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Effects of Phosphorus on Benthic Diatom Assemblage Network Structure

Eric M. Massa
Florida International University, emass016@fiu.edu

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Miami, Florida

EFFECTS OF PHOSPHORUS ON BENTHIC DIATOM ASSEMBLAGE NETWORK

STRUCTURE

A thesis submitted in partial fulfillment of

the requirements for the degree of

MASTER OF SCIENCE

in

BIOLOGY

by

Eric M. Massa

2019

To: Dean Michael R. Heithaus
        College of Arts, Sciences, and Education

This thesis, written by Eric M. Massa, and entitled Effects of Phosphorus on Benthic Diatom Assemblage Network Structure, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this thesis and recommend that it be approved

_____
Todd Crowl

_____
Jennifer Rehage

_____
Evelyn Gaiser, Major Professor

Date of Defense: June 25, 2019

The thesis of Eric M. Massa is approved.

_____
Dean Michael R. Heithaus
College of Arts, Sciences and Education

_____
Andrés G. Gil
Vice President for Research and Economic Development
and Dean of the University Graduate school

Florida International University, 2019

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DEDICATION

For family.

ACKNOWLEDGMENTS

ABSTRACT OF THE THESIS

EFFECTS OF PHOSPHORUS ON BENTHIC DIATOM ASSEMBLAGE NETWORK STRUCTURE

By

Eric M. Massa

Florida International University, 2019

Miami, Florida

Professor Evelyn Gaiser, Major Professor

Ecological network analysis helps identify how relationships among species differ over time and across sets of species. Microbial assemblages are ideal for evaluating changes in species interactions due to environmental changes because they are speciose and respond at multiple scales. To determine how phosphorus limitation influences diatom network structure, I analyzed relationships among 257 species of diatoms from benthic microbial (periphyton) mats from 10 years of annual samples from 136 sites. Expected evidence of changes in network structure in response to periphyton TP were not found, likely due to species replacement with increased TP. Analysis of species connection distributions and the effects of species removal on connections found frequency increases with TP for 23 species with significant roles in network structure, variable frequency for 15 species, and 13 species consistently decreased in frequency. This study brings a new methodology of study to the field of ecosystem restoration studies.

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LIST OF ABBREVIATIONS AND ACRONYMS

Analysis of Variance.. ANOVA

Comprehensive Everglades Restoration Plan..CERP

Ecological Network Analysis...ENA

Monitoring and Assessment Plan...MAP

Nestedness based on Overlap and Decreasing Fill.. NODF

Phosphorus...P

Palmar...PAL

Pennsuco...PEN

Principal Sampling Unit... PSU

Shark River Slough...SRS

Total Phosphorus..TP

Taylor Slough.. TSL

Water Conservation Area... WCA

Water Conservation Area 2.. WCA2

Water Conservation Area 3.. WCA3

I. Introduction

All species form assemblages of variable composition and complexity in nature, and collections of assemblages representing multiple ecological roles can form communities with resource limitation often determining the presence and strength of specific interdependencies between species (e.g., mutualisms) (Gotelli & McCabe, 2002; Muller et al., 2014). The three major challenges in community ecology relating to non-trophic interactions are why do species co-occur, how to determine the influence of any one species on other species in the community, and how environmental drivers alter patterns of co-occurrence (Gotelli & McCabe, 2002; Ives & Carpenter, 2007; Pellissier et al., 2018). Species co-occurrence and multiple types of interaction are studied in many systems, from how predator-prey relationships change in food webs to the interactions between microbial taxa in gut biomes (e.g., Allesina & Pascual, 2008; Tsai et al., 2015). There also exists a large body of work on the effects of diversity in one side of directional interactions on the other such as how pollinator diversity can alter plant communities (e.g., CaraDonna et al., 2017; Pocock et al., 2011). How the degree of species codependences and how the form those co-dependencies take change along gradients of resource availability to influence biodiversity and ecosystem function remains a significant challenge in ecology (e.g., Berry & Widder, 2014; Mills et al., 1993; Ovaskainen et al., 2017; Wiegand et al., 2007).

Microbial communities are ideal models to study how non-trophic interactions change to alter diversity patterns along resource gradients, especially interactions which are complex and mutualistic. First, unexpectedly specious microbial communities can exist in seemingly homogenous and resource-limited environments, partially driven by

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species inter-dependencies that enhance resource use efficiency (Cirri & Pohnert, 2019; Harpole et al., 2011; Hutchinson, 1961; Nemergut et al., 2013). Second, they tend to have meaningful patterns of species dominance and rarity, including foundation, or "core", species (those that have an outsized impact on local conditions relative to their abundance) whose abundance changes reliably along environmental gradients (Marazzi & Gaiser, 2018; Schöb et al., 2012). Changes in foundation species (especially extinctions) are known to drive diversity patterns and may have cascading effects on other parts of the community (Ellison et al., 2005; Hooper et al., 2012). Third, microbial communities and their emergent properties are influenced by resource limitation, as species interdependencies develop at the physiological level which enable and encourage coexistence (Cotner & Biddanda, 2002). This results in species which co-occur being at least partially reliant on the products of other species for resources.

Microbial interactions that produce emergent properties are particularly are evident in the formation of complex biofilms or mats (La Hée & Gaiser, 2012; Lee et al., 2013; Reid et al., 2000; Scinto & Reddy, 2003). For example, in limestone-rich regions of the world, distinctive microbial communities containing species with restricted biogeographic distributions form very thick, calcareous mats similar to marine stromatolites, (La Hée & Gaiser, 2012; Lee et al., 2013; Reid et al., 2000; Scinto & Reddy, 2003). Phosphorus availability structures these ecosystems, including community organization (e.g., Gaiser et al., 2006; Mazzei & Gaiser, 2018; Noe et al., 2001; Sokol et al., 2014). When exposed to above-ambient levels of phosphorus, endemic species disappear, leading to structural collapse of the calcareous mat structure through suspected changes in species co-dependencies (Evelyn E. Gaiser et al., 2006). In oligotrophic

ecosystems exposed to nutrient subsidies, these changes in microbial community resource-sharing dependencies have cascading effects throughout ecosystem resulting from a loss of rare species and the functional roles that those species play (Evelyn E. Gaiser et al., 2005). These functions include regulating water column nutrients and gasses, the production of enzymes and other metabolites, and producing biomass that is consumed by meiofauna and other consumers (Cirri & Pohnert, 2019; Ewe et al., 2006; Sabater et al., 2003). Due to their responsiveness to environmental conditions, their importance in ecosystems, and the ease with which they can be sampled, microbial communities have been used as ecological risk indicators in these ecosystems and around the world multiple states for (Stevenson, 1998; Stevenson et al., 2010).

The Everglades is an iconic example of a karst wetland and is host to extensive calcareous microbial mats (periphyton) that are responsible for over half of net primary productivity much in the ecosystem (Ewe et al., 2006; E. Gaiser, 2009). Periphyton is known for its emergent properties including the development of calcareous three-dimensional structure and is known to dissolve when exposed to excess phosphorus (Chick et al., 2008; Gaiser et al., 2006) The total phosphorus (TP) content of periphyton is used to detect phosphorus enrichment in the Everglades as P is rapidly taken up by the environment or adsorbed to the limestone bedrock of the area to the extent that levels of dissolved P in the water are usually below the detection thresholds of most analytical techniques, and periphyton is easily sampled at field sites for later laboratory analysis (E.E. Gaiser et al., 2004). Due to the wide variety of species which can live in periphyton and the high variability of species found in samples, these communities present an excellent opportunity for studying the interactions between species across environmental

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gradients. Once the effect of species in communities on other species are understood, the effects of changing conditions on emergent properties can be better understood through further analyses. These properties enable the prediction of diversity from understanding the roles of certain species on others.

Network approaches to ecological research have been useful for understanding how relationships between species differ over environmental gradients? (Ings et al., 2009; Olesen et al., 2008; Thompson, 2006). Network analysis can be utilized to predict community structure responses to changes of one or more environmental variables (Proulx et al., 2005; Spieles & Mitsch, 2000). One method of studying community dynamics is ecological network analysis (ENA), which has been used in studying the maintenance of community structure in microbial assemblages in medical and ecological studies (e.g., Fernandez et al., 2015; Koenig et al., 2011; Kurtz et al., 2015; Layeghifard et al., 2017). By applying ENA over different scales or gradients of interest, community dynamics can be revealed (Astegiano et al., 2017). While trophic ENA is widely known within the field of ecology (Layman et al., 2015), a rich library of work on non-trophic ENA also exists (Guimarães et al., 2017; Olesen et al., 2006). Much of the work on non-trophic ENA is related to plant-pollinator interactions (Alarcon, 2004; Olesen et al., 2008; Pocock et al., 2011) or the epidemiology of agricultural diseases (Jeger, 2000; Jeger et al., 2004; Margosian et al., 2009; Pautasso & Jeger, 2014). While some work has been done using ENA in researching aquatic microbial communities, there has been less work done in this area than in terrestrial ecology or medicine (e.g., Carey et al., 2017; Lima-Mendez et al., 2015; Morales-Castilla et al., 2015; Steele et al., 2011).

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Three ideas will be tested utilizing ENA to study how relationships between species change along environmental gradients. First, I expect that the algal assemblages present in calcareous periphyton will demonstrate a loss of network structure with increased TP. Under natural highly oligotrophic conditions, I expect that ENA will identify a pattern in connection between species which will be indicative of communities with oligotrophic communities, and that pattern deteriorating as conditions become more eutrophic. More specifically, that the probability distribution of species degree – the number of connections between one species and others – will follow a power law distribution (Equation 1) above some minimum threshold of species degree, x_{min}, and that as TP increases, species co-occurrence will become more random, the absolute value of the exponent should decrease, representing a "flattening" of the degree distribution. Power law distributions are commonly found in cases where the frequency of an event (such as species presence) is related to its magnitude (Bascompte & Jordano, 2014).

Equation 1: Power law probability distribution that a species with degree k will be found is proportional to k raised to the exponent -γ.

$$P(k) \propto k^{-\gamma}$$

Second, I expect to find a subset of species in the dataset which have an outsized influence on community structure of networks in which those species occur, or that network nestedness, used as a metric of species interdependence across samples, would decrease in response to nutrient enrichment, representing either a decrease in the importance of highly interdependent specialist species, or an increase in the presence of much less interdependent generalist species, or both.

Third and finally, I expect that those species identified in testing the second hypothesis will decrease in frequency with increasing TP. This means that I expect that there are species that would serve as a hub or important node, without which, networks would experience a change in the structure of the network. This was tested by comparing networks that contained each species with the same network with that species removed.

II. Methods

1. Field Region Description
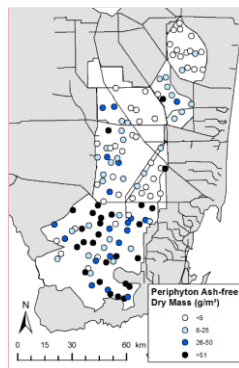
Figure 1. A map of all CERP MAP sites visited as part of CERP and the periphyton ash-free dry mass for the sample from that site for 2007. See table 1 for basin names and abbreviations.

As part of the Comprehensive Everglades Restoration Plan Monitoring and Assessment Plan (CERP MAP), the Everglades were divided into a series of basins, six of which are studied here (table 1). The majority of samples were collected in either SRS (40%) or WCA3 (43%).

Basin	Site samples	Cleaned dataset contains data from years
Palmar (PAL)	15	2007, 2008, 2009, 2011
Pennsuco (PEN)	1	2007
Shark River Slough (SRS)	399	2007 – 2016
Taylor Slough (TSL)	57	2007 – 2016
Water Conservation Area 2 (WCA2)	90	2007 – 2016
Water Conservation Area 3 (WCA3)	445	2007 – 2016

Table 1. Basins of the Everglades sampled in CERP MAP 2007-2016. Additionally, the number of site samples and the number of years that the basin in question is in the cleaned dataset.

2. Field Sampling

From 2007 to 2016, periphyton was collected annually during the wet season in a synoptic sampling program associated with CERP MAP through the Everglades that addresses the effectiveness of hydrologic restoration in restoring oligotrophic conditions of Everglades wetlands. Generalized random-tessellation stratification was used to select sampling sites, which were visited by helicopter (Stevens & Olsen, 2004). For this program, the Everglades was divided into basins and sampling within each basin was performed by dividing it into 800 m x 800 m Principal Sampling Units (PSUs) which were selected randomly for each year (Figure 1). At the sampling site, a 1 m^2 quadrat was randomly selected for sampling and periphyton surface cover as a percent of that quadrat, and visible composition are recorded. All periphyton in the quadrat is collected and volume measured with a perforated graduated cylinder. A representative 120-mL subsample of periphyton was collected and returned to the lab for further analysis.

3. Lab Analysis Methods

Periphyton subsamples were weighed before non-algal material (e.g., rocks, macrophytic material, gastropod shells) were removed from each sample before homogenizing in distilled water. Subsamples of the periphyton homogenate were taken for multiple analyses: a 10-mL subsample was oxidized for diatom analysis, a 40-mL sample was used to measure periphyton dry mass after drying in a 60°C oven for at least 48 hours, and the remaining is used for analysis of periphyton total phosphorus, also after drying in a 60°C oven for at least 48 hours. TP analysis is done by the Blue Carbon Analytical Laboratory at Florida International University, where the dried and ground TP sample was ashed, acidified with 5 mL of 0.2N HCl and heated at 80°C for thirty minutes before 10 mL of deionized water was added to each sample. Samples were capped tightly and vortexed before permitted to settle overnight. 1 mL of prepared sample is mixed with 9 mL of deionized water and 1 mL of a reagent prepared from ammonium molybdate, sulfuric acid, ascorbic acid, and potassium antimonyl tartrate before being analyzed with a Shimadzu UV-2101PC spectrophotometer (Shimadzu Corporation, n.d.). Oxidation of diatom samples was according to Hasle & Fryxell (1970), and a volumetric subsample was dried onto a coverslip and then permanently affixed to microscope slides using Naphrax (Phycotech Inc., n.d.).

Diatom counts were performed by selecting a random starting point on the slide and identifying all diatom valves (one-half of the complete silica shell that surrounds individual diatoms) that enter the field of view along a transect until 500 valves were identified plus any additional valves in the field of view at that point, recording the start and end coordinates on the stage. Relative abundances of each species were also

calculated by dividing the number of valves of each species found by the total number of

valves counted.

4. Data Analysis

Hypothesis	Network Measure	Expected Behavior
1: Algal assemblages present in calcareous periphyton demonstrate a small-world network structure under natural (P-limited) conditions.	Degree Distribution	Absolute value of the power law exponent, γ, decreases with increasing mean periphyton TP
2: A subset of the species consistently found in calcareous periphyton engineer the network cohesion of periphyton.	Nestedness	High nestedness in low P periphyton
	Mean First-order Ego Size	Significant change in value when important species are removed.
3: The presence of excess P is linked with the frequency decline of the same species providing cohesion to calcareous periphyton identified by testing (2).	Mean first-order ego size	Effect of removal decreases with increasing P in periphyton.
	Frequency of occurrence in network	Frequency of a species in the data generating a network decreases with increasing TP.

Table 2. Network measures tested and expected behaviors of those measures if the null hypothesis were to be rejected.

Species composition data were grouped by year and ranked by periphyton TP. To

determine the effects of periphyton mat TP on network structure, I used a sliding window

approach, where sets of 10 consecutive TP-ordered samples within the same year were

considered at a time when networks were generated (i.e., ranked samples 1-10, 2-11, 3-12

and so on). Species data were then transformed into a binary co-occurrence matrix by

means of matrix multiplication, and all values greater than 0 were set to 1 to represent a

cooccurrence of two species. Mean TP for each network of 10 sites was calculated and

used for analysis of how network measures changed with the sliding window gradient. Measures of how species co-occurrence was distributed (degree distribution) and the number of other species with which a species co-occurred within each network (first-order ego size) were used as the key response variables in these analyses (Bascompte & Jordano, 2014; Csárdi & Nepusz, 2006). First order ego size – the number of species within one link of the species being analyzed, including itself – was used as looking at ego sizes between networks incorporates both the number of species and the number of connections in each network.

Using the R library igraph, networks were constructed using the binary cooccurrence matrices (Csárdi & Nepusz, 2006). Utilizing the included functions for determining power-law fit of degree distributions above the minimum degree in the network per equation 1 and mean first-order ego size, measures of these descriptors of network structure were calculated. Using the e.divisive function from the R library ecp, changepoints in plots of these measurements compared with network TP were identified for each year (James & Matteson, 2014). Based on mean location of these changepoints, a two-way ANOVA with replication was performed to analyze differences in power law exponent and mean first-order ego size based on year and category of mean TP.

