formosa	Diatom	0.28	2.7	14.5	57.4
Group F (66.6%)	*Aulacoseira granulata*	Diatom	0.17	*	14.5	14.5
	Cyclotella comta	Diatom	0.13	*	8.6	23.0
	Small Microflagellates spp.	Flagellate	0.09	*	8.0	31.0
Group G *Major Group* (47.0%)	*Asterionella formosa*	Diatom	0.16	1.2	11.5	11.5
	Cryptomonas spp.	Flagellate	0.13	1.5	11.4	22.9
	Small Microflagellates spp.	Flagellate	0.10	4.7	10.1	33.0

[1] Group contains one sample. SIMPER in-group not calculatable.
* Group contains two samples. Not able to calculate standard deviation.

Selected results from the SIMPER comparison for between-group dissimilarity conducted on samples collected during the warm stratified lake function at station University Point are provided in Table 5-12. This table was truncated to provide only the results that compared each sample grouping to the majority of the sample in Group G. A complete table of results is provided in Appendix B.

The taxon contributing to the majority of the dissimilarity (28.6%) between sample Group A and G was a chlorophyte (*Planktosphaeria spp.*) which indicated an increase of *Planktosphaeria spp*. in samples from Group A. The comparison between Group B and G indicated a diatom (*Asterionella formosa*) contributed the greatest to the dissimilarity (7.6%) between these groups where there was an increase of *Asterionella formosa* in sample Group A. The SIMPER results indicate that *Willea spp*. is the taxon that is contributing the most dissimilarity between Group C while *Planktosphaeria spp.*, *Microcystis spp.*, and *Aulacoseira granulata* were the taxa contributing to the dissimilarity between Group D, E, and F respectively to Group H. Table 5-12 contains additional details regarding which sample group demonstrated an increase in these taxa.

Table 5-12. SIMPER Results University Point Warm Stratified Between-group Dissimilarity (Selected Results)

Group Comparison (% Dissimilarity)	Taxa	Phytoplankton Type	Group 1 Average Relative Biovolume	Group 2 Average Relative Biovolume	Dissimilarity / Standard Deviation	Contribution %	Cumulative %
Group A (1) to Group G (2) (63.7%)	*Planktosphaeria spp.*	Chlorophyte	0.72	0.03	5.4	28.6	28.6
	Aphanothece minutissimus	Blue-green	0.14	0.02	3.0	5.2	33.8
	Asterionella formosa	Diatom	0.09	0.16	1.6	4.1	37.9
Group B (1) to Group G (2) (71.4%)	*Asterionella formosa*	Diatom	0.38	0.16	1.7	7.6	7.6
	Planktosphaeria spp.	Chlorophyte	0.20	0.03	4.3	6.2	13.7
	Cryptomonas spp.	Flagellate	0.32	0.13	2.7	6.1	19.8
Group C (1) to Group G (2) (76.6%)	*Willea spp.*	Chlorophyte	0.34	0.00	10.6	9.4	9.4
	Tabellaria fenestrata	Diatom	0.28	0.03	4.3	7.2	16.6
	Planctonema spp.	Chlorophyte	0.23	0.00	10.6	6.4	23.0
Group D (1) to Group G (2) (75.8%)	*Planktosphaeria spp.*	Chlorophyte	0.66	0.03	3.9	17.0	17
	Tabellaria fenestrata	Diatom	0.57	0.03	4.7	14.6	31.6
	Fragilaria crotonensis	Diatom	0.20	0.02	2.8	4.8	36.4
Group E (1) to Group G (2) (70.0%)	*Microcystis spp.*	Blue-green	0.72	0.01	1.7	22.9	22.9
	Asterionella formosa	Diatom	0.28	0.16	1.1	5.8	28.7
	Cryptomonas spp.	Flagellate	0.22	0.13	1.4	3.2	31.8
Group F (1) to Group G (2) (61.5%)	*Aulacoseira granulata*	Diatom	0.17	0.01	4.0	7.8	7.8
	Asterionella formosa	Diatom	0.00	0.16	1.6	7.3	15.1
	Cyclotella comta	Diatom	0.13	0.00	3.3	6.0	21.2

A radial plot is provided (Figure 5-16) which illustrates the difference of biovolume of each of the top three taxa that are contributing to the dissimilarity between the sample groups (A through F) and the Major Group (G). The comparison between sample Group A as well as Group D and the Major Group shows the average relative biovolume of *Planktosphaeria spp* to be greater in Group A and Group D. Only one taxon, *Asterionella formosa*, showed the average relative biomass to be less from the Major group. This occurred for the comparisons with Group A and Group F and the Major Group.

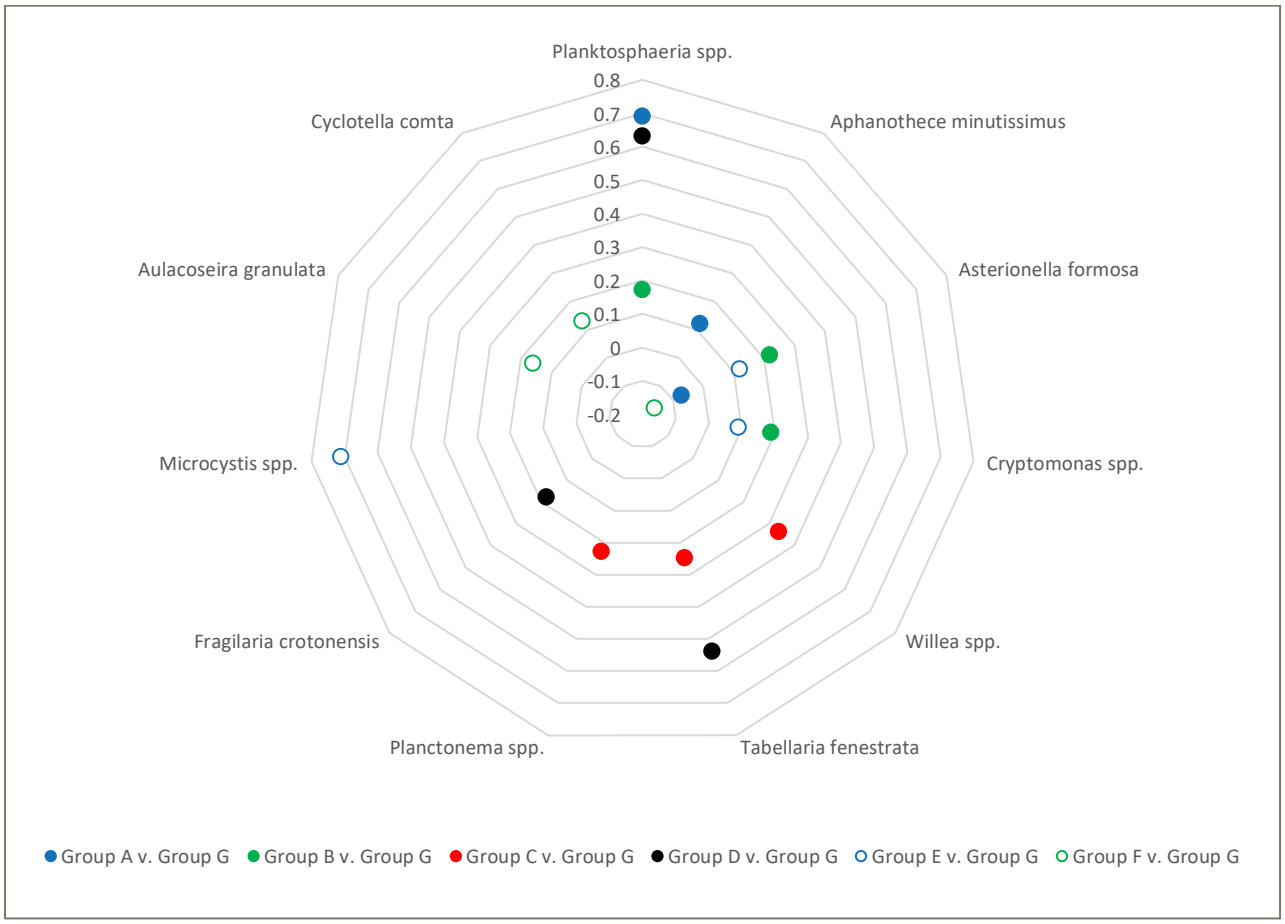

Figure 5-16. Average relative biovolume (mm^3/L) difference for taxa contributing to dissimilarity (University Point Warm Stratified)

5.3 Cold Clear Lake Function

5.3.1 Tubbs Hill

The results from the Bray-Curtis analysis and subsequent SIMPROF test for samples collected from Tubbs Hill during the cold clear lake function period are presented in Figure 5-17. The SIMPROF results indicate two distinct groups of samples. Only one sample that was collected in October of 2008 indicated a significant difference between the majority of the samples based on these results. The rest of the samples are included in Group B (Major Group).