Utilizing the Nestedness for Dummies software, Nestedness based on Overlap and Decreasing Fill (NODF) of all networks were calculated to serve as a measure of overall species interdependence in networks (Strona & Fattorini, 2014). These data were then imported to R and matched with the year and mean TP of networks used to calculate NODF index values. As with power-law exponents and mean first-order ego size, changepoints were calculated using the e.divisive function in R and a two-way ANOVA

with replication was utilized to identify significant differences between groups based on year and TP categorization.

To determine the effects of removing species on networks, mean first-order ego size of networks where a species occurred were compared with the mean first-order ego size of the same networks with the species in question removed and a dummy value added to represent a species which is present but not interacting with any other species in the network. A paired t-test was performed to determine if the difference between the two measurements was significantly different from 0, and if so, the species identity was recorded. For those species, changepoints for the difference between means were calculated and two-way ANOVA with replication was performed as with nestedness and mean first-order ego size. Additionally, the general effects of mean TP in each network on frequency of each of the identified species was observed to see if there was a relationship between mean TP and the frequency of diatom species occurrence in networks.

III. Results

There was no significant change in how species connections were distributed within networks with increasing mean periphyton TP. In other words, community structure did not completely break down with increasing TP. Second, nestedness (and thus, species interdependence) decreased in response to increasing mean periphyton TP, and the single removal of most species had little effect on how connected the rest of the network was. Third, most species identified as having a significant effect on network structure through removal were species whose frequencies increased with mean

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periphyton TP, including *Encyonema evergladianum*, which has previously been

identified as an indicator of low TP (Mazzei & Gaiser, 2017; Slate & Stevenson, 2007).

Year	Number of sites	Mean TP (µg/g)	Mean dry mass (g)	Minimum species richness	Maximum species richness
2007	86	147 (114)	233 (337)	7	34
2008	112	214 (130)	101 (220)	8	45
2009	107	144 (92)	182 (253)	7	32
2010	111	134 (79)	149 (203)	7	55
2011	87	163 (114)	268 (381)	6	37
2012	81	236 (102)	80 (126)	7	29
2013	94	112 (64)	139 (213)	7	35
2014	109	138 (126)	245 (354)	6	30
2015	112	208 (201)	315 (623)	5	31
2016	108	176 (159)	64 (81)	7	33

Table 3. Table describing the distribution of data utilized in analyses. Specifically, with regards to the number of sites, mean sample periphyton mat TP, mean periphyton sample dry mass, and minimum and maximum sample species richness.

1. Power-Law Structure of Degree Distribution.

Changepoint analysis suggests that if there is a difference between groups in the exponent of power-law descriptions of degree distribution above x_{min} in the networks generated, that those differences would be found based on mean changepoints of 100.34 +/- 41 µg/g and 206.77 +/- 56 µg/g TP (Figure 2). Indeed, mean exponent absolute values of 2.22 +/- 0.24, 2.22 +/- 0.27, and 2.20 +/- 0.25 were found for those groups, reflecting a small decrease in how rapidly probability distributions fell. However, two-way ANOVA analysis with replication of exponent values demonstrated that these differences between groups based on mat TP were not significant ($F(2, 17) = 0.808$, $p = 0.446$). Significant effects were observed based on year and the interaction between year and mat TP grouping.
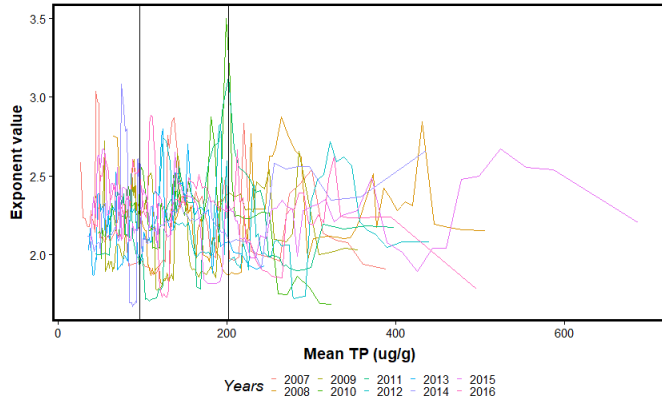
Figure 2. Power law exponent along a gradient of network mean TP (µg/g). The value (γ) that fits equation 1 for the degree distribution of each network above some minimum degree. Vertical lines indicate the mean first and last changepoint in the plot for all years. While breakpoints in individual years are significant for each year at $p < 0.05$, a two-way ANOVA with replication indicates that mean exponent value is not significantly different between groups across years (TP: $F_{(2, 17)} = 0.808$, $p = 0.446$, Years: $F_{(9, 17)} = 3.02$, $p < 0.01$).

2. Network Nestedness and Species Connection.
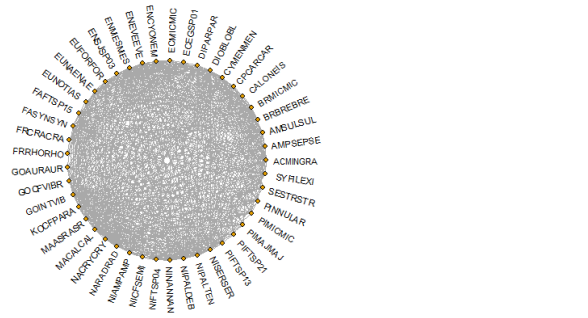

Figure 3. A network generated utilizing data from 2007 with a mean TP of 93.7 µg TP/g periphyton dry mass. The network contains 45 nodes (taxa) and 527 connections between nodes. Taxon codes are used as labels for each node.

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Figure 4. A network generated using data from 2007 with a mean TP of 273.6 µg TP/g periphyton dry mass. The network contains 55 nodes (taxa) and 826 connections between nodes. Taxon codes are used as labels for each node.

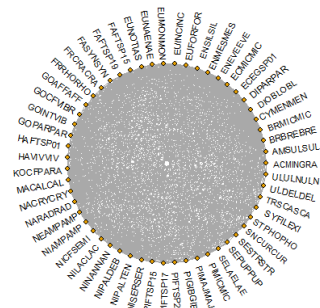Using changepoint analysis based on the relationship between NODF index values and mean TP in networks, mean locations of changepoints were identified as 83.12 +/- 48 µg/g and 195.36 +/- 65 µg/g TP (figure 3). Based on these changepoints, it was determined that the nestedness of networks compared to mean TP in samples used to generate networks differs significantly between low, medium, and high TP groups, per two-way ANOVA with replication and confirmed with a Tukey's post-hoc test ($F(2,17)$ = 65.30, $p < 0.01$). Mean NODF values of 57.30 +/- 6 µg/g, 54.24 +/-6 µg/g, and 51.53 +/- 7 µg/g TP were identified for low, medium, and high mean TP networks across all years. A decline in nestedness indicates that species are more vulnerable to extinction and thus that species composition in low-nestedness networks would be less consistent. A qualitative review of the species of networks at high mean TP supports this assertion.
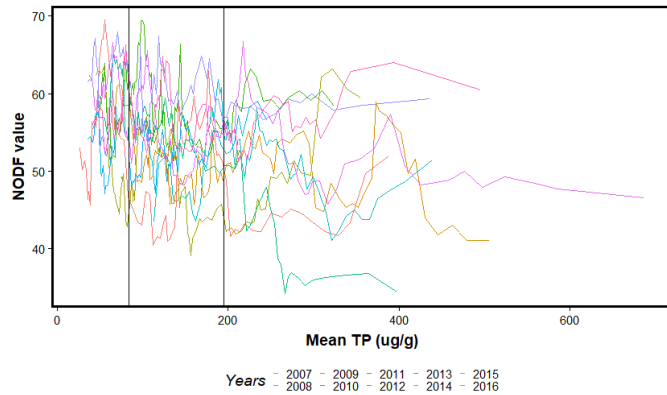
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Figure 5. NODF compared to mean TP (µg/g). NODF is a measurement of network nestedness indicative of species interdependence. Vertical lines indicate the mean first and last changepoint in the plot for all years. Changepoints in individual years are significant for each year at $p < 0.05$, and a mean initial and final breakpoint were calculated from those breakpoints. A two-way ANOVA with replication, followed by Tukey post-hoc test indicates that NODF differs significantly in all combinations of low, medium, and high TP groupings (TP: $F_{(2, 17)} = 65.30$, $p < 0.01$. Years: $F_{(9, 17)} = 56.69$, $p < 0.01$).
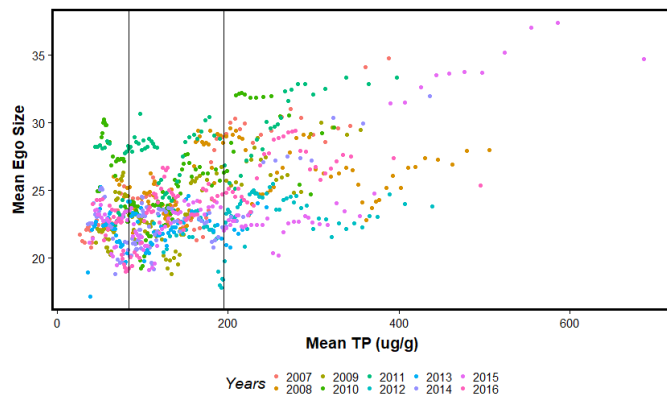
Figure 6. Mean first-order ego size compared to mean TP (µg/g). First-order ego size of a node is equal to the degree of the node plus one (the node itself). Vertical lines indicate the mean first and last changepoint in the plot for all years. Changepoints in individual years are significant for each year at $p < 0.05$, and a mean initial and final breakpoint were calculated from those breakpoints. A two-way ANOVA with replication indicates that mean first-order ego size differs significantly between low, medium, and high TP groupings, between years, and that the combination of those factors is significant. A Tukey post-hoc test looking at significant differences in means found significant differences between TP groupings at $p < 0.001$. (TP: $F_{(2,17)} = 183.94$, $p < 0.01$; Years: $F_{(9,17)} = 9.85$, $p < 0.01$)

In comparing the effects of removing species on networks by t-test between mean first-order ego size of networks with and without each species, a total of 51 species were identified where removal had a greater-than-expected effect on mean first-order ego size (table 1). Several of these species have previously been identified as important indicators of ecosystem health in response to TP. Two-way ANOVA with replication was performed on these 51 species to identify if there were significant differences in the effect of removal on first-order ego size, with Tukey's post-hoc test performed on those species where significant differences were found between groups. Species that had significant differences between TP groups identified include members of motile and non-motile genera of diatoms, such as *Achnanthidium*, *Fragilaria*, *Mastogloia*, and *Ulnaria*.
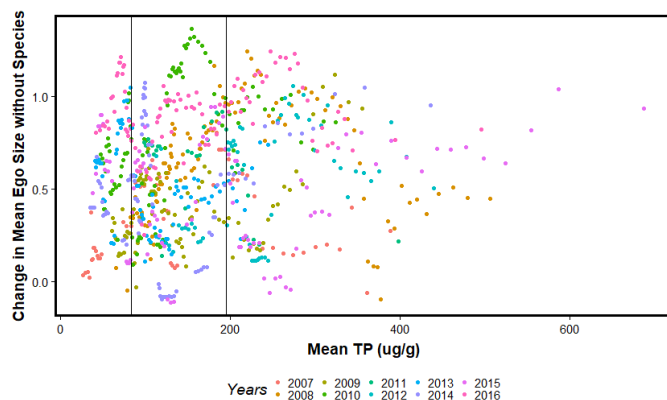
Figure 7. Change in mean ego size for networks that contain the species *Achnanthidium minutissimum* var. *gracillima* versus the same networks with the node representing *A. minutissimum* var. *gracillima* removed. Vertical lines represent mean first and last changepoints for plot in all years. Changepoints in individual years are significant at $p < 0.05$ and a mean initial and final changepoint were calculated from those breakpoints. A paired t test between networks with *A. minutissimum* var. *gracillima* in place and removed was performed, finding that the difference was significant ($p = 0.0141$). An ANOVA with replication determined that there exist statistically significant differences between low, medium, and high TP groups (Years: $F_{(9, 16)} = 37.90$, $p < 0.01$. TP: $F_{(2, 16)} = 13.57$, $p < 0.01$).

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3. Effects of TP on Frequency of Species

For species where removal from networks has confirmed statistically significant effects, species frequencies in network were compared to mean TP in samples used to generate the network. Overall trends in species frequency with increasing TP and trends within sections are noted in Table 4. Of the 51 taxa identified with statistically significant effects, species frequency increased with TP in 23 taxa (including *Achnanthidium minutissimum* var. *gracillima*, *Encyonopsis floridana*, *Nitzschia amphibia* and *Ulnaria delicatissima*), decreased in 13 taxa (including *Amphora sulcata*, *Brachysira microcephala*, *Encyonema evergladianum*, and *Nitzschia palea* var. *debilis*), and were variable in 15 taxa (including *Amphora ovalis*, *Brachysira brebissonii*, *Caponea caribbea*, and *Mastogloia lanceolata*) (Table 4).
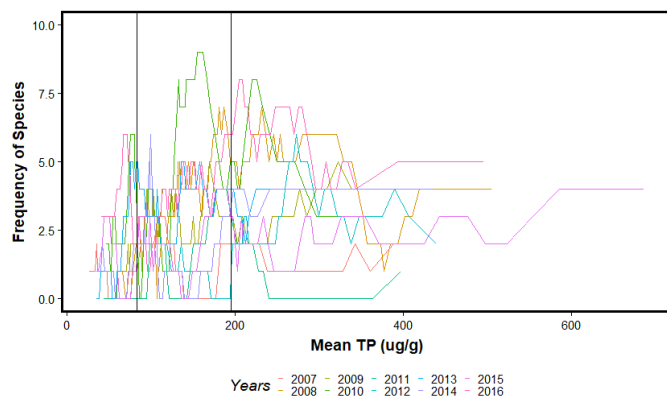
Figure 8. Frequency of diatom taxon *Achnanthidium minutissimum* var. *gracillima* in each network for years 2007-2016. Vertical lines indicate changepoints in community as determined by mean ego size in networks including all species. Counter to the hypothesis, there does not seem to be an overall trend of decreasing absolute frequency. Two-way ANOVA with replication identified statistically significant differences between groups based on TP ($F_{(2,17)} = 101.56$, $p < 0.01$) and year ($F_{(9,17)} = 75.43$, $p < 0.01$).
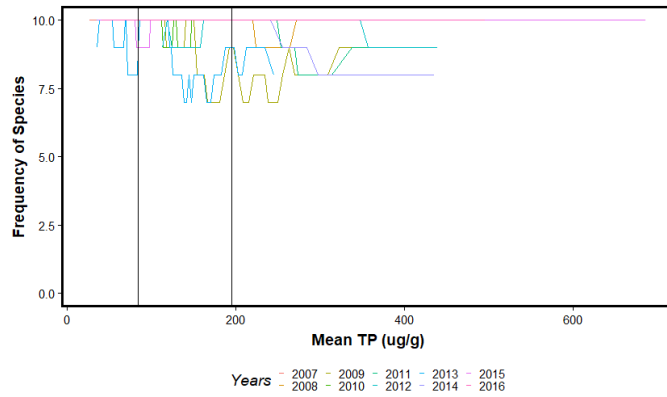
Figure 9. Frequency of diatom taxon *Brachysira microcephala* in each network along a mean TP (µg/g) gradient for years 2007-2016. Vertical lines indicate changepoints in community as determined by mean ego size in networks including all taxa. (TP: $F_{(2, 17)} = 37.21$, $p < 0.01$. Years: $F_{(9,17)} = 50.91$, $p < 0.01$)
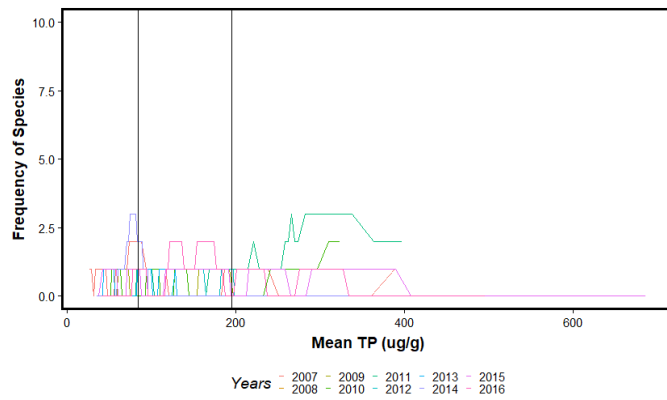
Figure 10. Frequency of diatom taxon *Caponea caribbea* in networks along a mean TP (µg/g) gradient for years 2007-2016. Vertical lines indicate changepoints in community as determined by mean ego size in networks including all taxa. Counter to the hypothesis, there does not seem to be an overall trend of decreasing frequency. (TP: $F_{(2, 17)} = .045$, $p = 0.96$, Years: $F_{(9,17)} = 18.52$, $p < 0.01$)