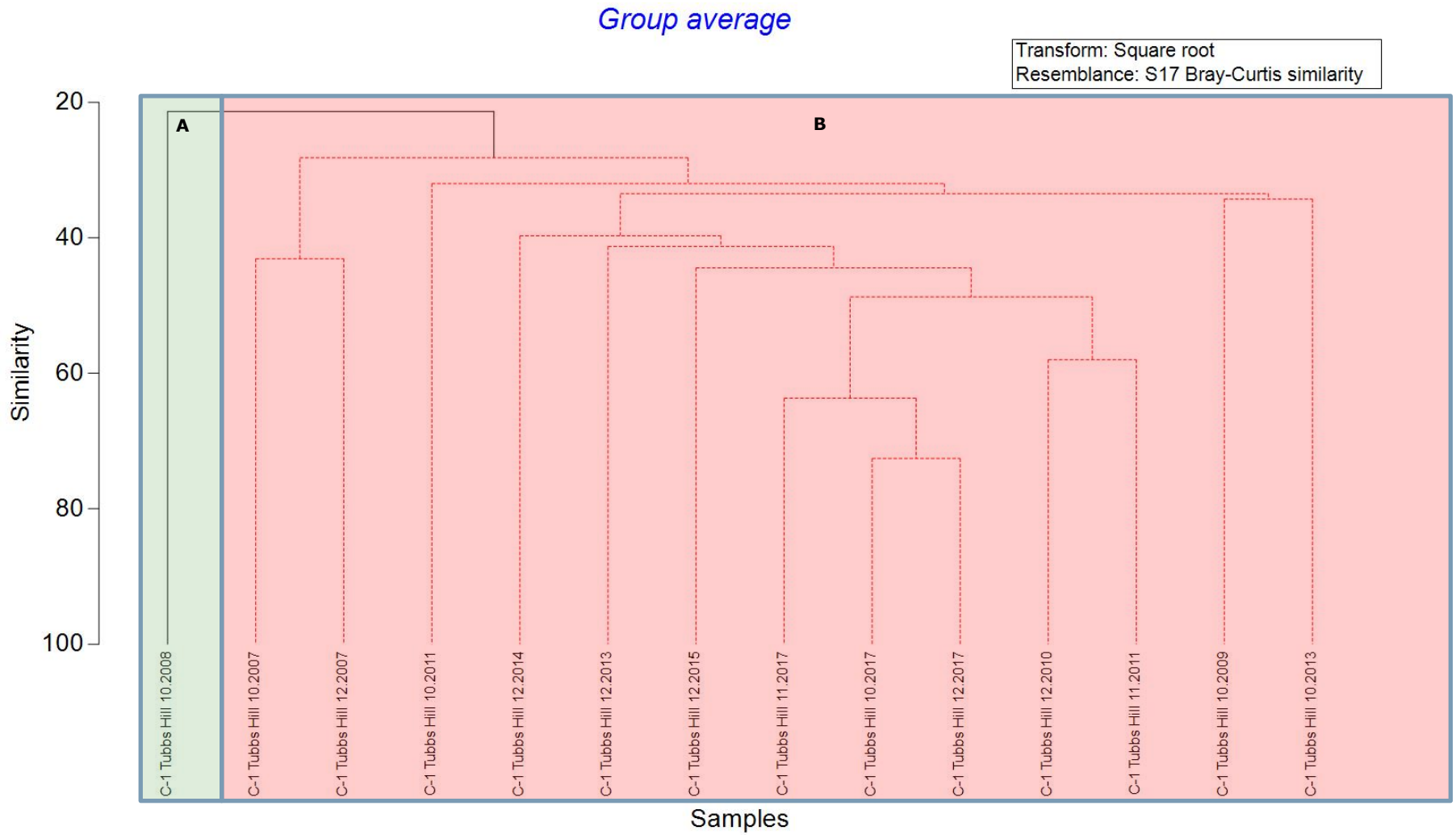

Figure 5-17. Tubbs Hill Cold Clear Sample Group Designations Based on Similarity Grouping

Results of the correlation analysis for samples collected during the cold clear lake function period at Tubbs Hill are provided in Figure 5-18. These results indicate two analytes have the best correlations with the positioning of the samples in the nMDS plot (sTIN, and sTIN:sRP). Individual axis correlations for each analyte are provided in Table 5-13. Figure 5-19 illustrates the concentrations of the four analytes for each sample. The Bray-Curtis sampling groupings are also provided to indicate the samples which demonstrated significant differences during the phytoplankton community analysis.

Table 5-13. Tubbs Hill Cold Clear correlation results for individual nMDS axes and combined vertex length

	Diss. Zn	Diss. Cd	Diss. Pb	TP	TN	Chl.	sTIN	sRP	TN:TP	sTIN: sRP
MDS1 (x)	0.15	0.03	0.07	-0.41	-0.31	0.00	0.41	-0.18	0.13	0.49
MDS2 (y)	0.24	0.23	0.07	0.04	0.34	0.39	-0.48	-0.33	0.21	-0.33
Vector	0.28	0.23	0.10	0.41	0.46	0.39	0.63	0.38	0.25	0.59

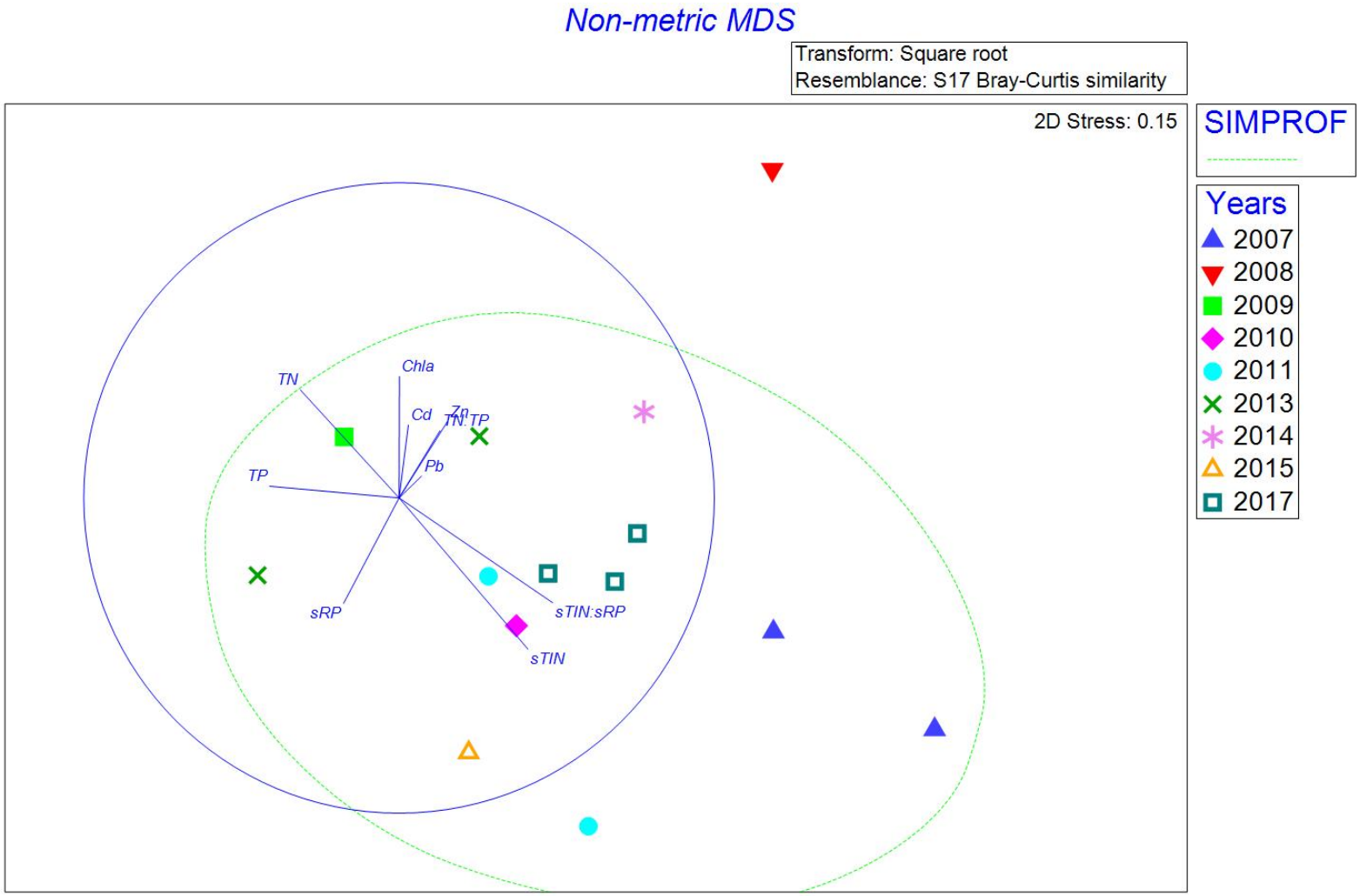

Figure 5-18. Tubbs Hill Cold Clear Vector Correlation Results

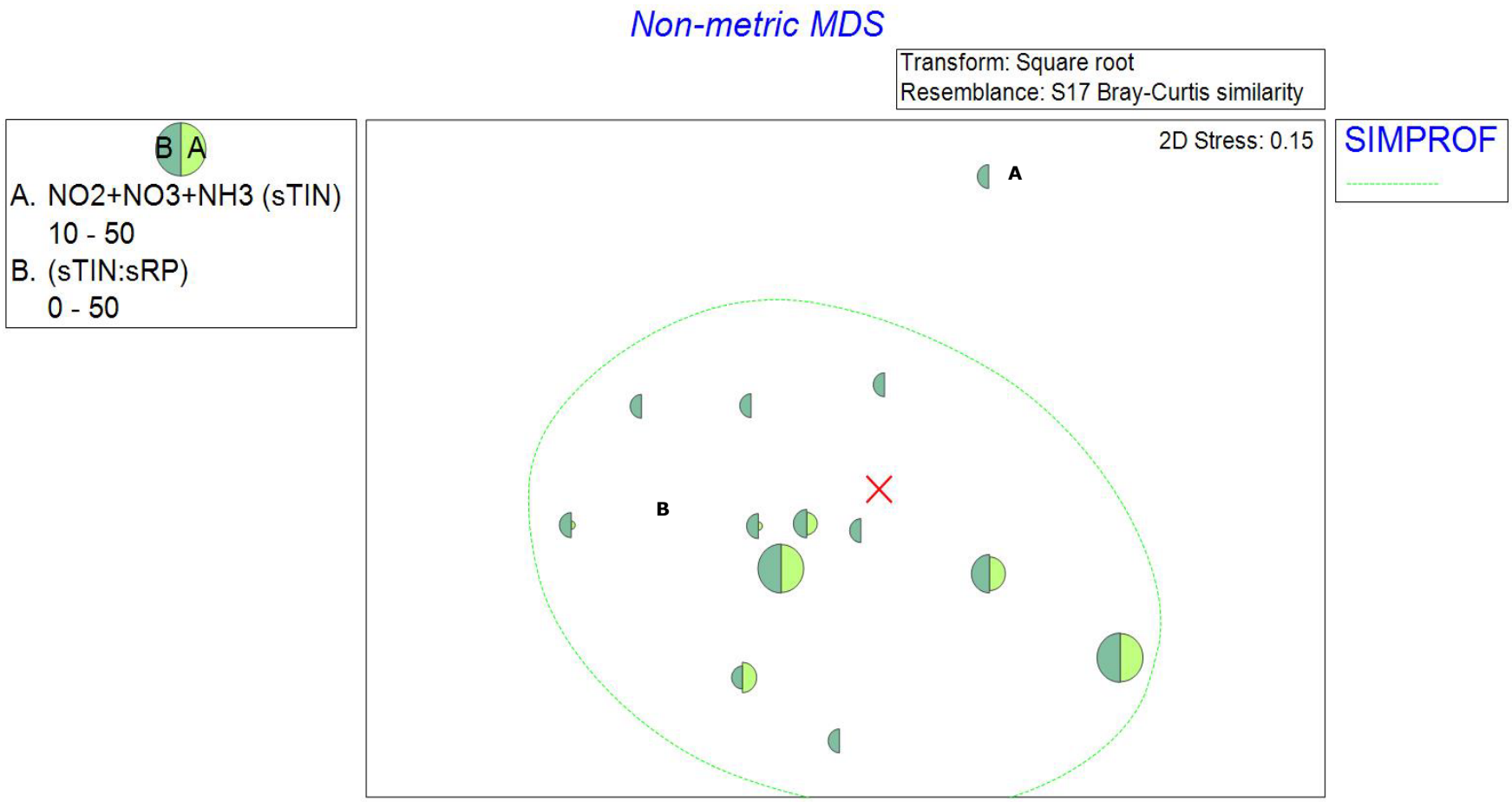

Figure 5-19. Tubbs Hill Cold Clear Sample Bubble Plot Indicating Concentration of Selected Analytes (Alpha codes indicate SIMPER Groupings)

The SIMPER results for in-group similarity for the samples collected during the cold clear lake function period at Tubbs Hill are provided in Table 5-14. Sample Group A only contained one sample so in-group similarity could not be assessed.

SIMPER results indicate that Small Microflagellates spp. was the taxon that contributed the greatest to the similarity within sample Group B (14.1%).

Table 5-14. SIMPER Results Tubbs Hill Cold Clear In-group Similarity

Group (% Similarity)	Taxa	Phytoplankton Type	Average Relative Biovolume	Similarity / Standard Deviation	Contribution %	Cumulative %
Group A[1]						
Group B *Major Group* (36.6%)	Small Microflagellates spp.	Flagellate	0.08	6.7	14.1	14.1
	Asterionella formosa	Diatom	0.09	1.0	12.4	26.5
	Cryptomonas spp.	Flagellate	0.10	0.91	12.3	38.8

[1] Group contains one sample. SIMPER in-group not calculatable.

Results from the SIMPER comparison for between-group dissimilarity conducted on samples collected during the cold clear lake function at station Tubbs Point are provided in Table 5-15. This table was not truncated and provides the results from the one possible comparison.

The taxon contributing to the majority of the dissimilarity (26.7%) between sample Group A and B was a blue-green alga (*Microcystis spp.*) which indicated an increase of *Microcystis spp*. in samples from Group A. The one sample that made up Group A showed an increase in the biovolumes of each of the three taxa that are driving the dissimilarity between these sample groups.