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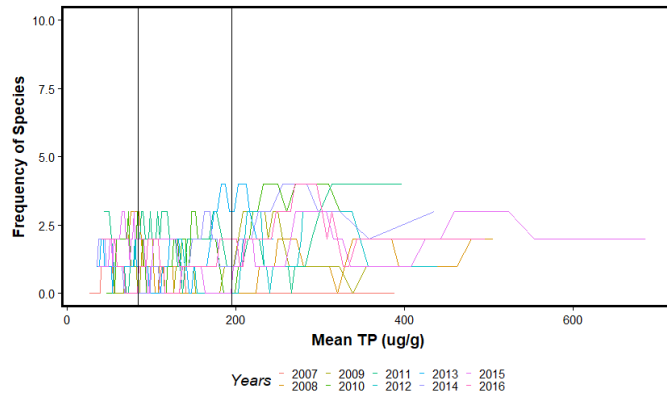
Figure 11. Frequency of diatom taxon *Encyonopsis subminuta* in each network along a mean TP (μg/g) gradient for years 2007-2016. Vertical lines indicate changepoints in community as determined by mean ego size in networks including all taxa. (TP: $F_{(2, 17)} = 70.91$, $p < 0.01$. Years: $F_{(9,17)} = 22.42$, $p < 0.01$)

Taxon Name	T-value	P-value	Average Frequency Low TP	Average Frequency Medium TP	Average Frequency High TP	Trend
Achnanthidium minutissimum var. *gracillima* (Meister) L. Bukhtiyarova	2.73	0.006	1.96	2.99	3.26	Increasing
Amphora ovalis (Kützing) Kützing	3.24	0.002	0.03	0.08	0	Variable
Amphora sulcata Schmidt	3.55	0.000	1.16	1.17	0.98	Decreasing
Aulacoseira cf. *distans* (Ehrenberg) Simonsen	2.27	0.024	0.14	0.14	0.05	Decreasing
Aulacoseira cf. *granulata* (Ehrenberg) Simonsen	2.70	0.008	0.14	0.08	0.1	Decreasing
Brachysira brebissonii R. Ross	2.66	0.008	0.4	0.37	0.71	Variable

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Brachysira microcephala (Grunow) Compère	-2.34	0.020	9.89	9.63	9.58	Decreasing
Caponea caribbea A.C. Podzorski	2.85	0.005	0.44	0.39	0.42	Variable
Craticula accomodiformis Lange-Bertalot	2.21	0.030	0.08	0.02	0	Decreasing
Cyclotella iris Brun & Héreibaud	4.46	0.000	0.05	0.02	0.03	Variable
Diploneis puella (Schumann) Cleve	2.82	0.005	0.15	0.19	0.18	Variable
Encyonopsis egsp01	2.68	0.007	0.51	0.38	0.64	Variable
Encyonopsis floridana Krammer	2.21	0.030	0	0.04	0.17	Increasing
Encyonopsis subminuta Krammer & E. Reichardt	3.89	0.000	1.02	1.02	1.91	Increasing
Encyonema cf. *evergladianum* Krammer	2.31	0.022	0.14	0.35	0.35	Increasing
Encyonema evergladianum Krammer	-2.22	0.027	9.95	9.84	9.42	Decreasing
Encyonema ftsp04	2.26	0.025	0.09	0.18	0.01	Variable
Encyonema silesiacum var. *elegans* Krammer	2.17	0.030	2.19	2.74	4.1	Increasing
Encyonema silesiacum (Bleisch) D.G. Mann	3.21	0.001	0.19	0.67	1.23	Increasing
Encyonema sjsp03	4.29	0.000	2	1.46	1.28	Decreasing
Eunotia egsp01	2.09	0.037	0.16	0.24	0.45	Increasing

Eunotia flexuosa (Brébisson ex Kützing) Kützing	2.37	0.018	0.27	0.91	2.24	Increasing
Eunotia formica Ehrenberg	2.02	0.044	0.21	0.31	0.39	Increasing
Eunotia naegelii Migula	2.73	0.006	0.28	0.74	2.07	Increasing
Fragilaria cf. *tenera* (W.Smith) Lange-Bertalot	1.97	0.050	0.08	0.26	0.48	Increasing
Fragilaria ftsp16	2.07	0.039	0.04	0.26	0.28	Increasing
Fragilariforma virescens var. *capitata* (Østrup) Czarnecki	1.97	0.049	0.09	0.28	0.25	Variable
Frustulia crassinervia (Brébisson ex W. Smith) Lange-Bertalot & Krammer	2.83	0.005	0.46	0.26	0.55	Variable
Gomphonema affine Kützing	2.38	0.018	0.31	0.19	0.45	Variable
Gomphonema cf. vibrioides E. Reichardt & Lange-Bertalot	3.58	0.000	0.58	1.34	1.63	Increasing
Gomphonema gracile Ehrenberg	2.41	0.016	0.44	0.34	0.29	Decreasing
Mastogloia calcarea S.S. Lee, E. E. Gaiser, B. Van de Vijver, M.B. Edlund and Spaulding	-2.13	0.033	8.37	8.86	9.14	Increasing
Mastogloia lanceolata Thwaites ex W. Smith	2.60	0.010	0.33	0.39	0.23	Variable
Navicula densilineolata (Lange-Bertalot) Lange-Bertalot	3.27	0.001	0.05	0.06	0.17	Increasing

Navicula radiosafallax Lange-Bertalot	2.28	0.023	0.21	0.49	0.63	Increasing
Nitzschia amphibia Grunow	3.01	0.003	0.98	1.86	3.24	Increasing
Nitzschia cf. semirobusta Lange-Bertalot	2.55	0.011	0.33	0.76	0.5	Variable
Nitzschia ftsp16	2.19	0.029	0.24	0.33	0.16	Variable
Nitzschia intermedia Hantzsch ex Cleve et Grunow	2.15	0.033	0.21	0.32	0.37	Increasing
Nitzschia microcephala Grunow	2.28	0.026	0	0.08	0	Variable
Nitzschia nana Grunow	2.30	0.022	0.3	0.43	1.46	Increasing
Nitzschia palea var. *debilis* (Kützing) Grunow	-2.01	0.044	9.5	9.18	8.82	Decreasing
Nitzschia palea var. *tenuirostris* Grunow	3.09	0.002	1.03	1.18	1.91	Increasing
Pseudostaurosira brevistriata (Grunow) D.M. Williams & Round	2.37	0.018	0.8	0.56	0.49	Decreasing
Pinnularia microstauron (Ehrenberg) Cleve	2.31	0.021	0.57	0.55	0.98	Variable
*Rhopalodia gibba (*Eherenberg) O. Müller	2.16	0.031	0.09	0.25	0.43	Increasing
Sellaphora laevissima (Kützing) D.G. Mann	3.28	0.001	2.42	1.53	0.95	Decreasing

Sellaphora seminulum (Grunow) D.G. Mann	2.52	0.012	0.28	0.24	0.08	Decreasing
Sellaphora stroemii (Hustedt) H. Kobayasi	3.74	0.000	0.74	0.63	0.46	Decreasing
Synedra filiformis var. *exilis* Cleve	3.08	0.002	0.2	0.36	0.53	Increasing
Ulnaria delicatissima (W. Smith) Aboal & P.C. Silva	2.54	0.012	0.17	0.29	0.97	Increasing

Table 4. Species with significant differences in mean first-order ego size between otherwise identical networks with and without the species. Results of t-test comparing the mean first-order ego size of networks containing the specified taxon before and after they were removed, average frequency in low, medium, and high TP, and the pattern that the frequency of species follows with increasing TP (increasing, decreasing, or peaking at medium TP).

IV. Discussion

I found that while networks as a whole do not collapse with increasing TP, there exists a core of species, including previously identified indicator species, which have their role in network structure altered by eutrophication. In some cases, species frequency behaves as expected based on previous abundance-based works, with decreasing frequency in response to increased TP. However, for some species, frequency behaves different to the trends observed for species abundance. This can be either species frequency increasing or displaying variable behavior with eutrophication.

Several diatom species have previously been identified as important indicators of ecosystem health in response to TP in the Everglades Ecosystem (Gaiser et al., 2015; Slate & Stevenson, 2007). Among these are *Achnanthidium minutissimum* var. *gracillima*, *Amphora sulcata*, *Encyonema evergladianum*, and *Nitzschia palea* var. *tenuirostris*, all of which have previously been found to be indicative of low TP, but my

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analysis found that these species peaked in absolute frequency at a higher TP than previously reported optima (if any). This could be due to the difference in analytical methodology, as Slate & Stevenson (2007) utilized historical TP data to calculate species optima, Gaiser et al. (2006) identifies changepoints based on TP content of periphyton, and Gaiser (2009) assigned stoplight categories based on the distance between mean values in environmental variables and site values. However, this paper uses species frequency in each network and assigns changepoints based on a statistical analysis of how those frequencies change along the TP gradient. While these studies differ on the location of borders between TP categories and how to identify those points, they do suggest that significant differences in periphyton communities exist and can be identified with multiple methodologies.

TP Category	Gaiser et al. 2006 (Experimental multivariate regression)	Gaiser 2009 (Gradient study)	This work (Networks and species frequency)
Low	< 178 µg/g TP	< 100 µg/g TP	< 83 µg/g
Medium	178 – 458 µg/g TP	100-200 µg/g TP	83-195 µg/g
High	> 458 µg/g TP	> 200 µg/g TP	> 195 µg/g

Table 5. Summary of periphyton TP changepoints in the Everglades from previous works (E. Gaiser, 2009; Evelyn E. Gaiser et al., 2006), as well as the changepoints found in this study.

1. Power-Law Structure of Degree Distribution

Results indicated that there is no significant effect of TP on the strength with which degree distribution, the number of connections per species versus the probability of finding a species with that number of connections, decays according to Equation 1 above x_{min} (Bascompte & Jordano, 2014; Csárdi & Nepusz, 2006). Measurements of network characteristics are often interdependent with one characteristic having the

potential to drastically alter others (Bascompte & Jordano, 2014). The power-law equation is indicative of the shape of the degree distribution, with the exponent describing how quickly the probability of a species having a specified degree falls in response to increasing the species degree being sought. As there was no significant effect of TP on the exponent alpha with which probability decays, I cannot reject the null hypothesis that mat TP has no effect on the degree distribution of the networks analyzed in this paper. This conclusion thus does not suggest that there is a change in the overarching structure of networks with increasing TP. Based on this, I suggest that there exists no relationship between mean network TP and overall network structure. While specific diatom species are known to play crucial roles in the production and maintenance of the calcareous periphyton of the Everglades (e.g., *Mastogloia calcarea*, *Encyonema evergladianum*), it does not appear that there is an over-arching structure to the network that also changes with increasing TP (Evelyn E. Gaiser et al., 2006; La Hée & Gaiser, 2012; Slate & Stevenson, 2007).

2. Network Nestedness and Species Connection

Significant changes in network nestedness occur with increasing TP and demonstrates that there are changes occurring within networks as nutrient loads increase beyond those found under natural conditions in the Everglades. The decrease in NODF with increasing TP suggests that networks are becoming less nested and that species interdependence is thus decreasing (Bascompte & Jordano, 2014; James et al., 2012). Exact causes and consequences of this change are not in the scope of this study, but causes could range from species being less dependent on exudates found in the matrix of the periphyton to changes in metabolic pathways resulting from increased P availability

to cosmopolitan generalist species being better able to thrive in nutrient enriched environs and harsher conditions combining to drastically alter which species are present.

3. Effects of TP on Frequency of Species

Most species which were identified as being significantly affected by TP in periphyton increased in frequency along the TP gradient. This is consistent with the general concept of the Everglades as a P-limited ecosystem (Gaiser et al., 2011). However, it does raise questions of how community structure has changed with increasing TP and how that plays into the dissociation and dissolution of periphyton mats. Other research suggests that much of the reason that periphyton dissolves is that the heterotrophic bacterial component of the mat community becomes more active with increased TP and digest the mucilage that holds the various components of the mat together (Gaiser et al., 2015; Johnson et al., 1997).

4. Implications

There have been several different approaches to using periphyton to identify environmental P thresholds in the Everglades since the early 21st century (e.g., Gaiser, 2009; Gaiser et al., 2006, 2004). This work demonstrates that even without the benefits of using abundance data, environmental thresholds are still detectable, and that currently accepted thresholds can even be considered too high, as species frequency is significantly altered in several core species at levels below current accepted abundance-based thresholds. Based on this, one could argue that current Everglades restoration goals do not go far enough to fend off further changes to the periphyton community in the Everglades.

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One potential avenue of further research in this area include looking at how the addition of other components of the periphyton community alters the results reported, especially the heterotrophic bacteria which are implicated in the process of periphyton dissociation. However, this would require the use of expensive molecular techniques to determine the identity of species of bacteria that cannot be cultured (e.g., Pham & Kim, 2012; Steele et al., 2011), and significant thought on how to accurately standardize count data from this project with count data for soft algae and the suggested molecular work, which do not have the same definition of counting units due to differences in the type of data being generated (single diatom valves versus a variety of colony or filament structures and cell sizes versus how molecular data can take a variety of forms). Similar research in other systems includes soil microbiomes, the microbial communities in lungs of cystic fibrosis patients, and human gut flora (de Menezes et al., 2015; Fernandez et al., 2015; Kurtz et al., 2015; van der Gast et al., 2011)

Other avenues of research may include the effect of three-dimensional mat structure, other environmental variables such as pH, water depth, and precipitation patterns, and even different numbers of samples in network generation on network structure. Each of these may provide distinct useful insights into the way that non-trophic interaction networks influence both communities and emergent properties thereof.

The results I have found suggest that the network dynamics studied do not have strong responses to the mean TP of networks, and thus that a causal relationship between whole-network dynamics and mat cover does not exist. Future utilization of ENA in this context may depend on a more species-centric focus.

5. Context

The Everglades is a highly P-sensitive system which responds dramatically to even low levels of eutrophication (Evelyn E. Gaiser et al., 2006; Noe et al., 2001). Previous works have demonstrated that periphyton cover, one of the major measures of ecosystem health, is negatively impacted by almost any level of P enrichment, and with significant effects at levels above 178 µg TP/g periphyton dry mass and more dramatic effects at 458 µg TP/g periphyton dry mass (Evelyn E. Gaiser et al., 2006). This work suggests that such thresholds may be too generous and community health is already being impacted at lower levels (83 versus 178 µg TP/g periphyton dry mass). Additional work focusing on the effects of species abundance, as well as how other environmental variables effect changes on periphyton communities will aid in further quantifying exactly how TP alters the community which produces the periphyton mats of the Everglades.

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APPENDICES

Appendix A: Analyses and Figures Code

A.1 Network Graph Labeling

```
library(igraph)

g <- g.sim_storage[[70]]

# laid out as a circle to begin with

la <- layout.circle(g)

par(mar=c(8,6,6,6))

plot(g, layout=la, vertex.size=5, vertex.label="")

# Apply labels manually

#Specify x and y coordinates of labels, adjust outward as desired

x = la[,1]*1.3

y = la[,2]*1.3

#create vector of angles for text based on number of nodes (flipping the orientation of the

words half way around so none appear upside down)

angle = ifelse(atan(-(la[,1]/la[,2]))*(180/pi) < 0,  90 + atan(-(la[,1]/la[,2]))*(180/pi), 270

+ atan(-la[,1]/la[,2])*(180/pi))

#Apply the text labels with a loop with angle as srt

for (i in 1:length(x)) {

  text(x=x[i], y=y[i], labels=V(g)$name[i], adj=NULL, pos=NULL, cex=.7, col="black",

srt=angle[i], xpd=T)

}
```

```
# Code sourced from

https://gist.github.com/ajhmohr/5337a5c99b504e4a243fad96203fa74f
```

```
library(ggplot2)

library(ggthemes)

theme_Publication <- function(base_size=14, base_family="helvetica") {

 library(grid)

 library(ggthemes)

 (theme_foundation(base_size=base_size, base_family=base_family)

  + theme(plot.title = element_text(face = "bold",

                    size = rel(1.2), hjust = 0.5),

      text = element_text(), panel.background = element_rect(colour = NA),

      plot.background = element_rect(colour = NA), axis.title = element_text(face =

"bold",size = rel(1)),

      axis.title.y = element_text(angle=90,vjust =2), axis.title.x = element_text(vjust = -

0.2),

      axis.text = element_text(), axis.line = element_line(colour="black"),

      axis.ticks = element_line(), panel.border = element_rect(colour = "black", fill =

NA, size = 2),

      panel.grid.major = element_blank(), panel.grid.minor = element_blank(),

      legend.key = element_rect(colour = NA), legend.position = "bottom",

      legend.direction = "horizontal", legend.key.size= unit(0.2, "cm"),

      legend.spacing = unit(0, "cm"), legend.title = element_text(face="italic"),
```

```
       plot.margin=unit(c(10,5,5,5),"mm"),

        strip.background=element_rect(colour="#f0f0f0",fill="#f0f0f0"),

       strip.text = element_text(face="bold")

  ))

 }
```