Table 5-15. SIMPER Results Tubbs Hill Cold Clear Between-group Dissimilarity (Selected Results)

Group Comparison (% Dissimilarity)	Taxa	Phytoplankton Type	Group 1 Average Relative Biovolume	Group 2 Average Relative Biovolume	Dissimilarity / Standard Deviation	Contribution %	Cumulative %
Group A (1) to Group B (2) (78.7%)	*Microcystis spp.*	Blue-green	0.65	0.01	7.6	26.7	26.7
	Chlamydocapsa spp.	Chlorophyte	0.38	0.00	8.5	15.8	42.6
	Aulacoseira granulata	Diatom	0.11	0.00	8.5	4.6	47.1

A radial plot is provided (Figure 5-20) which illustrates the difference of biovolume of each of the top three taxa that are contributing to the dissimilarity between the sample groups (A) and the Major Group (B). This comparison showed the average relative biomass of *Microcystis spp*. to be greater in Group A.

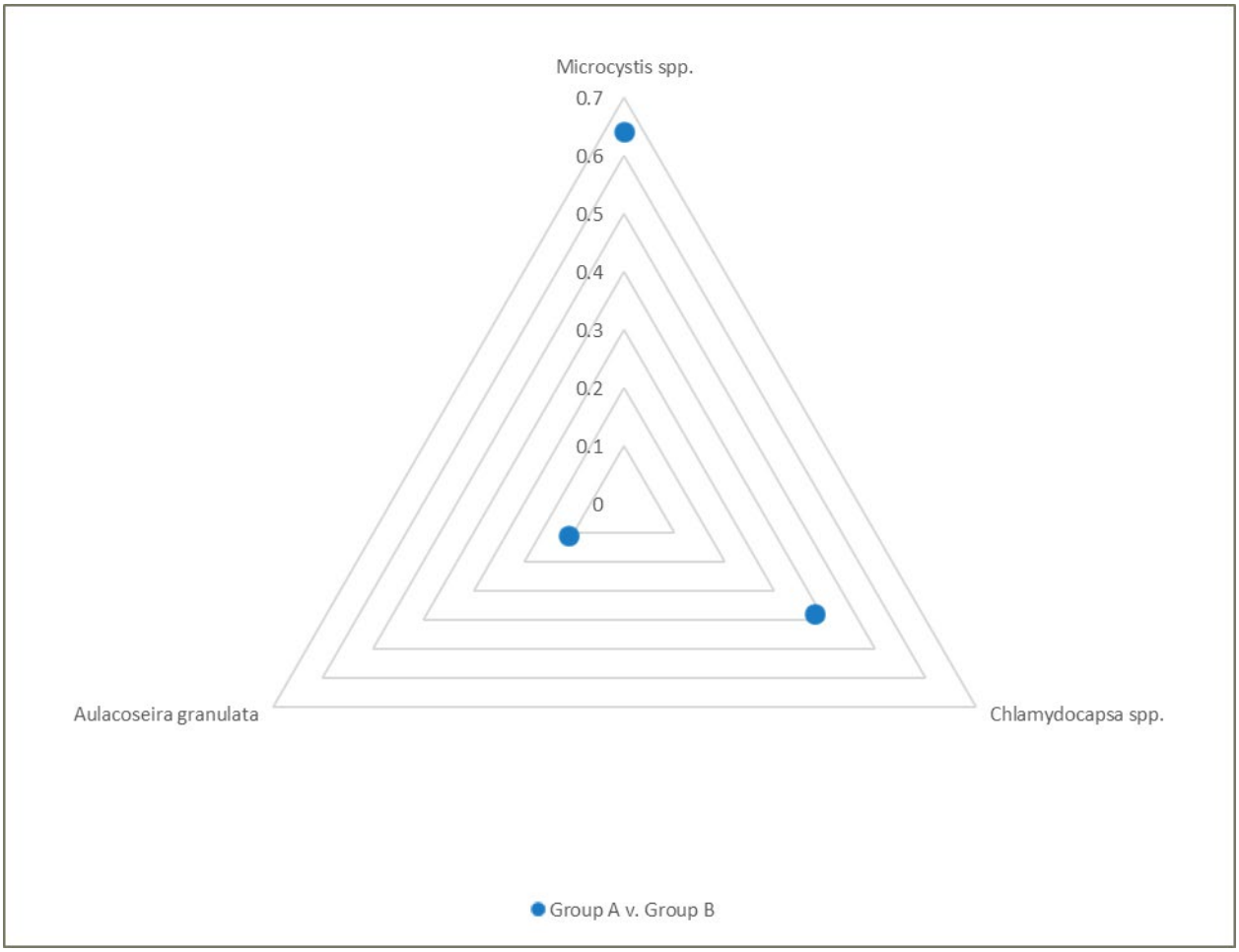

Figure 5-20. Average relative biovolume (mm3/L) difference for taxa contributing to dissimilarity (Tubbs Hill Cold Clear)

5.3.2 University Point

The results from the Bray-Curtis analysis and subsequent SIMPROF test for samples collected from University Point during the cold clear season are presented in Figure 5-21. The SIMPROF results indicate two distinct groups of samples. Only one sample that was collected in December of 2011 indicated a significant difference between the majority of the samples based on these results. The rest of the samples are included in Group B (Major Group).

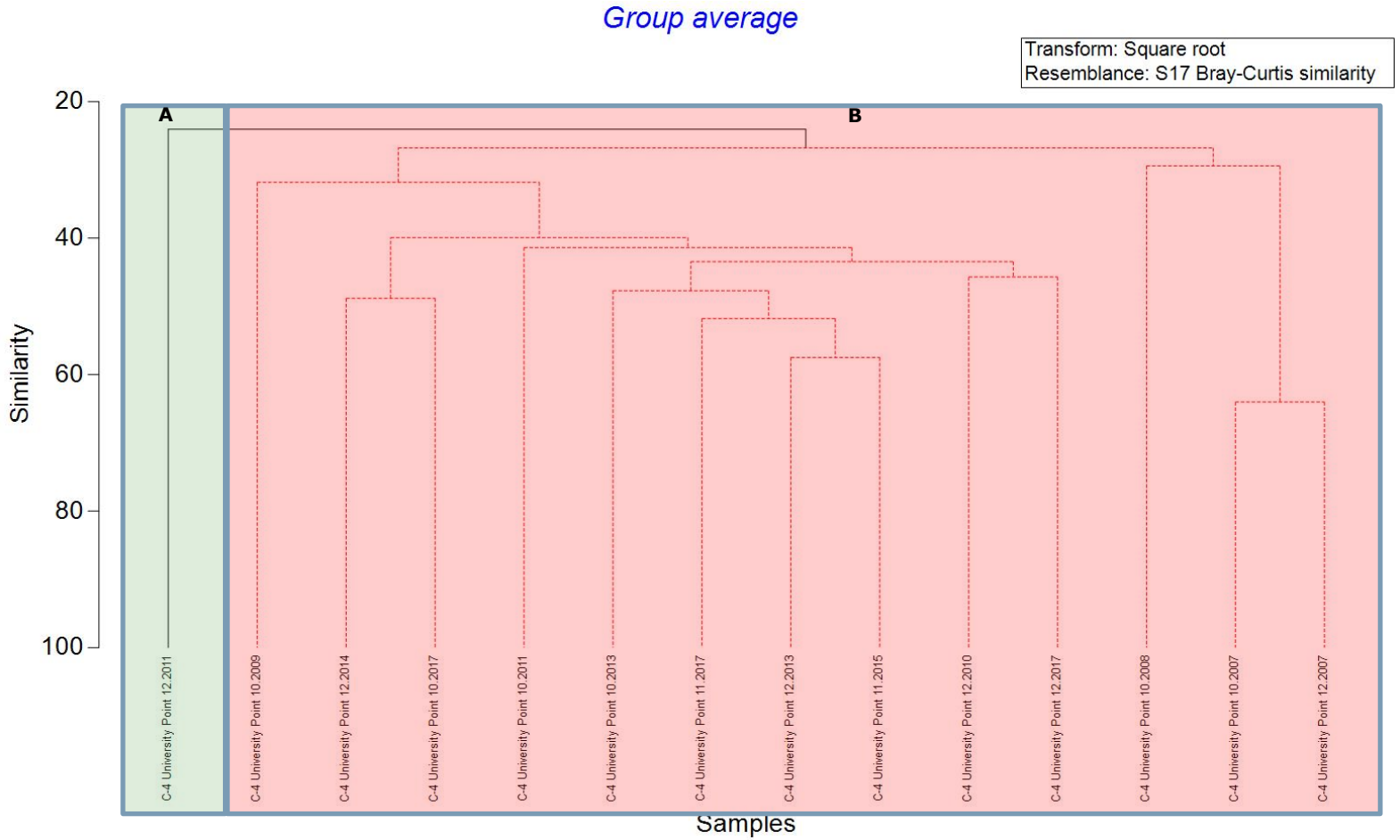

Figure 5-21. University Point Cold Clear Sample Group Designations Based on Similarity Grouping

Results of the correlation analysis for samples collected during the cold clear lake function period at University Point are provided in Figure 5-22. These results indicated four analytes have the best correlations with the positioning of the samples in the nMDS plot (sTIN, dissolved zinc, sTIN:sRP, and total nitrogen). Individual axis correlations for each analyte are provided in Table 5-16. Figure 5-23 illustrates the concentrations of the five analytes for each sample. The Bray-Curtis sampling groupings are also provided to indicate the samples which demonstrated significant differences during the phytoplankton community analysis.

Table 5-16. University Point Cold Clear correlation results for individual nMDS axes and combined vertex length

	Diss. Zn	Diss. Cd	Diss. Pb	TP	TN	Chl.	sTIN	sRP	TN:TP	sTIN: sRP
MDS1 (x)	0.61	0.15	0.23	0.32	0.53	-0.25	0.68	0.07	0.13	0.70
MDS2 (y)	-0.20	-0.12	0.09	-0.38	-0.36	-0.06	-0.17	-0.09	0.07	-0.17
Vector	0.64	0.19	0.25	0.50	0.64	0.26	0.70	0.11	0.15	0.72

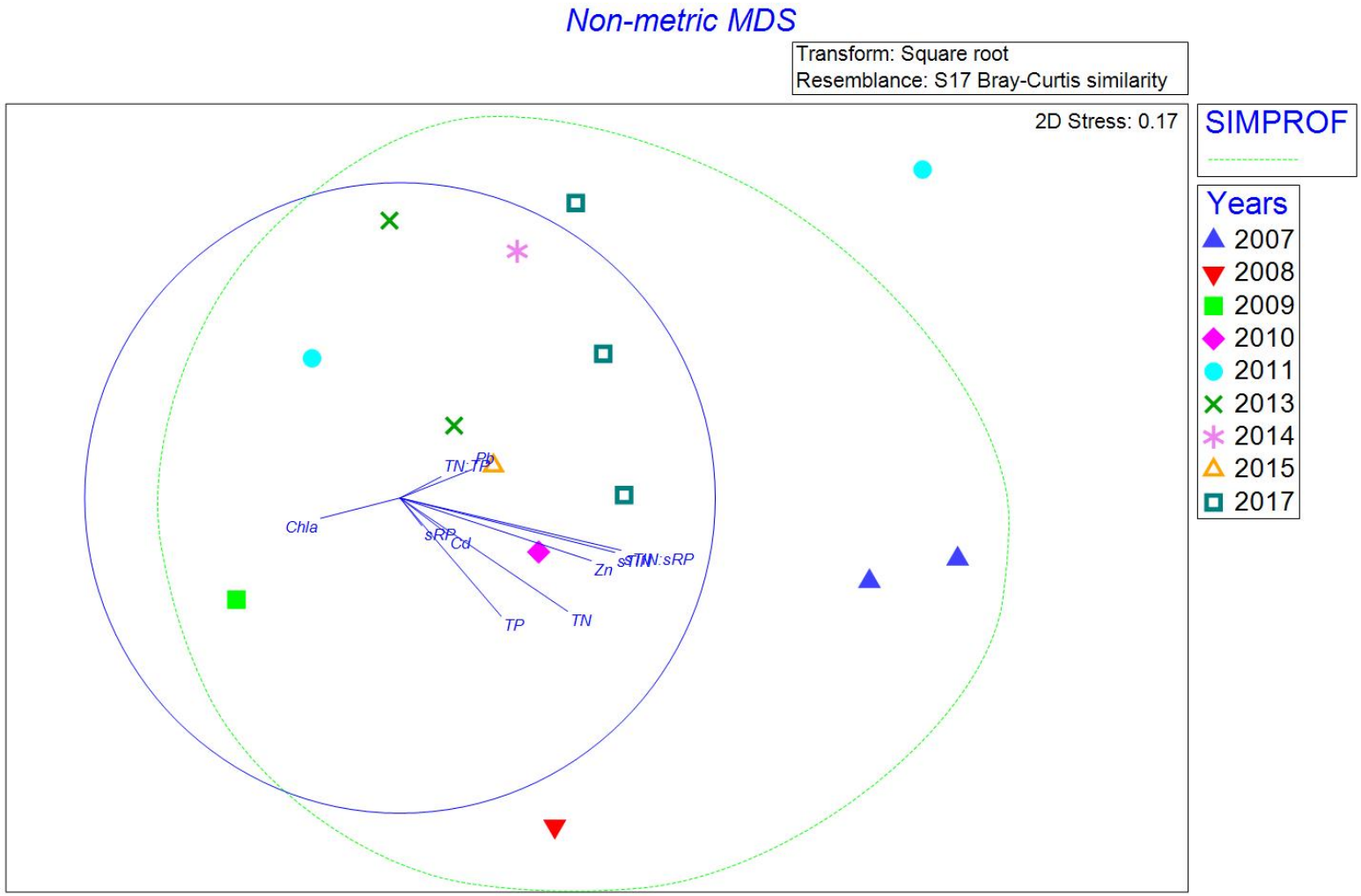

Figure 5-22. University Point Cold Clear Vector Correlation Results

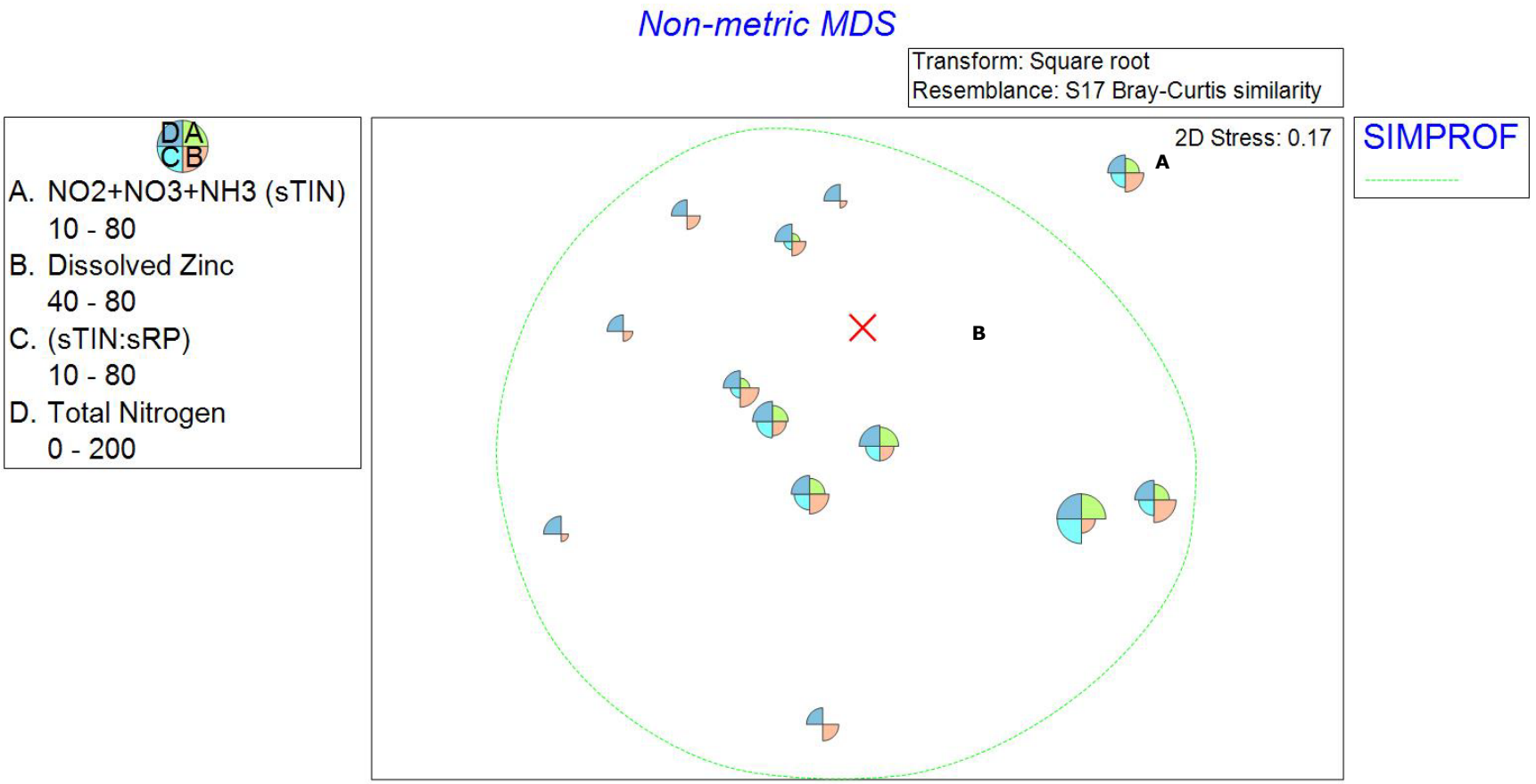

Figure 5-23. University Point Cold Clear Sample Bubble Plot Indicating Concentration of Selected Analytes (Alpha codes indicate SIMPER Groupings)

The SIMPER results for in-group similarity for the samples collected during the cold clear lake function period at University Point are provided in Table 5-17. Sample Group A only contained one sample therefore in-group similarity could not be assessed.

SIMPER results indicate that *Cryptomonas spp.* was the taxon that contributed the greatest to the similarity within sample Group B (17.7%).

Table 5-17. SIMPER Results University Point Cold Clear In-group Similarity

Group (% Similarity)	Taxa	Phytoplankton Type	Average Relative Biovolume	Similarity/ Standard Deviation	Contribution %	Cumulative %
Group A[1]						
Group B *Major Group* (35.5%)	*Cryptomonas spp.*	Flagellate	0.15	1.3	17.7	17.7
	Small Microflagellates spp.	Flagellate	0.09	4.8	14.5	32.2
	Asterionella formosa	Diatom	0.08	1.8	12.4	44.6

[1] Group contains one sample. SIMPER in-group not calculatable.

Results from the SIMPER comparison for between-group dissimilarity conducted on samples collected during the cold clear lake function at station University Point are provided in Table 5-18. This table was not truncated and provides the results from the one possible comparison.