#adapted from https://rpubs.com/Koundy/71792

<div align="center">A.3 Importing Data from Excel</div>

```
# Run to perform data import, clean

#' *Import Phase*

# Set working directory to folder containing data CSVs

setwd("~/R/Research Scripts/Thesis/OriginalData/CSVs/")

# Creates character vector containing all filenames for folder which end in .csv.

# e.g. "Data-2007 summary.csv"

filenames <- list.files(path = getwd() , pattern = "*.csv")

# Create character vector containing full file path to each data file.

fullpath = file.path(getwd(),filenames)

# Print file paths. Can be commented out without issue.

print(fullpath)

# Creates dataframe containing all data from each .csv file.

dataset <- do.call("rbind",lapply(filenames,FUN=function(files) {

 read.csv(files, stringsAsFactors = FALSE, na.strings = c("NA", "#DIV/0!",

"#VALUE!"))}))

# End of importing phase
```

```
#' *Data Cleaning Phase*

# Create dataset that only contains quantitative samples

dataset2 <- dataset[(dataset$Sample.Quantitative...Y.or.N. == "Y"),]

# Remove columns containing extraneous/redundant information (replicate number,

#  sample plot area, whether sample is quantitative or not. Also remove relative

#  abundance columns)

dataset3 <- dataset2[ ,-c(3, 14, 19, 53:441)]

# Remove samples from Loxahatchee and Lake Okeechobee due to differing underlying

geology/soil.

dataset4 <- dataset3[!dataset3$Location == "LOX" & dataset3$Location != "LKO",]

# Remove samples which do not have positive value (or are NA).

dataset5 <- dataset4[which(as.numeric(dataset4$TP.µg.g) >= 0), ]

#'*Write Combined Dataset for backup purposes.*

write.csv(dataset5, "~/R/Research

Scripts/Thesis/OriginalData/CombinedQuantData.csv", row.names = FALSE)

setwd(dir = "~")

#'End of ImportAndClean.R

# The output is manually inspected and cleaned for compatibility with planned

# abundance-related analyses.
```

A.4 Generating Network Matrices

```
#'*Group data and create matrices (binary)*

# Libraries

library(igraph)
```

```
# Functions

is.wholenumber <-  function(x, tol = .Machine$double.eps^0.5)  abs(x - round(x)) < tol

#' *From the is.integer() man page*

# Import data from manually cleaned dataset, which has also been ordered by year and

TP

data1 <- read.csv("C:/Users/Eric/Desktop/OrderedQuantData-Cleaned1.csv")

# Create complete separate dataframes for environmental and species co-occurrence.

env.data.raw <- data1[ ,c(1:69,(length(data1)-2):length(data1))]

spp.data.raw <- data1[ ,70:(length(data1)-3)]

for (iter in 1:length(colnames(spp.data.raw))){

 x <- colnames(spp.data.raw)[iter]

 output <- substr(x, 1, nchar(x) - 2)

 colnames(spp.data.raw)[iter] <- output

}

window.size <- 9

n.loop <- as.numeric(nrow(env.data.raw)) - window.size

#Create empty lists

year.analysis <- as.list(rep(NA, n.loop))

spp.data_storage <- as.list(rep(NA, n.loop))

tp.min_storage <- as.list(rep(NA, n.loop))

tp.max_storage <- as.list(rep(NA, n.loop))

env.data_storage <- as.list(rep(NA, n.loop))

g.sim_storage <- as.list(rep(NA, n.loop))
```

```r
lay.sim_storage <- as.list(rep(NA, n.loop))

co.occ_storage <- as.list(rep(NA, n.loop))

mean.surface_storage <- as.list(rep(NA, n.loop))

for (i in 1:n.loop) {

 #get start and end row numbers for window

 #For tracking rough location of faults, providing assurance that program hasn't hung.

 if (is.wholenumber(i/50) == TRUE) {

   print(i)

   print(Sys.time())

 }

 window.init <- as.numeric(i)

 window.end <- as.numeric(i+window.size)

 #Determine if only data from one year is represented. If not, skip to next line, try again.

 if (length(unique(env.data.raw[c(window.init:window.end), 1])) == 1 &&

is.na(unique(env.data.raw[c(window.init:window.end), 1])) == FALSE) {

   #Get raw data

   year.analysis[[i]] <- unique(env.data.raw[c(window.init:window.end), 1])

   data.temp <- spp.data.raw[c(window.init:window.end), ]

   spp.data <- as.matrix(data.temp)

   spp.data_storage[[i]] <- spp.data

   env.data <- env.data.raw[c(window.init:window.end), ]

   tp.min_storage[[i]] <- min(env.data[ ,38])

   tp.max_storage[[i]] <- max(env.data[ ,38])
```

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```r
    mean.surface_storage[[i]] <- mean(env.data[ ,16])

    env.data_storage[[i]] <- env.data

    #Create square matrix for undirected network with values at interaction points.

    m <- t(spp.data) %*% spp.data

    #Convert to binary presence/absence matrix.

    m[m > 0] <- 1

    #storage of matrix.

    co.occ_storage[[i]] <- m

    #Create graph

    g <- graph_from_adjacency_matrix(m, mode = "upper")

    #simplify by removing nodes with zero links whatsoever.

    g <- igraph::delete.vertices(simplify(g),

                    degree(g) == 0)

    g.sim <- delete_edges(g, E(g)[which_loop(g) == TRUE])

    # Layout graph

    lay.sim <- layout_in_circle(g.sim)

    #Store graph and network layout.

    g.sim_storage[[i]] <- g.sim

    lay.sim_storage[[i]] <- lay.sim

  }

}

#remove empty networks here.

for (i in rev(1:length(g.sim_storage))){
```

```r
  if (class(g.sim_storage[[i]]) == "logical") {

    print(i)

    env.data_storage[[i]] <- NULL

    g.sim_storage[[i]] <- NULL

    lay.sim_storage[[i]] <- NULL

    co.occ_storage[[i]] <- NULL

    tp.max_storage[[i]] <- NULL

    tp.min_storage[[i]] <- NULL

    spp.data_storage[[i]] <- NULL

    year.analysis[[i]] <- NULL

  }

}

setwd(dir = "~")

#End of GroupMatrices.R
```

A.5 Generate igraph Networks and Layouts, Calculate Mean TP in Networks, and Mean

First-Order Egos

```r
#Libraries

library(igraph)

library(ggplot2)

#Function

is.wholenumber <-

  function(x, tol = .Machine$double.eps^0.5)  abs(x - round(x)) < tol

n.loop <- length(g.sim_storage)
```

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```r
#Create variable lists

degr <- as.list(rep(NA, n.loop))

degr.dist <- as.list(rep(NA, n.loop))

egos <- as.list(rep(NA, n.loop))

egos.size <- as.list(rep(NA, n.loop))

mean.egos <- as.list(rep(NA, n.loop))

for (i in 1:n.loop){

  g <- g.sim_storage[[i]]

  if (is.wholenumber(i/50) == TRUE){

    print(i)

    print(Sys.time())

  }

  if (length(g) > 1) {

    degr[[i]] <- degree(g, normalized = TRUE)

    degr.dist[[i]] <- degree.distribution(graph = g, cumulative = TRUE)

    egos.size[[i]] <- ego_size(g)

    mean.egos[[i]] <- mean(egos.size[[i]])

  }

}

years <- unlist(year.analysis)

min.TPs <- unlist(tp.min_storage)

max.TPs <- unlist(tp.max_storage)

ego.means <- unlist(mean.egos)
```

```
TP.means <- list()

for (i in 1:length(env.data_storage)) {

  TP.means[[i]] <- mean(env.data_storage[[i]]$TP.Âµg.g, na.rm = TRUE)

}

mean.TPs <- unlist(TP.means)

dat <- cbind.data.frame(years, min.TPs, max.TPs, mean.TPs, ego.means) #, adhes, assos,

gords, vcons, sw)

dat.complete <- dat[complete.cases(dat),]

setwd(dir = "~")

#End of datcompletegen.R
```

A.6 Perform Power-law Analysis and Related Figure

```
#libraries

library(tidyverse)

library(broom)

library(ecp)

#Power Law Analysis

powerlaw.output <- NULL

for (i in 1:length(g.sim_storage)){

  g <- g.sim_storage[[i]]

  if (class(g) == "igraph"){

    print(i)

    d <- igraph::degree(g)

    powerlaw <- fit_power_law(d, xmin = min(d), implementation = "plfit")
```

```
  alpha <- powerlaw[[2]]

  pvalue <- powerlaw[[6]]

  powerlaw.output <- rbind(powerlaw.output, c(alpha, pvalue))

 } else {

  powerlaw.output <- rbind(powerlaw.output, c(NA, NA))

 }

}

colnames(powerlaw.output) <- c("alpha", "p value")

#' *add year, mean tp to powerlaw.output*

powerlaw.data <- data.frame(cbind(powerlaw.output, years, mean.TPs))

#'*Changepoints*

#powerlaw.changepoints <- matrix(powerlaw.output)

vline.loc <- list()

vline.p <- list()

x1.storage <- list()

for (i in 1:length(unique(powerlaw.data$years))){

 year <- unique(powerlaw.data$years)[i]

 x1 <- matrix(rbind(powerlaw.data[,1][which(powerlaw.data$years == year)]))

 y1 <- e.divisive(x1, sig.lvl = 0.05, min.size = length(x1)/5)

 #print(length(as.numeric(as.character(NODF.analysis[,6][which(NODF.analysis$Year

== year)]))))

 vline.loc[[i]] <- y1$estimates

 vline.p[[i]] <- y1$p.values
```

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```
  x1.storage[[i]] <- x1

}

vline.loc

vline.vector <- vector()

#Create matrix/data frame with

vline.list <- list()

for (i in 1:length(vline.loc)) {

 for (j in 1:5){

  if(is.na(vline.loc[[i]][j]) != TRUE) {

   if (vline.loc[[i]][j] != 1 && vline.loc[[i]][j] != (length(x1.storage[[i]]) + 1)) {

     #attach to end of vector in positon [j]

     vline.vector[j] <- vline.loc[[i]][j]

    } else {

     #attach NA to end of vector in position [j]

     vline.vector[j] <- NA

    }

   } else {

    #attach NA to end of vector in position [j]

    vline.vector[j] <- NA

   }

  #attach vector to end of list in position [i]

  vline.list[[i]] <- vline.vector

 }
```

```
}

break1 <- numeric()

break2 <- numeric()

break3 <- numeric()

break4 <- numeric()

break5 <- numeric()

for (i in 1:length(vline.list)){

 year <- unique(powerlaw.data$years)[i]

 indx1 <- vline.list[[i]][1]

 indx2 <- vline.list[[i]][2]

 indx3 <- vline.list[[i]][3]

 indx4 <- vline.list[[i]][4]

 indx5 <- vline.list[[i]][5]

 break1[i] <- powerlaw.data$mean.TPs[which(dat.complete$years == year)][indx1]

 break2[i] <- powerlaw.data$mean.TPs[which(dat.complete$years == year)][indx2]

 break3[i] <- powerlaw.data$mean.TPs[which(dat.complete$years == year)][indx3]

 break4[i] <- powerlaw.data$mean.TPs[which(dat.complete$years == year)][indx4]

 break5[i] <- powerlaw.data$mean.TPs[which(dat.complete$years == year)][indx5]

}

vlines <- data.frame(years = unique(dat.complete$years),

              break1 = break1, break2 = break2,

              break3 = break3, break4 = break4,

              break5 = break5)
```

```r
vlines

vlines[6,5] <- vlines[6,4]

vlines[6,4] <- NA

vlines[10,5] <- vlines[10,4]

vlines[10,4] <- NA

vlines.order <- vlines[c(1,3,5)]

colnames(vlines.order) <- c("years", "first", "last")

#average change point by TP

mean.first <- mean(vlines.order$first, na.rm = TRUE)

mean.last <- mean(vlines.order$last, na.rm = TRUE)

#standard deviation for changepoints

sd.first <- sd(vlines.order$first, na.rm = TRUE)

sd.last <- sd(vlines.order$last, na.rm = TRUE)

print(paste("mean.first =", mean.first))

print(paste("sd.first =", sd.first))

print(paste("mean.last =", mean.last))

print(paste("sd.last =", sd.last))

#split data by year, into groups where TP < mean.first, mean.first < TP < mean.last, and

mean.last < TP

#Then anova by low, medium, high

dat.aov <- powerlaw.data

low <- dat.aov[which(dat.aov$mean.TPs < mean.first), ]

low$mean.TPs <- rep("LOW", length(low$mean.TPs))
```

```
high <- dat.aov[which(dat.aov$mean.TPs > mean.last), ]

high$mean.TPs <- rep("HIGH", length(high$mean.TPs))

med <- dat.aov[which(dat.aov$mean.TPs >= mean.first & dat.aov$mean.TPs <=

mean.last),]

med$mean.TPs <- rep("MED", length(med$mean.TPs))

dat.aov.twoway <- rbind(low, med, high)

#remove rows where alpha is NA

for (i in rev(1:nrow(dat.aov.twoway))) {

 if (is.na(dat.aov.twoway[i,1]) == TRUE) {

   dat.aov.twoway <- dat.aov.twoway[-i, ]

 }

 }

powerlaw.anova <- anova(lm(alpha ~ as.factor(years) * as.factor(mean.TPs),

dat.aov.twoway))

powerlaw.anova.summary <- summary(anova(lm(alpha ~ as.factor(years) *

as.factor(mean.TPs), dat.aov.twoway)))

powerlaw.data$Years <- as.factor(powerlaw.data$years)

# Figure

var.title <- "Power law exponent compared to mean TP (ug/g)"

ggplot(data = powerlaw.data, aes(x = mean.TPs, y = alpha, color = Years)) +

 geom_line() +   labs(x = "Mean TP (ug/g)", y = "Exponent value") +

 #theme(plot.title = element_text(hjust = 0.5)) + #ggtitle(var.title) +

 geom_vline(aes(xintercept = mean.first)) +
```

```
  geom_vline(aes(xintercept = mean.last)) +  theme_Publication()
```

#scale_colour_Publication() +

#End of powerlaw.R

A.7 Generating Matrices for Nestedness Analysis and Analyzing Nestedness Results

#Degree Distribution, nestedness

#libraries

```
library(igraph)

library(xlsx)

library(ggplot2)

library(ecp)
```

#set working directory

```
setwd("~/R/Nestedness/")

numrows <- length(colnames(spp.data_storage[[1]]))

numcols <- length(spp.data_storage)

numreps <- numrows * numcols

networks2 <- list()

k <- 0

networks2.names <- NULL

for (i in 1:length(env.data_storage)) {

 testing <- env.data_storage[[i]]

 if (is.null(dim(testing)) == FALSE) {

  networks2[[i]] <- t(spp.data_storage[[i]])

  if(is.wholenumber(i / 5) == TRUE) {
```

```
    print(i)

  }

 }

}

#Convert to binary presence/absence matrix.

networks3 <- list()

for (i in 1:length(networks2)){

  networks3[[i]] <- networks2[[i]]

  networks3[[i]][networks3[[i]] > 0] <- 1

}

for (i in 1:length(networks3)) {

  year <- unique(env.data_storage[[i]]$ï..Date)

  mean.tp <- mean(env.data_storage[[i]]$TP.µg.g)

  write.table(networks3[[i]], file = paste(year,mean.tp,"nestedness.txt", sep = "-"))

}
```

Run static download of Nestedness for Dummies (Strona & Fattorini, 2014) on folder

containing the output of the loop immediately above.

Copy outputs into document titled "output.networks.cleaned.xlsx" in folder specified

below.

Import output.networks

NODF.overall <-

read.xlsx("C:/Users/Eric/Documents/R/Nestedness/output.networks.xlsx", sheetIndex =

1)

```
#Add two columns with year and TP from splitting first column.