The taxon contributing to the majority of the dissimilarity (10.7%) between sample Group A and B was a flagellate (*Cryptomonas spp.*) which indicated an decrease of *Cryptomonas spp*. in samples from Group A. The one sample that made up Group A showed an increase in the biovolumes of *Tabellaria fenestrata* and *Asterionella formosa* which was also contributing to the dissimilarity between these sample groups.

Table 5-18. SIMPER Results University Point Cold Clear Between-group Dissimilarity (Selected Results)

Group Comparison (% Dissimilarity)	Taxa	Phytoplankton Type	Group 1 Average Relative Biovolume	Group 2 Average Relative Biovolume	Dissimilarity/ Standard Deviation	Contribution %	Cumulative %
Group A (1) to Group B (2) (76.0%)	*Cryptomonas spp.*	Flagellate	0.00	0.15	1.5	10.7	10.7
	Tabellaria fenestrata	Diatom	0.09	0.03	3.9	6.9	17.6
	Asterionella formosa	Diatom	0.14	0.08	1.6	3.9	21.5

A radial plot is provided (Figure 5-24) which illustrates the difference of biovolume of each of the top three taxa that are contributing to the dissimilarity between the sample groups (A) and the Major Group (B). This comparison showed the average relative biovolume for *Asterionella formosa* and *Tabellaria fenestrata* to be greater while also indicating the average relative biovolume of *Cryptomonas spp*. to be less in Group A.

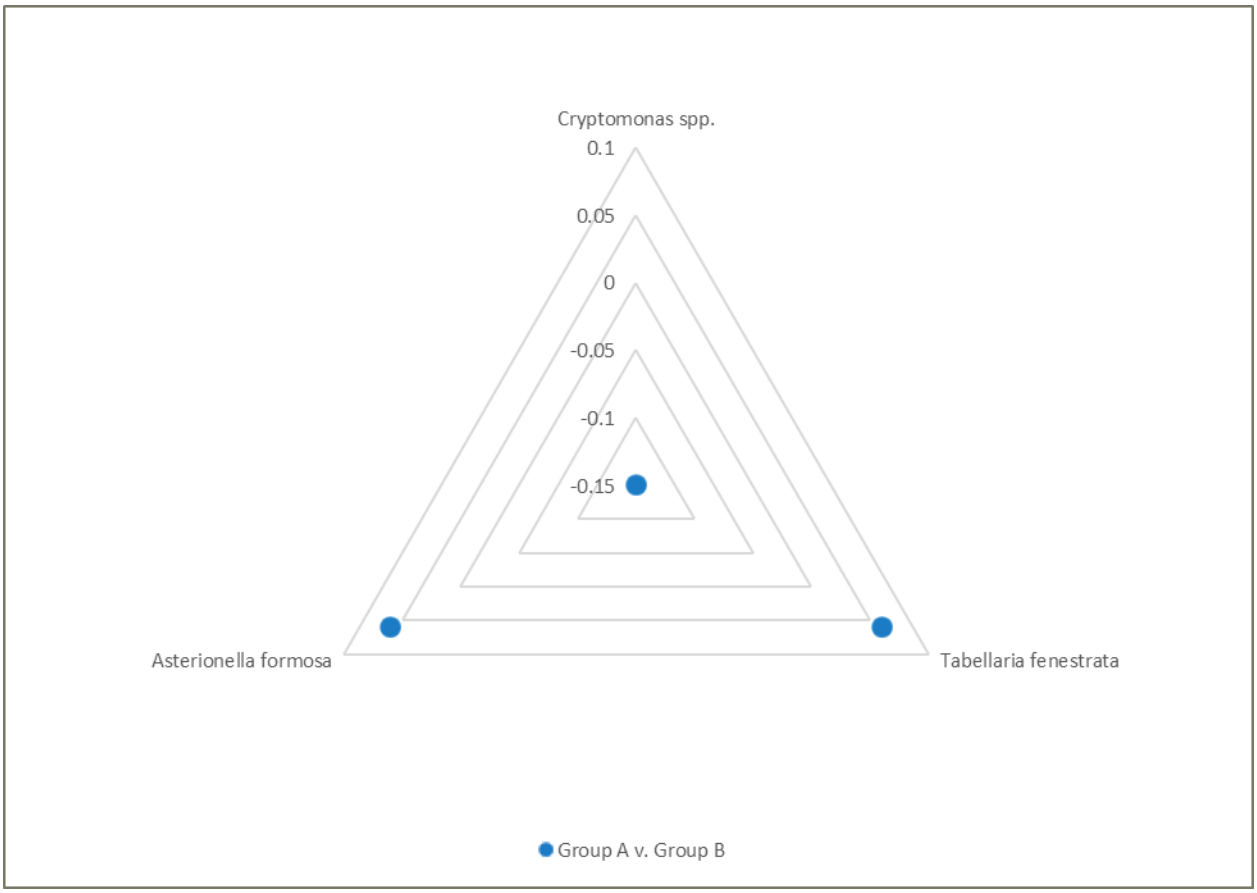

Figure 5-24. Average relative biovolume (mm3/L) difference for taxa contributing to dissimilarity (University Point Cold Clear)

6. DISCUSSION

Measured concentrations of selected analytes appear to correspond with seasonal function in Coeur d'Alene Lake. Typically, these analytes are elevated during the runoff lake function from February to June in northern Idaho and appear to be at higher concentrations at the southern station (University Point).

Asterionella formosa was the primary phytoplankton component based on average biovolume for samples collected at Tubbs Hill and University Point sampling locations during the runoff and warm stratified lake functions. During the cold clear lake function *Microcystis spp*. became the primary component for samples collected at Tubbs Hill while during this lake function both *Tabellaria flocculosa* and *Cryptomonas spp.* were the primary phytoplankton taxa components at University Point.

Investigation of phytoplankton communities based on biovolume indicate significant changes that appear to correspond with specific years. Table 6-1 summarizes the number of phytoplankton community samples that were significantly different than the majority of the phytoplankton samples collected during a specific lake function by year. The SIMPER results indicate that these significant dissimilarities typically correspond with an increase in biovolume of specific phytoplankton taxa which would indicate a bloom. It is important to note that although the phytoplankton communities at both stations during the cold clear season appear to have less samples with significant differences, this season was characterized by far fewer samples.

Correlation analyses performed with selected environmental variables indicated that chlorophyll best described the dissimilarity between phytoplankton community at Tubbs Hill and University Point during the runoff lake function as well as at University Point during the warm stratified lake function. Dissolved cadmium, total nitrogen, and chlorophyll demonstrated the best correlation with the phytoplankton communities at Tubbs Hill during the warm stratified lake function. Soluble total inorganic nitrogen (sTIN) and the ratio between sTIN and soluble reactive phosphorus (sRP) demonstrated good correlations with the phytoplankton communities at Tubbs Hill and University Point stations during the cold clear lake function. In addition to these environmental factors, dissolved zinc and total nitrogen also showed good correlations with phytoplankton communities at University Point station during the cold clear lake function.

The month of June is a transitional month for Coeur d'Alene Lake and samples collected during this month were included in both the runoff and warm stratified analyses. Only one sample collected in June at Tubbs Hill station (2008) showed a significantly different community than the majority. This sample was significantly different than the majority for both lake function seasons. Four samples collected at University Point station indicated significant differences than the majority (2008, 2009, 2011, and 2012). The sample collected in 2008 was the only sample that showed a significant difference in both the runoff and warm stratified lake function season. The sample collected in 2009 showed a significant difference during the runoff function season while samples collected in 2011 and 2012 indicated a significant difference during the warm stratified lake function season. The summation presented in Table 6-1 include samples collected during the June transitional month in both the runoff and warm stratified function seasons.

When these data are summed by year regardless of lake function, the data appears to indicate the greatest significant change to the phytoplankton communities at both Tubbs Hill and University Point sampling stations occurred during 2008 with 2007 and 2009 also indicating significant years (Table 6-1).

Data from University Point also indicated that the phytoplankton communities at this station tend to demonstrate significant differences more often that at Tubbs Hill station and from 2010 – 2015 the phytoplankton communities at Tubbs Hill sampling station showed no significant differences during this analysis. With the current data it is difficult to determine if these patterns are an indication that 2007-2009 are abnormal years or that there is a temporal trend that is driving these communities to be more similar. It would be interesting to determine if there are phytoplankton data prior to 2007 to investigate whether phytoplankton data from these previous years are more consistent with the more recent data.

Table 6-1. Occurrence of samples indicating a significant difference from the Major Group

Sampling Station – Lake Function	Significantly Different phytoplankton Community Samples – Count (percent of samples)										
	2007	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**	**2017**
Tubbs Hill											
Runoff	ND	4 (100%)	3 (60%)	---	---	---	---	---	---	---	---
Warm Stratified	6 (100%)	5 (100%)	1 (20%)	---	---	---	---	---	---	1 (25%)	2 (50%)
Cold Clear	---	1 (100%)	---	---	---	ND	---	---	---	ND	---
Annual Summary	**6 (75%)**	**10 (100%)**	**4 (36%)**	---	---	---	---	---	---	**1 (14%)**	**2 (17%)**
University Point											
Runoff	ND	4 (100%)	4 (100%)	3 (75%)	3 (75%)	2 (50%)	---	---	---	---	---
Warm Stratified	5 (100%)	5 (100%)	---	---	1 (33%)	1 (25%)	---	---	---	---	1 (25%)
Cold Clear	---	---	---	---	1 (50%)	ND	---	---	---	ND	---
Annual Summary	**5 (71%)**	**9 (90%)**	**4 (44%)**	**3 (33%)**	**5 (56%)**	**3 (38%)**	---	---	---	---	**1 (9%)**

Most of the samples collected at each station during the three lake functional periods had phytoplankton communities that were not significantly different from each other. The sample group that contains these samples are referred to as the "Major Group." A SIMPER analysis was performed to compare the Major Groups from each station during each lake function. The results provided in Table 6-2 summarizes the three major taxa that are contributing to the dissimilarity between samples collected at Tubbs Hill and University Point during the three lake function periods. Bolded taxa identifications indicate the taxa was also identified as one of the top three taxa driving the similarity within both Major Groups from the comparison. Dissimilarity between Major Groups in these cases reflect a significant change in the biovolume of one of the primary taxa. Similarity of the Major Groups are provided in the in-group similarity tables from Section 5.

Table 6-2. Major Group comparison between Tubbs Hill and University Point during specific lake function

Lake Function (% Dissimilarity)	Taxa	Phytoplankton Type	Tubbs Hill Average Relative Biovolume	University Point Average Relative Biovolume	Dissimilarity/ Standard Deviation	Contribution %	Cumulative %
Runoff (49.3%)	***Asterionella formosa***	Diatom	0.29	0.18	1.04	8.1	8.1
	Chroococcus spp.	Blue-green	0.08	0.10	1.15	4.8	12.9
	Aulacoseira italica	Diatom	0.08	0.04	0.95	4.4	17.3
Warm Stratified (52.4%)	***Asterionella formosa***	Diatom	0.19	0.16	1.29	6.0	6.0
	Chroococcus spp.	Blue-green	0.08	0.07	1.22	4.4	10.4
	Gymnodinium spp.	Flagellate	0.06	0.06	1.13	4.1	14.4
Cold Clear (62.9%)	***Cryptomonas spp.***	Flagellate	0.10	0.15	1.23	6.4	6.4
	Tabellaria fenestrata	Diatom	0.04	0.03	0.47	3.6	10.0
	Euglena spp.	Euglenoid	0.02	0.05	0.50	3.4	13.4

Bold Taxa were one of the top three taxa driving the similarity within both Major Groups.

Figure 6-1 illustrates the difference between average relative biovolume between the top three taxa driving dissimilarity between Tubbs Hill and University Point stations. Positive values indicate the average relative biovolume of the indicated taxa is greater at Tubbs Hill station. Negative values indicate the average relative biovolume of the indicated taxa is less at the Tubbs Hill station. These results indicate that the average relative biovolume of *Asterionella formosa* at Tubbs Hill station is greater than at University Point and is the major contributor of dissimilarity during the runoff and warm stratified. While the relative biovolume of *Cryptomonas spp.* is less at Tubbs Hill than at University Point during the cold clear lake function.