#Get data for columns

NODF.year.tp <- matrix(unlist(strsplit(as.character(NODF.overall[ ,1]), split = "-|\\s")),

ncol = 3, byrow = TRUE)

colnames(NODF.year.tp) <- c("Year","TP", "remove")

#Year + TP columns columns

NODF.analysis <- data.frame(cbind(NODF.overall,NODF.year.tp[,1:2]))

head(NODF.analysis)

vline.loc <- list()

vline.p <- list()

x2.storage <- list()

for (i in 1:length(unique(NODF.analysis$Year))){

  year <- unique(NODF.analysis$Year)[i]

  x2 <-

matrix(rbind(as.numeric(as.character(NODF.analysis[,2][which(NODF.analysis$Year ==

year)]))))

  y2 <- e.divisive(x2, sig.lvl = 0.05, min.size = length(x2)/5)

  #print(length(as.numeric(as.character(NODF.analysis[,6][which(NODF.analysis$Year

== year)]))))

  vline.loc[[i]] <- y2$estimates

  vline.p[[i]] <- y2$p.values

  x2.storage[[i]] <- x2

}
```

```
vline.loc

#Create matrix/data frame with

vline.vector <- vector()

vline.list <- list()

for (i in 1:length(vline.loc)) {

  for (j in 1:length(vline.loc[[i]])){

    if (vline.loc[[i]][j] != 1 && vline.loc[[i]][j] != (length(x2.storage[[i]] + 1))){

      #attach to end of vector in positon [j]

      vline.vector[j] <- vline.loc[[i]][j]

    } else {

      #attach NA to end of vector in position [j]

      vline.vector[j] <- NA

    }

  }

  #attach vector to end of list in position [i]

  vline.list[[i]] <- vline.vector

}

break1 <- numeric()

break2 <- numeric()

break3 <- numeric()

break4 <- numeric()

break5 <- numeric()

for (i in 1:length(vline.list)){
```

```
  year <- unique(NODF.analysis$Year)[i]

 indx1 <- vline.list[[i]][1]

 indx2 <- vline.list[[i]][2]

 indx3 <- vline.list[[i]][3]

 indx4 <- vline.list[[i]][4]

 indx5 <- vline.list[[i]][5]

 break1[i] <- as.numeric(as.character(NODF.analysis$TP[which(NODF.analysis$Year

== year)]))[indx1]

 break2[i] <- as.numeric(as.character(NODF.analysis$TP[which(NODF.analysis$Year

== year)]))[indx2]

 break3[i] <- as.numeric(as.character(NODF.analysis$TP[which(NODF.analysis$Year

== year)]))[indx3]

 break4[i] <- as.numeric(as.character(NODF.analysis$TP[which(NODF.analysis$Year

== year)]))[indx4]

 break5[i] <- as.numeric(as.character(NODF.analysis$TP[which(NODF.analysis$Year

== year)]))[indx5]

}

vlines <- data.frame(Year = unique(NODF.analysis$Year),

            break1 = break1, break2 = break2,

            break3 = break3, break4 = break4,

            break5 = break5)

vlines.2 <- vlines[,c(1,3:5)]

colnames(vlines.2) <- NULL
```

```
vlines.order <- data.frame()

for(i in 1:nrow(vlines.2)){

  vlines.order[i, c(1:4)] <- vlines.2[i, c(1, base::order(vlines.2[i,c(2:4)])+1)]

}

vlines.order[7,4] <- vlines.order[7,3]

vlines.order[7,3] <- NA

vlines.order[8,4] <- vlines.order[8,3]

vlines.order[8,3] <- NA

vlines.order[10,4] <- vlines.order[10,3]

vlines.order[10,3] <- NA

colnames(vlines.order) <- c("years", "first", "last")

#average change point by TP

mean.first <- mean(vlines.order$first, na.rm = TRUE)

mean.last <- mean(vlines.order$last, na.rm = TRUE)

#standard deviation of changepoints

sd.first <- sd(vlines.order$first, na.rm = TRUE)

sd.last <- sd(vlines.order$last, na.rm = TRUE)

mean.first

sd.first

mean.last

sd.last

#split data by year, into groups where TP < mean.first, mean.first < TP < mean.last, and

mean.last < TP
```

```
#Then anova by low, medium, high

dat.aov <- NODF.analysis[,c(2,3,4)]

colnames(dat.aov) <- c("NODF", "years", "mean.TPs")

low <- dat.aov[which(as.numeric(as.character(dat.aov$mean.TPs)) < mean.first), ]

low$mean.TPs <- rep("LOW", length(low$mean.TPs))

high <- dat.aov[which(as.numeric(as.character(dat.aov$mean.TPs)) > mean.last), ]

high$mean.TPs <- rep("HIGH", length(high$mean.TPs))

med <- dat.aov[which(as.numeric(as.character(dat.aov$mean.TPs)) >= mean.first &

            as.numeric(as.character(dat.aov$mean.TPs)) <= mean.last),]

med$mean.TPs <- rep("MED", length(med$mean.TPs))

dat.aov.twoway <- rbind(low, med, high)

#remove rows where alpha is NA

for (i in rev(1:nrow(dat.aov.twoway))){

 if (is.na(dat.aov.twoway[i,1]) == TRUE) {

   dat.aov.twoway <- dat.aov.twoway[-i, ]

 }

}

anova(lm(as.numeric(as.character(NODF)) ~ as.factor(years) * as.factor(mean.TPs),

dat.aov.twoway))

library(lsmeans)

model <- lm(as.numeric(as.character(NODF)) ~ as.factor(years) * as.factor(mean.TPs),

dat.aov.twoway)

lsmeans(model, pairwise ~ mean.TPs, adjust ="tukey")
```

```r
summary(anova(lm(as.numeric(as.character(NODF)) ~ as.factor(years) *

as.factor(mean.TPs), dat.aov.twoway)))

dat.aov$Years <- as.factor(dat.aov$years)

var.title <- "NODF compared to mean TP (ug/g)"

#Figure

ggplot(data = dat.aov, aes(x = as.numeric(as.character(mean.TPs)), y =

as.numeric(as.character(NODF)),

                color = Years)) + geom_line() +   labs(x = "Mean TP (ug/g)", y =

"NODF value") +

 theme(plot.title = element_text(hjust = 0.5)) + #ggtitle(var.title) +

 geom_vline(aes(xintercept = mean.first)) +

 geom_vline(aes(xintercept = mean.last)) +  theme_Publication()

setwd(dir = "~")

#End of NestednessDegDist.R
```

A.8 Ego Size Comparison

```r
#libraries

library(ggplot2)

library(igraph)

specieslist <- as.list(rep(NA, length(colnames(spp.data.raw))))

names(specieslist) <- colnames(spp.data.raw)

for (species in names(specieslist)) {

 output <- c()

 for (iter in 1:length(g.sim_storage)){
```

```
  if (class(g.sim_storage[[iter]]) == "igraph") {

   if (species %in% V(g.sim_storage[[iter]])$name == TRUE) {

     num <- which(V(g.sim_storage[[iter]])$name == species)

     output[iter] <- num

   }

   if (species %in% V(g.sim_storage[[iter]])$name == FALSE) {

     output[iter] <- 9999

   }

  } else {

   output[iter] <- 9999

  }

 }

 print(species)

 specieslist[[species]] <- output

}

#Species removal testing

#Store measures of unaltered networks

#For species with high degree (>50% of network order in all networks they occur in),

#  iteratively remove one species from each network and take ego sizes etc.

#storage of results

iterative.removal_storage <- list()

for (i in 1:length(names(specieslist))){

 species.node <- specieslist[[i]]
```

```r
  output <- list()

  print(names(specieslist)[i])

  for (j in 1:length(species.node)){

   network.node <- species.node[j]

   if (network.node != 9999) {

    g <- g.sim_storage[[j]]

    g.compare <- delete.vertices(g, V(g)[network.node])

    #Get code for network size, mean ego size here

    g.compare.order <- gorder(g.compare)

    g.compare.egos <- ego_size(g.compare)

    g.compare.mean.egos <- mean(g.compare.egos)

    output[[j]] <- cbind(g.compare.order, g.compare.mean.egos)

    #iterative.removal_storage[[i]][j] <-

   } else { output[[j]] <- cbind(NA, NA)}

  }

  iterative.removal_storage[[i]] <- output

  names(iterative.removal_storage[[i]]) <- names(specieslist[[i]])

}

#now have list of species with network size, mean ego size

dat.basic <- dat.complete[,c(1,4,5)]

species.ttest <- list()

species.summary <- list()

specis.anovas <- list()
```

```
dat.aov.storage <- list()

OldMeanEgo <- numeric()

species.plot <- list()

for (i in 1:length(g.sim_storage)) {

  OldMeanEgo[i] <- mean(ego_size(g.sim_storage[[i]]))

}

for (i in 1:length(iterative.removal_storage)) {

  species.dat <- data.frame(matrix(unlist(iterative.removal_storage[[i]]), byrow = TRUE,

ncol = 2))

  dat.aov1 <- cbind(species.dat, mean.TPs, years, OldMeanEgo)

  colnames(dat.aov1) <- c("NetOrder", "NewMeanEgo", "mean.TPs", "years",

"OldMeanEgo")

  differences <- dat.aov1$OldMeanEgo - dat.aov1$NewMeanEgo

  dat.aov <- cbind(dat.aov1, differences)

  colnames(dat.aov) <- c(colnames(dat.aov1), "Differences")

  low <- dat.aov[which(as.numeric(as.character(dat.aov$mean.TPs)) < mean.first), ]

  low$TPs <- rep("LOW", length(low$mean.TPs))

  high <- dat.aov[which(as.numeric(as.character(dat.aov$mean.TPs)) > mean.last), ]

  high$TPs <- rep("HIGH", length(high$mean.TPs))

  med <- dat.aov[which(as.numeric(as.character(dat.aov$mean.TPs)) >= mean.first &

                 as.numeric(as.character(dat.aov$mean.TPs)) <= mean.last),]

  med$TPs <- rep("MED", length(med$mean.TPs))

  dat.aov.twoway <- rbind(low, med, high)
```

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```
  for (j in rev(1:nrow(dat.aov.twoway))){

   if (is.na(dat.aov.twoway[j,1]) == TRUE) {

    dat.aov.twoway <- dat.aov.twoway[-j, ]

   }

  }

  dat.aov.twoway$Years <- as.factor(dat.aov.twoway$years)

  dat.aov.storage[[i]] <- dat.aov.twoway

if (length(unique(species.dat[,1])) > 10){

   species.ttest[[i]] <- t.test((dat.aov.twoway$NewMeanEgo+1),

dat.aov.twoway$OldMeanEgo)

   species.plot[[i]] <-  ggplot(data = dat.aov.twoway, aes(x =

as.numeric(as.character(mean.TPs)), y = Differences, color = Years)) + geom_point() +

labs(x = "Mean TP (ug/g)", y = "Change in Mean Ego Size without Species") +

theme(plot.title = element_text(hjust = 0.5)) +

    geom_vline(aes(xintercept = mean.first)) +

    geom_vline(aes(xintercept = mean.last)) + theme_Publication()

 } else {

   species.ttest[[i]] <- NA

   species.plot[[i]] <- NA

 }

 names(species.ttest)[i] <- names(specieslist)[i]

 names(species.plot)[i] <- names(specieslist)[i]

}
```

```r
count <- 0

indic.spp <- numeric()

indic.tvalue <- numeric()

indic.pvalue <- numeric()

for (i in 1:length(species.ttest)) {

 if (class(species.ttest[[i]]) != "logical"){

   pvalue <- species.ttest[[i]]$p.value

   if (pvalue < 0.05) {

    count <- count + 1

    indic.spp[count] <- i

    indic.tvalue[count] <- species.ttest[[i]]$statistic

    indic.pvalue[count] <- pvalue

   }

 }

}

names.spp <- character()

for (i in 1:length(indic.spp)){

 spp <- indic.spp[i]

 names.spp[i] <- names(specieslist)[spp]

 }

results <- as.data.frame(cbind(names.spp, indic.tvalue, indic.pvalue))

write.csv(results, file = "results.csv")

egoplot.y <- unlist(OldMeanEgo)
```

```
egoplot.x <- mean.TPs

ego.df <- as.data.frame(cbind(egoplot.x, egoplot.y))#, as.factor(as.character(Years)))

ego.df <- cbind(ego.df, as.factor(as.character(years)))

colnames(ego.df) <- c("x.ego", "y.ego", "Years")

ggplot(data = ego.df, aes(x = x.ego, y = y.ego,

                    color = Years)) + geom_point() + labs(x = "Mean TP (ug/g)", y =

"Mean Ego Size") +

  geom_vline(aes(xintercept = mean.first)) +

  geom_vline(aes(xintercept = mean.last)) + theme_Publication()

#End of iterativeremoval.R
```

A.9 Species Frequency

```
#Frequency of species

#Overarching variables

species.frequencies <- matrix(data = NA, nrow = length(spp.data_storage), ncol =

ncol(spp.data_storage[[1]]))

mean.TP <- numeric()

#Start with spp.data_storage

for (i in 1:length(spp.data_storage)){

  dat <- spp.data_storage[[i]]

  frequencies <- numeric()

    for (j in 1:ncol(dat)){
```

```r
    frequencies[j] <- length(unique(which(dat[,j] > 0)))

  }

 species.frequencies[i,] <- frequencies

}

colnames(species.frequencies) <- colnames(spp.data_storage[[1]])

#Now have matrix where each species' frequencies in network are represented by column

in matrix.

# Need to match with year and TP values.

for (i in 1:length(env.data_storage)){

 dat <- env.data_storage[[i]]

 mean.TP[i] <- mean(dat[,38], na.rm = TRUE)

 print(i)

}

Years <- unlist(year.analysis)

freq.dat <- as.data.frame(cbind(species.frequencies, mean.TP, Years))

freq.dat$Years <- as.factor(Years)

#split data by year, into groups where TP < mean.first, mean.first < TP < mean.last, and

mean.last < TP

#Then anova by low, medium, high

dat.aov <- freq.dat

low <- dat.aov[which(dat.aov$mean.TP < mean.first), ]

low$mean.TPs <- rep("LOW", length(low$mean.TP))

high <- dat.aov[which(dat.aov$mean.TP > mean.last), ]
```

```r
high$mean.TPs <- rep("HIGH", length(high$mean.TP))

med <- dat.aov[which(dat.aov$mean.TP >= mean.first & dat.aov$mean.TP <=

mean.last),]

med$mean.TPs <- rep("MED", length(med$mean.TP))

dat.aov.twoway <- rbind(low, med, high)

#remove rows where alpha is NA

for (i in rev(1:nrow(dat.aov.twoway))){

  if (is.na(dat.aov.twoway[i,1]) == TRUE) {

    dat.aov.twoway <- dat.aov.twoway[-i, ]

  }

}

summary.list <- list()

anova.list <- list()

lm.list <- list()

for (j in 1:length(indic.spp)){

i <- indic.spp[j]

species <- colnames(dat.aov.twoway)[i]

lm.list[[i]] <- lm(dat.aov.twoway[,i] ~ Years * as.factor(mean.TPs), dat.aov.twoway)

anova.list[[i]] <- anova(lm.list[[i]])

summary.list[[i]] <- summary(anova.list[[i]])

}

anova(lm(ACMINGRA ~ as.factor(Years) * as.factor(mean.TPs), dat.aov.twoway))
```

```
summary(anova(lm(ACMINGRA ~ as.factor(years) * as.factor(mean.TPs),

dat.aov.twoway)))

#Figure

var.title <- "Frequency of species in network compared to mean TP (ug/g)"

ggplot(data = freq.dat, aes(x = mean.TPs, y = ECSUBSUB, color = Years)) +

 geom_line() +   labs(x = "Mean TP (ug/g)", y = "Frequency of Species") +

 theme(plot.title = element_text(hjust = 0.5)) + #ggtitle(var.title) +

 geom_vline(aes(xintercept = mean.first)) +

 geom_vline(aes(xintercept = mean.last)) + #labs(fill = "Years") +

 ylim(0,10) + theme_Publication()
```