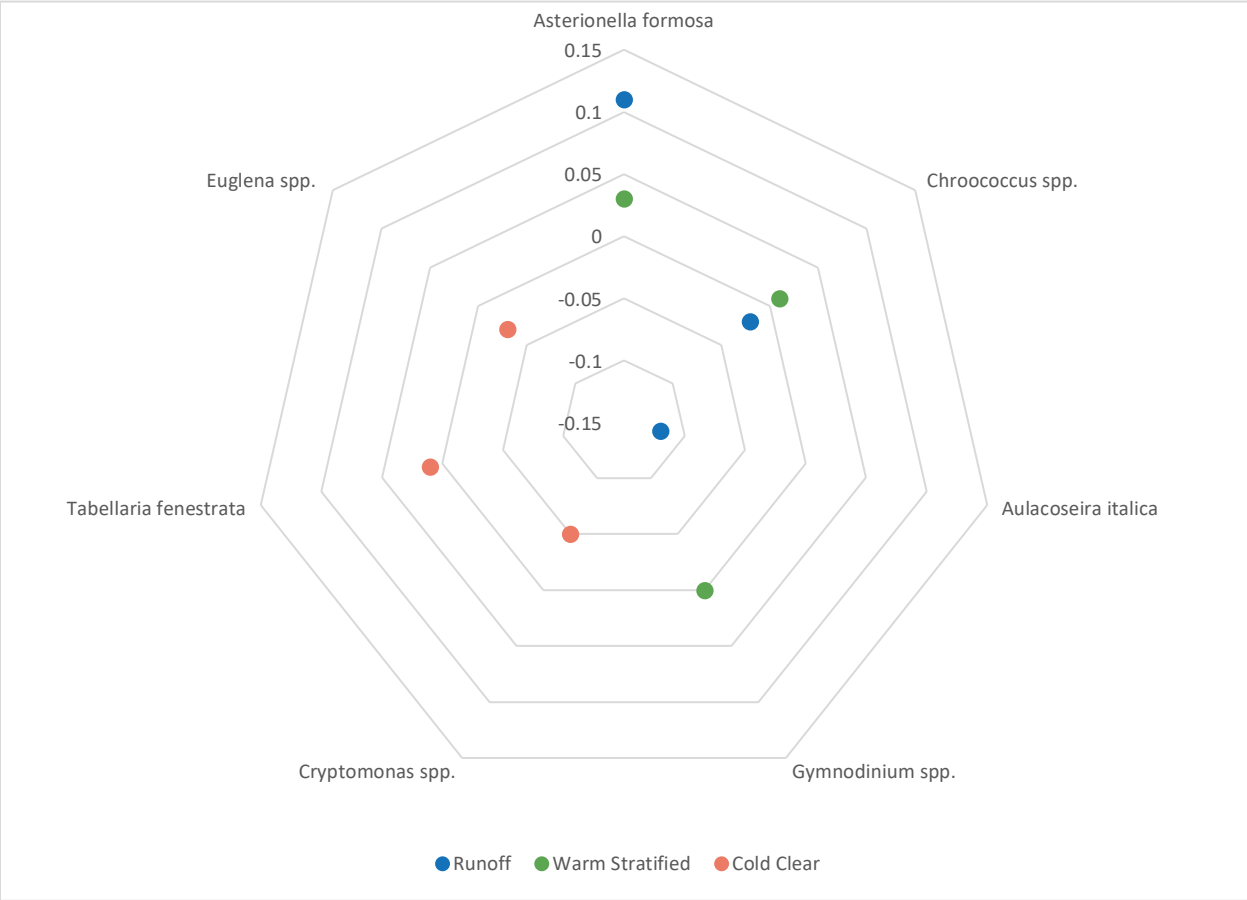

Figure 6-1. Average relative biovolume (mm^3/L) difference between Major Groups defined for Tubbs Hill and University Point during lake functions.

7. RECOMMENDATIONS

The intention of this report was to focus on developing trends between phytoplankton communities over time and correlating dissimilarity within these communities to environmental factors at the Tubbs Hill and University Point monitoring stations. The seasonal patterns of Northern Idaho create distinct lake functional periods that could influence the phytoplankton communities. This report focuses on investigating these periods individually, however, future studies should be made that will give better understanding of how this system is functioning. The following section offers some recommended studies that could help refine the understanding of influences to phytoplankton communities in Coeur d'Alene Lake.

The first step to determining how lake function influences phytoplankton communities, and in turn the food web in Coeur d'Alene Lake, is to determine a hydrological model of the lake and how water flow changes during the different lake function periods. Coeur d'Alene Lake is fed by two primary river systems, the Coeur d'Alene river which enters the lake in the east, and the Saint Joe river which enters the lake in the south. The outflow of the lake is the Spokane river in the north which ultimately feeds into the Columbia River. The Coeur d'Alene river flows out of the Silver Valley which has a long history of mineral mining while the Saint Joe river mainly flows through wilderness or agricultural use areas. Distribution of contamination from the various river inputs will be determined by influences to the river prior to the lake and the general hydrological mixing of these waters in the lake. The water quality of these two rivers systems could be very different and determining how these waters blend in Coeur d'Alene Lake, as well as how smaller inputs influence individual bays could be very informative for determining the causes associated with changes in the phytoplankton community.

After determining the hydrological patterns in the lake, these results could be compared to the changes in the phytoplankton communities during the year. If the river input from the agricultural areas are adding more nutrients to the system and these waters are typically found to be surficial water during the runoff function in the south but well blended and diluted during the warm stratified function in the north, can these relationships better predict phytoplankton community responses throughout the year?

Also, phytoplankton data has been collected from many other sampling locations in Coeur d'Alene Lake. These data have been collected from individual stations less frequently than at Tubbs Hill or University Point stations but are representative of individual bays. The hydrological analysis will likely determine that the lake water quality is somewhat heterogeneous and that conclusions made based on samples collected at two sampling stations in the middle of the lake (Tubbs Hill and University Point) may not be reflective of the phytoplankton communities in the bays. To get a more refined picture of the phytoplankton communities in the lake as a whole, data collected at individual bays should be investigated further. Initial efforts should be made to determine the proportionality based on biovolume of the major phytoplankton taxa as well as average total biovolume at Tubbs Hill and University Point for each lake function and year. These results would then be compared to the same data summarized at each bay. Following this, further, more refined comparisons could be made to investigate how similar these phytoplankton communities are to the phytoplankton communities in the middle of the lake.

Following an assessment of community similarity, these data could further be assessed by determining trophic quality and quantity of the phytoplankton at each location. Some phytoplankton are either too large, small, not nutritious, or even toxic contributors to a food web within a water body. This assessment could be performed to relate biovolume and cell counts to whether a phytoplankton would be deemed "edible" or a functional part of the food web in Coeur d'Alene Lake.

The current study attempted to correlate selected environmental variables with the dissimilarity demonstrated between phytoplankton communities based on biovolume. As with all correlation analysis, these results should be used to guide further studies and not be interpreted as causal agents of these dissimilarity. Causal studies could be performed in controlled laboratory exposures using individual environmental factors as the independent variables. Phytoplankton samples could be collected in the field and a split of this sample could have the phytoplankton communities identified and biovolumes assessed. The rest of the sample could then be exposed to different concentrations of a specific environmental factor and assessed again for phytoplankton community and biovolumes. If properly designed, this study could demonstrate differences in the phytoplankton community in response to an environmental factor.

This investigation has identified phytoplankton taxa that are both greatly representative of the community as a whole and also taxa that drive dissimilarity between samples within specific functional groups. *Asterionella formosa* is one of the most consistently represented taxa in samples collected from Coeur d'Alene Lake. The relative average biovolume of this taxon often drives similarity of samples from within Bray-Curtis defined groups. *Microcystis spp., Anabaena spp.* and *Tabellaria fenestrate* as well as some other taxa showed major contributions to the dissimilarity between samples. Often these taxa were not present or present in small quantities in the Major Groups but showed higher relative biovolume concentrations in the samples that were different from the Major Group. Further investigations could consider these taxa as indicators of deviations from typical conditions in the lake.

As new data is collected, it can be added to the interpretation to which would refine the Major Group. During this analysis, a large perturbation event (2007 – 2009) occurred that dominated the analyses. An investigation of the smaller trends could be made by conducting an analysis of the Major Group without the presence of the phytoplankton communities that were collected during a large perturbation event.

8. REFERENCES

Clarke, K. R., Gorley, R. N., Somerfield, P. J., & Warwick, R. M. (2014). *Changes in Marine Communities: An Approach to Statistical Analysis and Interpretation* (3rd ed.).

APPENDIX A

ANALYTICAL DATA

APPENDIX B

SIMPER RESULTS