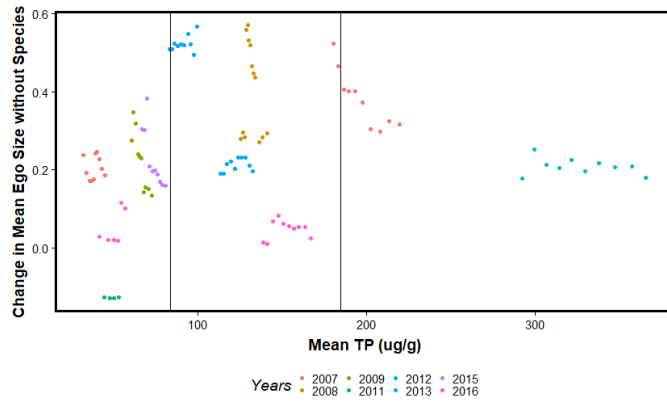Appendix B: Species Removal Effect on Mean Ego Size – Plots and ANOVA tables
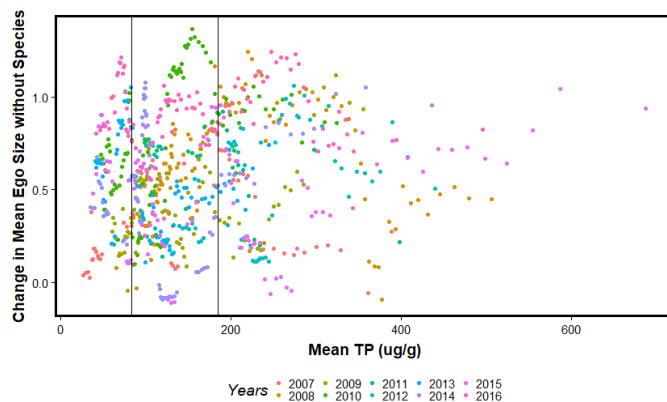
Figure B1. Plot of Effect of species removal on mean ego size in network along a mean Total Phosphorus gradient (µg/g) for taxon *Achnanthidium minutissimum* var. *gracillima*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: Differences

|	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	22.675	2.51946	37.2529	< 2.2e-16 ***
TP	2	2.283	1.14154	16.8789	6.751e-08 ***
Year:TP	16	5.577	0.34855	5.1536	2.557e-10 ***
Residuals	752	50.859	0.06763		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
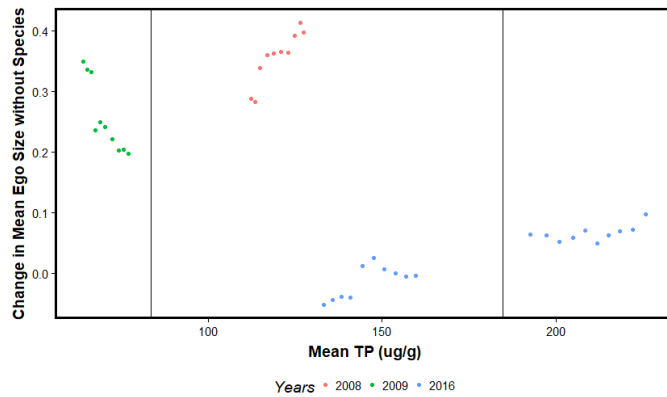
Figure B2. Plot of Effect of species removal on mean ego size in network along a mean Total Phosphorus gradient (µg/g) for taxon *Amphora ovalis*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: Differences

|	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	2	0.83874	0.41937	265.857	< 2.2e-16 ***
TP	1	0.03191	0.03191	20.232	6.888e-05 ***
Residuals	36	0.05679	0.00158		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
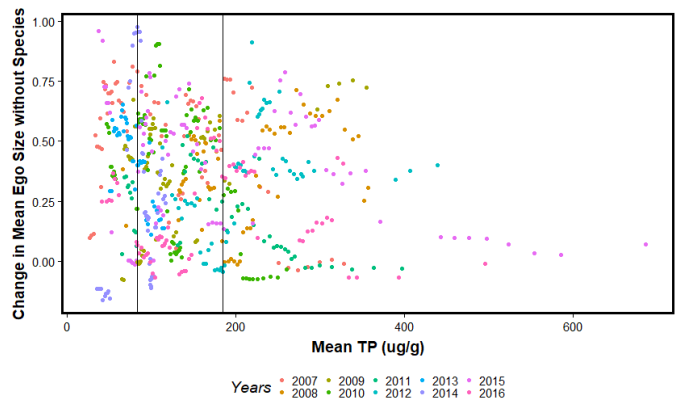
Figure B3. Plot of Effect of species removal on mean ego size in network along a mean Total Phosphorus gradient (µg/g) for taxon *Amphora sulcata*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: Differences

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	5.1711	0.57457	11.0970	4.559e-16 ***
TP	2	0.5053	0.25265	4.8795	0.007913 **
Year:TP	15	5.0936	0.33958	6.5584	4.335e-13 ***
Residuals	585	30.2897	0.05178		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

70
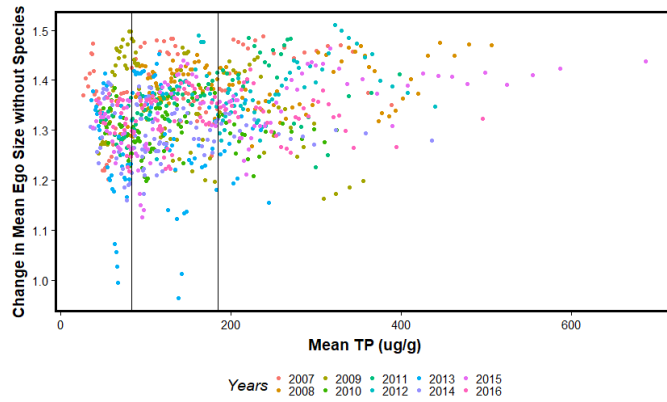
Figure B4. Plot of Effect of species removal on mean ego size in network along a mean Total Phosphorus gradient (µg/g) for taxon *Aulacoseira* cf. *distans*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: Differences

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	7	1.86844	0.266921	30.6340	< 2.2e-16 ***
TP	2	0.06473	0.032363	3.7142	0.0281447 *
Year:TP	2	0.14369	0.071845	8.2456	0.0005116 ***
Residuals	91	0.79290	0.008713		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
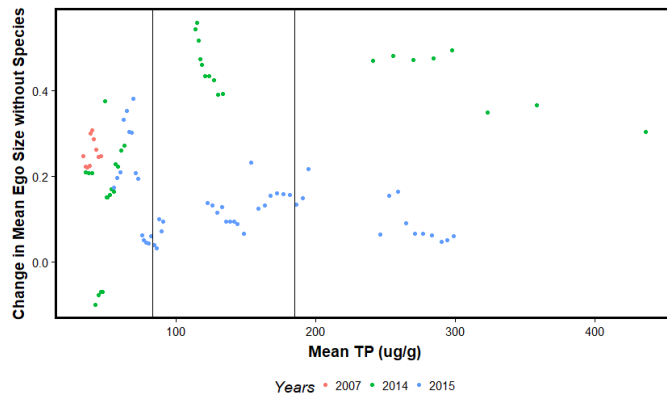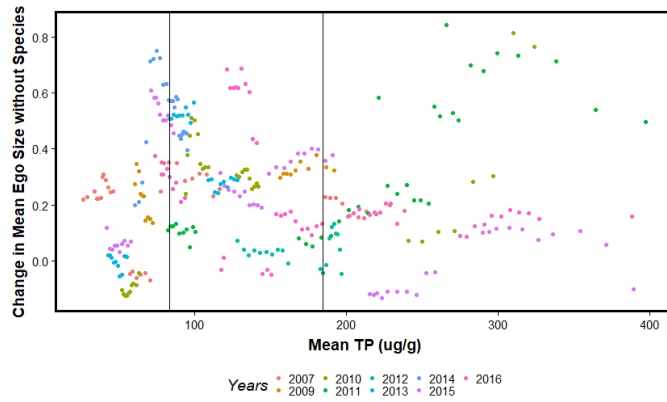
Figure B5. Plot of Effect of species removal on mean ego size in network along a mean Total Phosphorus gradient (µg/g) for taxon *Aulacoseira* cf. *granulata*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: Differences

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	2	0.57288	0.28644	37.190	2.127e-12 ***
TP	2	0.16257	0.08129	10.554	7.878e-05 ***
Year:TP	2	0.71382	0.35691	46.339	1.989e-14 ***
Residuals	87	0.67009	0.00770		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
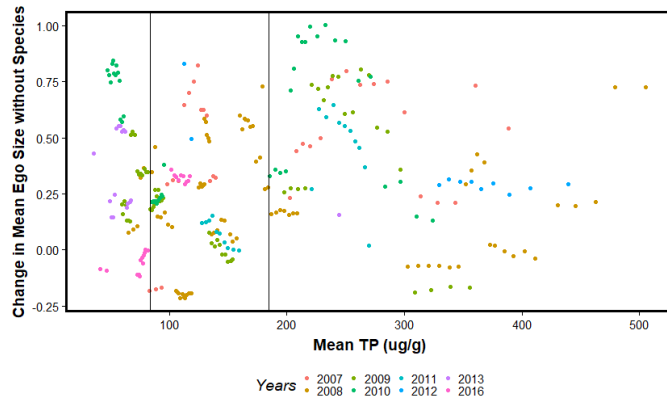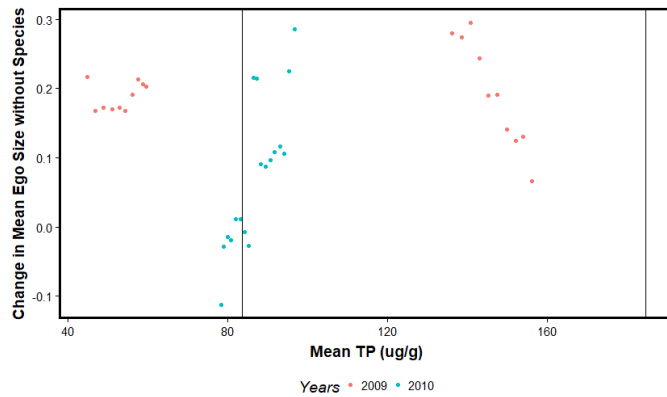
Figure B6. Plot of Effect of species removal on mean ego size in network along a mean Total Phosphorus gradient (µg/g) for taxon *Brachysira brebissonii*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: Differences

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	7	5.8333	0.83332	16.7018	< 2.2e-16 ***
TP	2	0.6152	0.30759	6.1649	0.002417 **
Year:TP	10	4.5138	0.45138	9.0468	8.106e-13 ***
Residuals	264	13.1721	0.04989		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
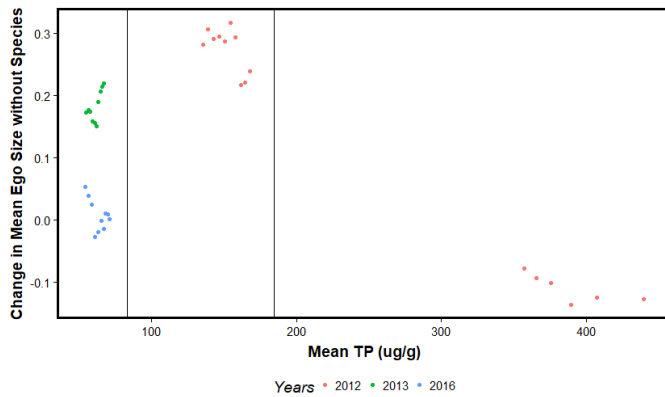
Figure B7. Plot of Effect of species removal on mean ego size in network along a mean Total Phosphorus gradient (µg/g) for taxon *Brachysira microcephala*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: Differences

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	1.2714	0.141263	34.0459	< 2.2e-16 ***
TP	2	0.1001	0.050060	12.0650	6.763e-06 ***
Year:TP	17	0.3334	0.019611	4.7265	1.035e-09 ***
Residuals	888	3.6845	0.004149		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
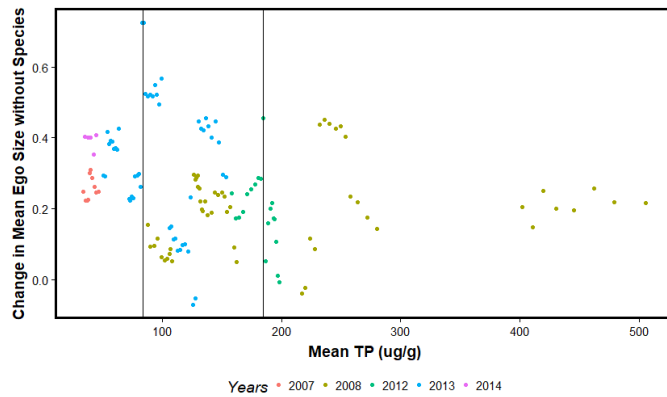
Figure B8. Plot of Effect of species removal on mean ego size in network along a mean Total Phosphorus gradient (µg/g) for taxon *Caponea caribbea*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: Differences

|	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	8	3.0010	0.37513	15.572	< 2.2e-16 ***
TP	2	0.6295	0.31473	13.065	3.627e-06 ***
Year:TP	13	3.9996	0.30766	12.771	< 2.2e-16 ***
Residuals	300	7.2269	0.02409		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Figure B9. Plot of Effect of species removal on mean ego size in network along a mean Total Phosphorus gradient (µg/g) for taxon *Craticula accomodiformis*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: Differences

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	1	0.125967	0.125967	25.8751	1.326e-05 ***
TP	1	0.044461	0.044461	9.1327	0.004746 **
Year:TP	1	0.046997	0.046997	9.6537	0.003802 **
Residuals	34	0.165521	0.004868		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
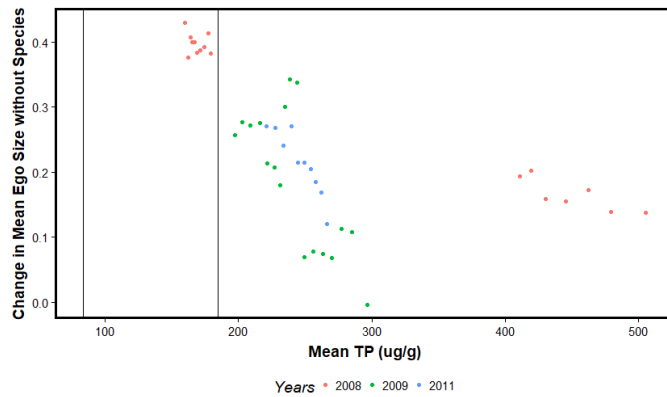
Figure B10. Plot of Effect of species removal on mean ego size in network along a mean Total Phosphorus gradient (μg/g) for taxon *Cyclotella iris*. Vertical lines indicate thresholds at 83 and 195 μg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: Differences

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	2	0.16291	0.08145	102.24	1.265e-14 ***
TP	1	0.55396	0.55396	695.30	< 2.2e-16 ***
Residuals	32	0.02550	0.00080		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
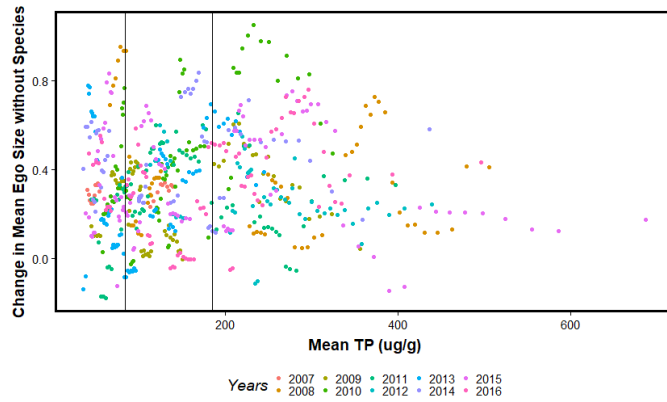
Figure B11. Plot of Effect of species removal on mean ego size in network along a mean Total Phosphorus gradient (µg/g) for taxon *Diploneis puella*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: Differences

|	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	4	0.60641	0.151603	8.3398	5.199e-06 ***
TP	2	0.00501	0.002503	0.1377	0.871499
Year:TP	1	0.13588	0.135875	7.4746	0.007144 **
Residuals	128	2.32682	0.018178		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
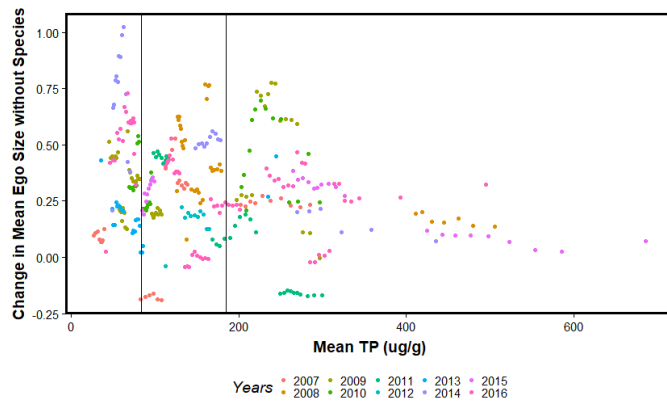
Figure B12. Plot of Effect of species removal on mean ego size in network along a mean Total Phosphorus gradient (µg/g) for taxon *Ensyonopsis* egsp01. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: Differences

|	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	4.2143	0.46825	18.837	< 2.2e-16 ***
TP	2	0.5015	0.25075	10.087	5.626e-05 ***
Year:TP	13	4.5968	0.35360	14.225	< 2.2e-16 ***
Residuals	324	8.0541	0.02486		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Figure B13. Plot of Effect of species removal on mean ego size in network along a mean Total Phosphorus gradient (µg/g) for taxon *Encyonopsis floridiana*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: Differences

|	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	2	0.11929	0.059647	10.967	0.0001595 ***
TP	1	0.22198	0.221975	40.812	1.345e-07 ***
Residuals	40	0.21756	0.005439		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Figure B14. Plot of Effect of species removal on mean ego size in network along a mean Total Phosphorus gradient (µg/g) for taxon *Encyonopsis subminuta*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: Differences

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	2.5701	0.28557	7.2352	4.972e-10 ***
TP	2	0.8683	0.43413	10.9991	2.028e-05 ***
Year:TP	16	6.2330	0.38956	9.8700	< 2.2e-16 ***
Residuals	612	24.1554	0.03947		

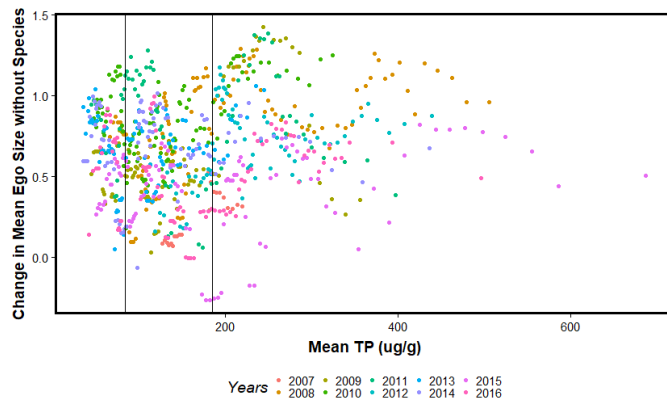
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

81
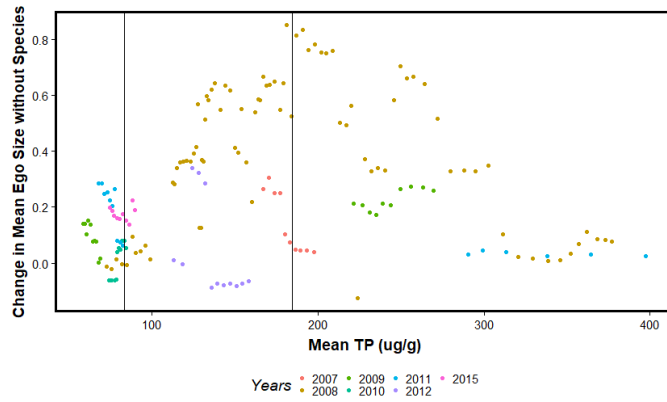
Figure B15. Plot of Effect of species removal on mean ego size in network along a mean Total Phosphorus gradient (µg/g) for taxon *Encyonema* cf. *evergladianum*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: Differences

|	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	6	3.2245	0.53742	14.4280	6.431e-13 ***
TP	2	0.2506	0.12528	3.3634	0.037285 *
Year:TP	5	0.6739	0.13479	3.6186	0.004066 **
Residuals	148	5.5128	0.03725		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
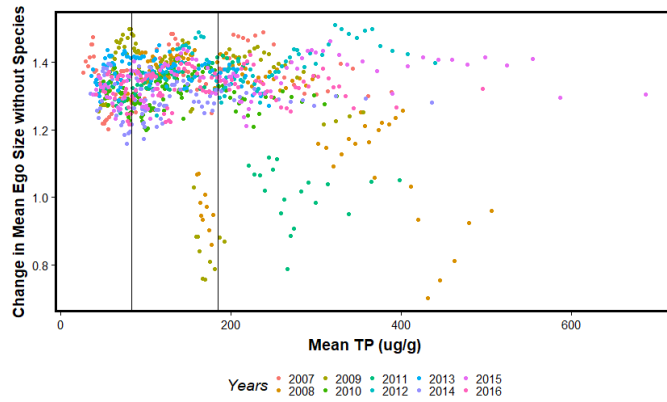
Figure B16. Plot of Effect of species removal on mean ego size in network along a mean Total Phosphorus gradient (µg/g) for taxon *Encyonema evergladianum*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: Differences

|	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	1.3251	0.147232	15.2633	< 2.2e-16 ***
TP	2	0.1781	0.089064	9.2332	0.0001075 ***
Year:TP	17	1.2524	0.073668	7.6371	< 2.2e-16 ***
Residuals	888	8.5658	0.009646		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
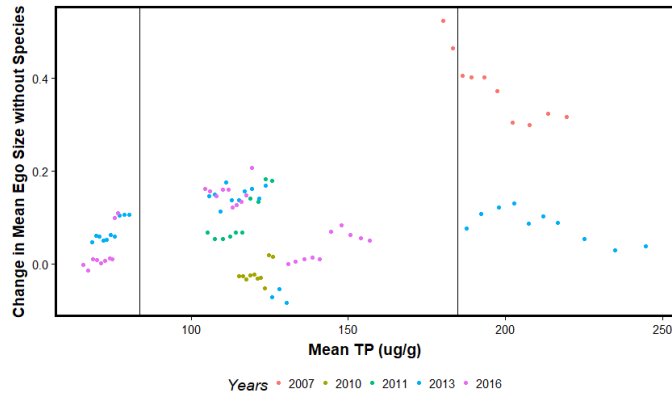
Figure B17. Plot of Effect of species removal on mean ego size in network along a mean Total Phosphorus gradient (µg/g) for taxon *Encyonema* ftsp04. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: Differences

|	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	4	0.97145	0.242862	74.8108	< 2.2e-16 ***
TP	2	0.04588	0.022941	7.0668	0.001457 **
Year:TP	2	0.02274	0.011369	3.5022	0.034602 *
Residuals	84	0.27269	0.003246		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Figure B18. Plot of Effect of species removal on mean ego size in network along a mean Total Phosphorus gradient (µg/g) for taxon *Encyonema silesiacum* var. *elegans*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: Differences

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	20.301	2.2556	40.674	< 2.2e-16 ***
TP	2	8.754	4.3771	78.928	< 2.2e-16 ***
Year:TP	16	5.487	0.3429	6.184	4.643e-13 ***
Residuals	776	43.034	0.0555		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
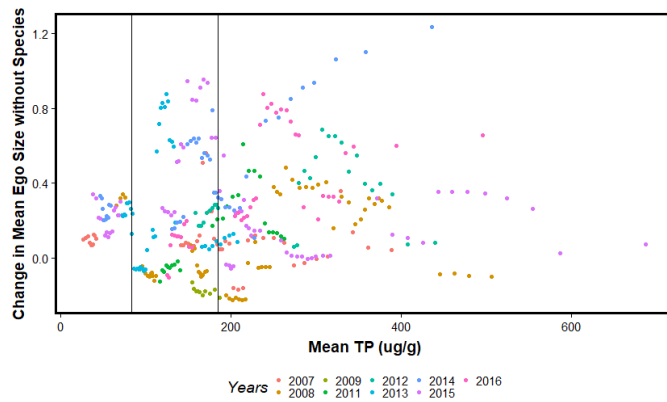
Figure B19. Plot of Effect of species removal on mean ego size in network along a mean Total Phosphorus gradient (µg/g) for taxon *Encyonema silesiacum*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: Differences

|	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	8	7.7772	0.97216	23.925	< 2e-16 ***
TP	2	0.2588	0.12938	3.184	0.04265 *
Year:TP	12	6.2001	0.51668	12.716	< 2e-16 ***
Residuals	343	13.9371	0.04063		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
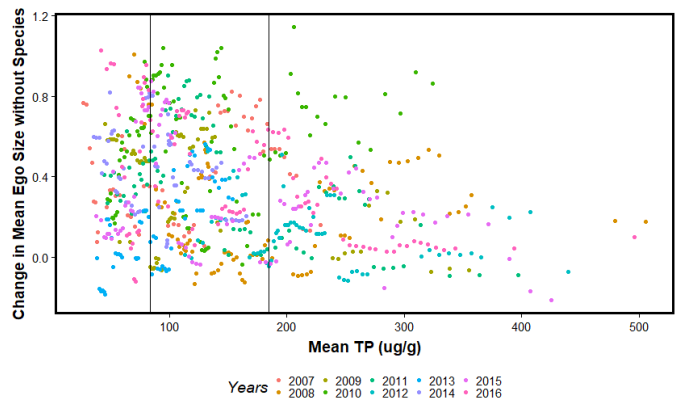
Figure B20. Plot of Effect of species removal on mean ego size in network along a mean Total Phosphorus gradient (µg/g) for taxon *Encyonema* sjsp03. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: Differences

|	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	13.607	1.51188	27.9534	< 2.2e-16 ***
TP	2	2.038	1.01890	18.8387	1.057e-08 ***
Year:TP	15	7.487	0.49914	9.2287	< 2.2e-16 ***
Residuals	724	39.158	0.05409		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
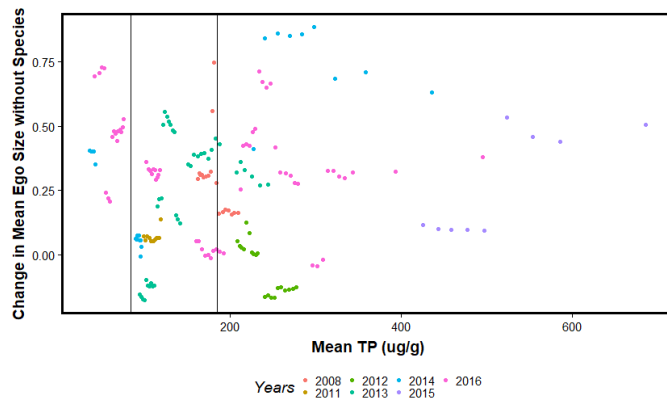
Figure B21. Plot of Effect of species removal on mean ego size in network along a mean Total Phosphorus gradient (µg/g) for taxon *Eunotia* egsp01. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: Differences

|	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	6	3.0497	0.50828	16.833	3.448e-15 ***
TP	2	1.2706	0.63529	21.039	6.935e-09 ***
Year:TP	4	2.0421	0.51053	16.907	1.117e-11 ***
Residuals	169	5.1031	0.03020		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
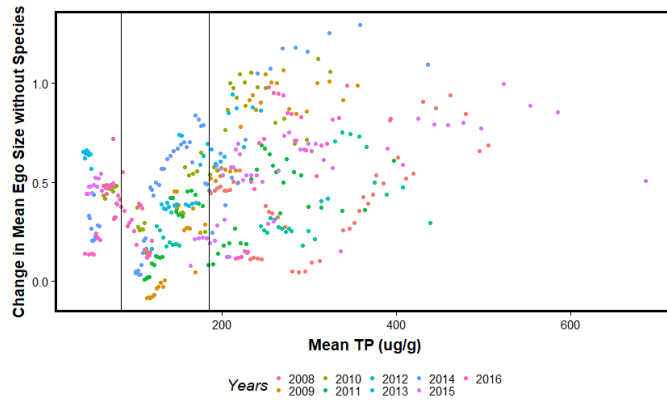
Figure B22. Plot of Effect of species removal on mean ego size in network along a mean Total Phosphorus gradient (µg/g) for taxon *Eunotia flexuosa*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: Differences

|	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	8	2.9264	0.3658	8.9829	2.142e-11 ***
TP	2	11.3457	5.6728	139.3059	< 2.2e-16 ***
Year:TP	11	3.0266	0.2751	6.7567	1.786e-10 ***
Residuals	418	17.0219	0.0407		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
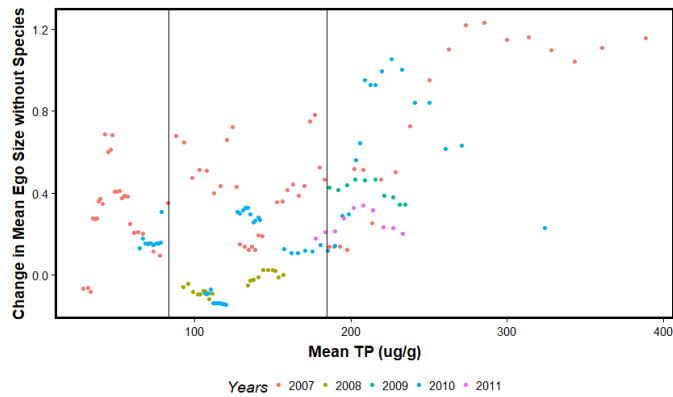
Figure B23. Plot of Effect of species removal on mean ego size in network along a mean Total Phosphorus gradient (µg/g) for taxon *Eunotia formica*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: Differences

|	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	4	4.3823	1.09556	20.5235	1.279e-13 ***
TP	2	5.0625	2.53127	47.4190	< 2.2e-16 ***
Year:TP	3	0.5657	0.18858	3.5327	0.01627 *
Residuals	157	8.3808	0.05338		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
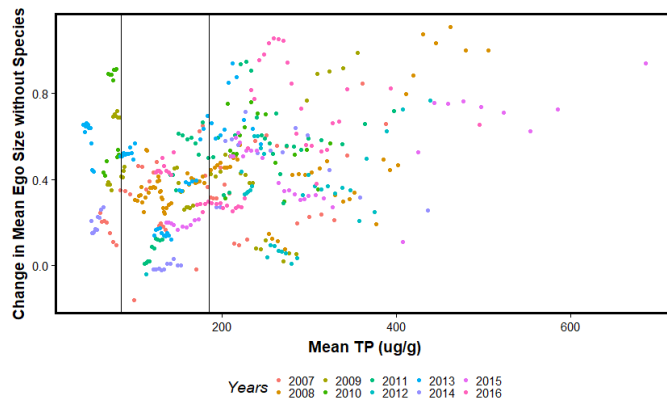
Figure B24. Plot of Effect of species removal on mean ego size in network along a mean Total Phosphorus gradient (µg/g) for taxon *Eunotia naegelii*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: Differences

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	3.7604	0.41783	11.0022	1.962e-15 ***
TP	2	3.7695	1.88477	49.6300	< 2.2e-16 ***
Year:TP	12	1.9885	0.16571	4.3634	1.446e-06 ***
Residuals	418	15.8742	0.03798		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
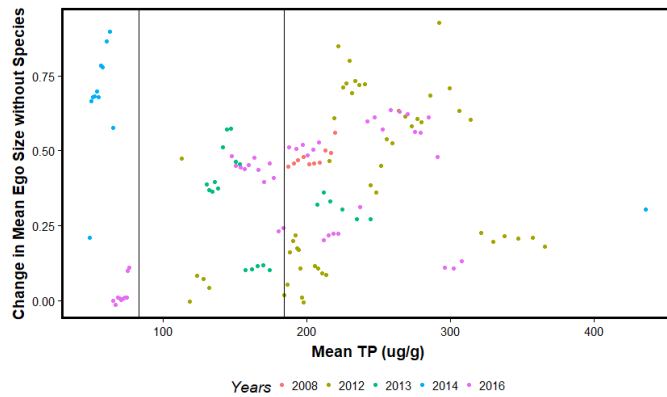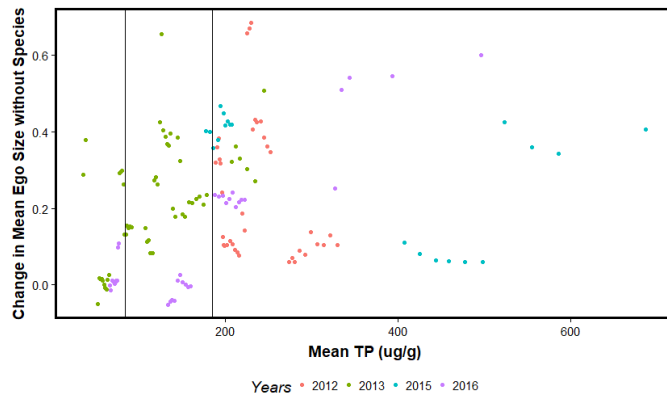
Figure B25. Plot of Effect of species removal on mean ego size in network along a mean Total Phosphorus gradient (μg/g) for taxon *Fragilaria* cf. *tenera*. Vertical lines indicate thresholds at 83 and 195 μg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: Differences

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	4	1.0995	0.27487	7.0930	3.409e-05 ***
TP	2	1.0335	0.51675	13.3347	5.444e-06 ***
Year:TP	3	0.8834	0.29448	7.5989	0.0001017 ***
Residuals	129	4.9991	0.03875		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
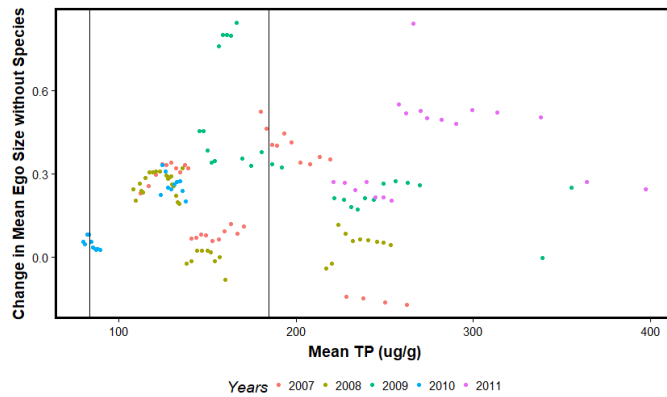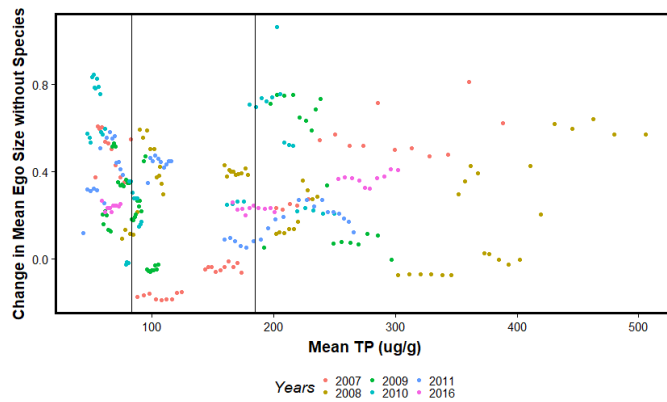
Figure B26. Plot of Effect of species removal on mean ego size in network along a mean Total Phosphorus gradient (µg/g) for taxon *Fragilaria* ftsp16. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: Differences

|	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	4	1.53321	0.38330	15.921	1.245e-10 ***
TP	2	0.52855	0.26427	10.977	3.937e-05 ***
Year:TP	2	0.32112	0.16056	6.669	0.001749 **
Residuals	130	3.12986	0.02408		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Figure B27. Plot of Effect of species removal on mean ego size in network along a mean Total Phosphorus gradient (µg/g) for taxon *Fragilariforma virescens* var. *capitata*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: Differences

|	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	3	0.40196	0.13399	6.4435	0.0004085 ***
TP	2	0.82015	0.41008	19.7205	2.93e-08 ***
Year:TP	3	0.32595	0.10865	5.2250	0.0019040 **
Residuals	138	2.86963	0.02079		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Figure B28. Plot of Effect of species removal on mean ego size in network along a mean Total Phosphorus gradient (µg/g) for taxon *Frustulia crassinervia*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: Differences

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	5	1.3827	0.27654	8.2012	3.324e-07 ***
TP	2	1.4322	0.71610	21.2374	2.895e-09 ***
Year:TP	10	4.5830	0.45830	13.5917	< 2.2e-16 ***
Residuals	258	8.6995	0.03372		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
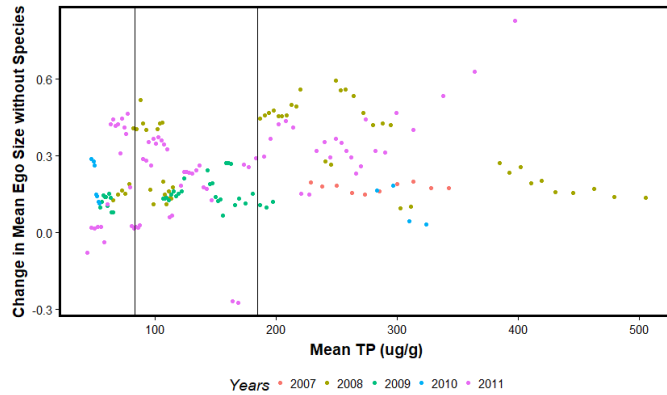
Figure B29. Plot of Effect of species removal on mean ego size in network along a mean Total Phosphorus gradient (µg/g) for taxon *Gomphonema affine*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: Differences

|	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	4	0.7504	0.187604	9.6942	4.254e-07 ***
TP	2	0.4182	0.209096	10.8047	3.787e-05 ***
Year:TP	5	0.2621	0.052417	2.7086	0.02201 *
Residuals	173	3.3479	0.019352		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
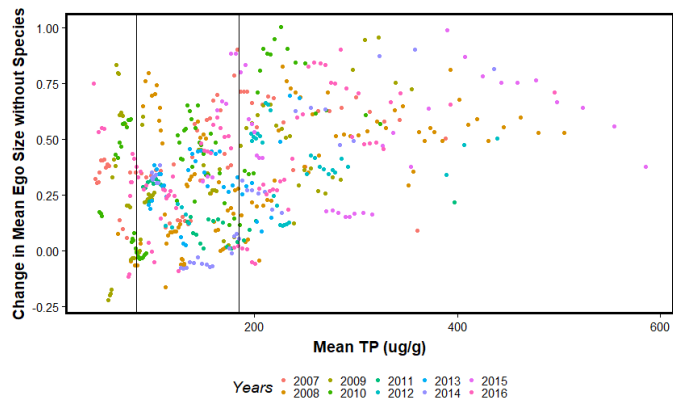
Figure B30. Plot of Effect of species removal on mean ego size in network along a mean Total Phosphorus gradient (µg/g) for taxon *Gomphonema* cf. *vibrioides*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: Differences

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	3.1186	0.34651	7.0936	9.842e-10 ***
TP	2	5.5397	2.76985	56.7032	< 2.2e-16 ***
Year:TP	13	1.9638	0.15107	3.0925	0.0001887 ***
Residuals	534	26.0849	0.04885		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
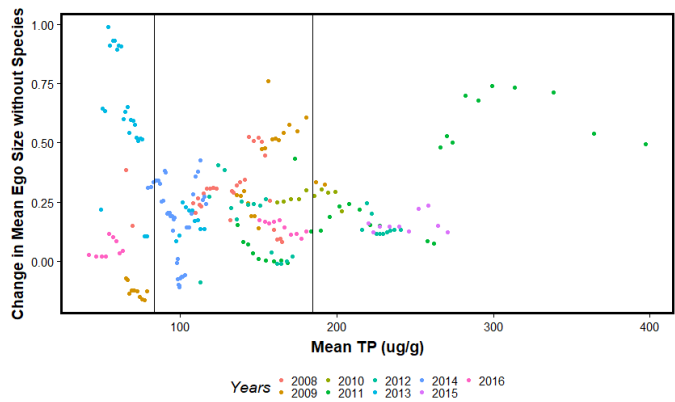
Figure B31. Plot of Effect of species removal on mean ego size in network along a mean Total Phosphorus gradient (µg/g) for taxon *Gomphonema gracile*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: Differences

|	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	5	2.1211	0.42422	19.595	3.526e-16 ***
TP	2	1.4234	0.71172	32.874	3.030e-13 ***
Year:TP	8	4.9191	0.61489	28.402	< 2.2e-16 ***
Residuals	224	4.8495	0.02165		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
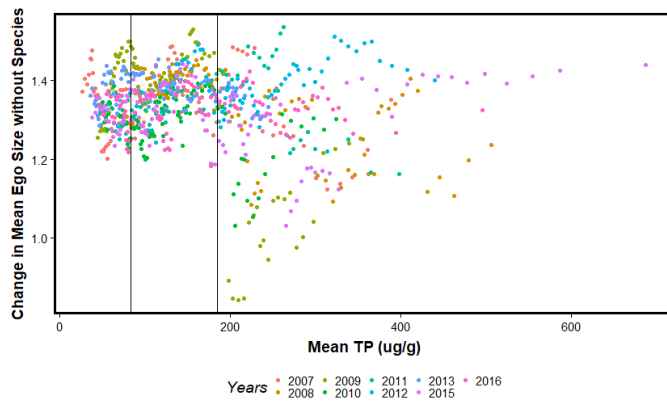
Figure B32. Plot of Effect of species removal on mean ego size in network along a mean Total Phosphorus gradient (µg/g) for taxon *Mastogloia calcarea*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: Differences

|	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	8	0.5996	0.07495	14.187	< 2.2e-16 ***
TP	2	0.8935	0.44676	84.566	< 2.2e-16 ***
Year:TP	15	1.6987	0.11325	21.437	< 2.2e-16 ***
Residuals	791	4.1788	0.00528		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Figure B33. Plot of Effect of species removal on mean ego size in network along a mean Total Phosphorus gradient (µg/g) for taxon *Mastogloia lanceolata*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: Differences

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	8	2.9231	0.36539	15.0910	< 2.2e-16 ***
TP	2	0.3341	0.16705	6.8994	0.001258 **
Year:TP	7	3.8663	0.55234	22.8120	< 2.2e-16 ***
Residuals	206	4.9878	0.02421		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
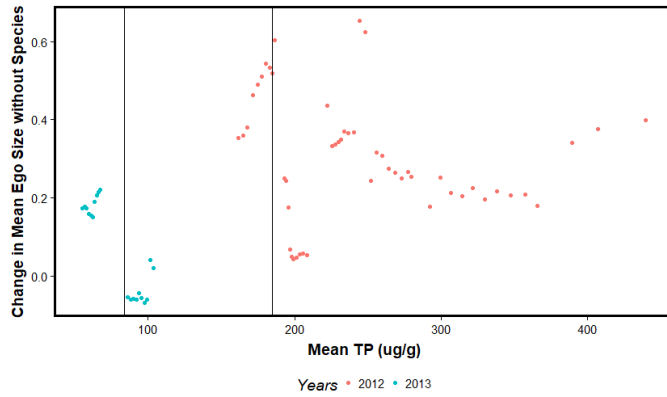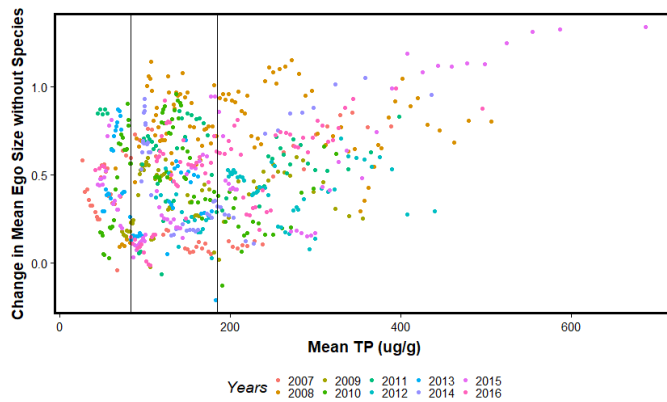
Figure B34. Plot of Effect of species removal on mean ego size in network along a mean Total Phosphorus gradient (µg/g) for taxon *Navicula densilineolata*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: Differences

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	1	0.76378	0.76378	53.866	3.728e-10 ***
TP	2	0.53074	0.26537	18.716	3.489e-07 ***
Residuals	67	0.95000	0.01418		

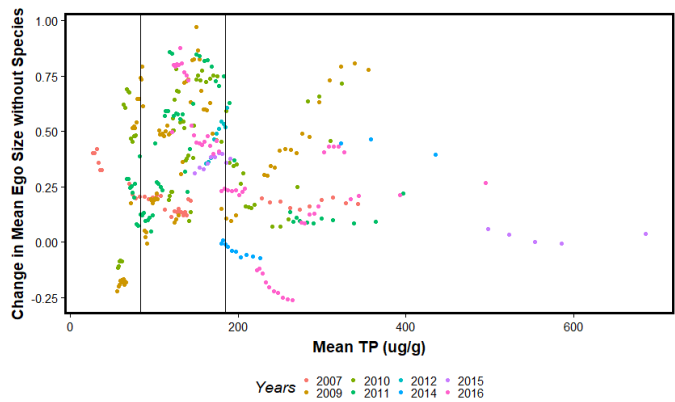---

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

101
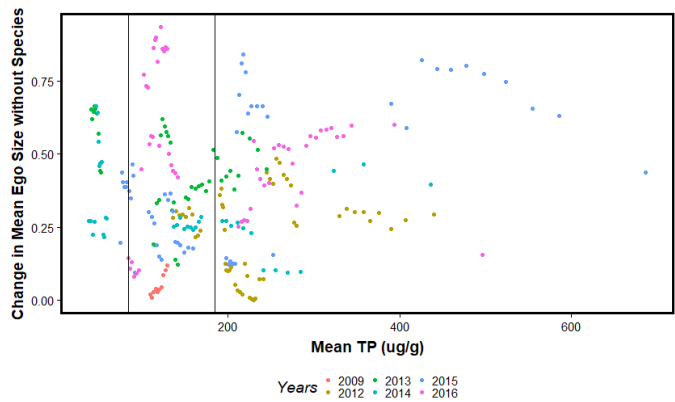
Figure B35. Plot of Effect of species removal on mean ego size in network along a mean Total Phosphorus gradient (µg/g) for taxon *Navicula radiosafallax*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: Differences

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	5	3.6491	0.72981	25.7406	< 2.2e-16 ***
TP	2	0.3405	0.17025	6.0047	0.002858 **
Year:TP	6	1.4255	0.23758	8.3794	2.998e-08 ***
Residuals	237	6.7196	0.02835		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Figure B36. Plot of Effect of species removal on mean ego size in network along a mean Total Phosphorus gradient (µg/g) for taxon *Nitzschia amphibia*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: Differences

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	10.972	1.21914	23.2188	< 2.2e-16 ***
TP	2	1.218	0.60905	11.5994	1.121e-05 ***
Year:TP	15	7.488	0.49919	9.5072	< 2.2e-16 ***
Residuals	654	34.339	0.05251		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
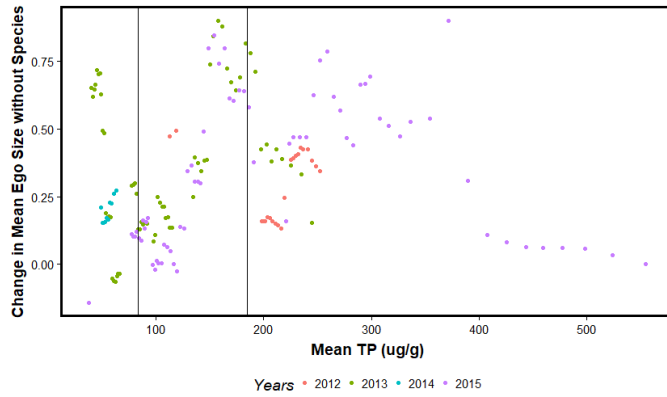
Figure B37. Plot of Effect of species removal on mean ego size in network along a mean Total Phosphorus gradient (µg/g) for taxon *Nitzschia* cf. *semirobusta*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: Differences

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	7	2.5101	0.35858	6.9192	1.159e-07 ***
TP	2	3.3827	1.69137	32.6364	1.360e-13 ***
Year:TP	10	3.2240	0.32240	6.2210	1.109e-08 ***
Residuals	311	16.1175	0.05182		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
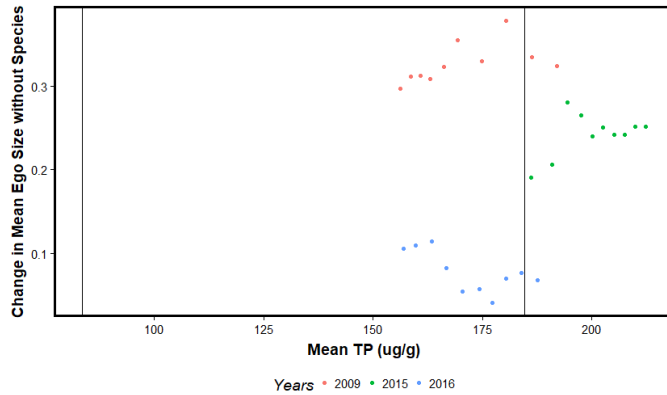
Figure B38. Plot of Effect of species removal on mean ego size in network along a mean Total Phosphorus gradient (µg/g) for taxon *Nitzschia* ftsp16. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: Differences

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	5	0.2135	0.04270	0.7439	0.5917673
TP	2	0.3471	0.17355	3.0235	0.0515839 .
Year:TP	2	0.9788	0.48940	8.5263	0.0003102 ***
Residuals	151	8.6672	0.05740		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Figure B39. Plot of Effect of species removal on mean ego size in network along a mean Total Phosphorus gradient (µg/g) for taxon *Nitzscia intermedia*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: Differences

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	3	0.2846	0.094859	1.6015	0.191241
TP	2	0.5704	0.285196	4.8151	0.009342 **
Year:TP	3	0.3380	0.112655	1.9020	0.131491
Residuals	157	9.2990	0.059229		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
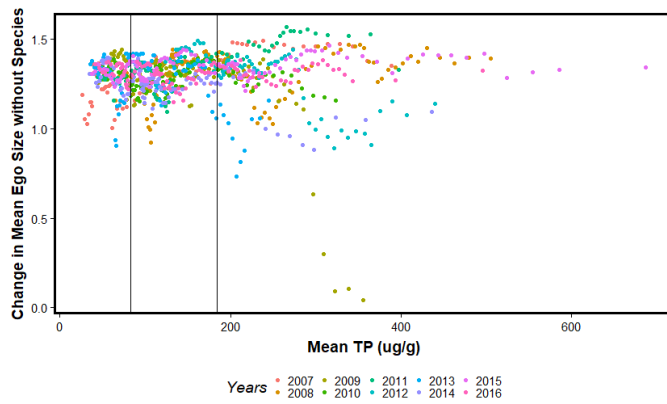
Figure B40. Plot of Effect of species removal on mean ego size in network along a mean Total Phosphorus gradient (µg/g) for taxon *Nitzschia microcephala*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: Differences

|	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	2	0.32337	0.161687	238.3503	<2e-16 ***
TP	1	0.00002	0.000015	0.0227	0.8816
Year:TP	1	0.00011	0.000107	0.1578	0.6946
Residuals	25	0.01696	0.000678		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Figure B41. Plot of Effect of species removal on mean ego size in network along a mean Total Phosphorus gradient (µg/g) for taxon *Nitzschia nana*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: Differences

|	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	13.5546	1.50607	25.0108	< 2.2e-16 ***
TP	2	4.0590	2.02952	33.7035	7.047e-14 ***
Year:TP	11	2.7981	0.25437	4.2243	8.068e-06 ***
Residuals	288	17.3425	0.06022		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Figure B42. Plot of Effect of species removal on mean ego size in network along a mean Total Phosphorus gradient (µg/g) for taxon *Nitzschia palea* var. *debilis*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: Differences

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	1.1245	0.124940	9.4512	7.96e-14 ***
TP	2	0.1297	0.064859	4.9063	0.007602 **
Year:TP	17	3.7626	0.221330	16.7428	< 2.2e-16 ***
Residuals	888	11.7389	0.013219		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
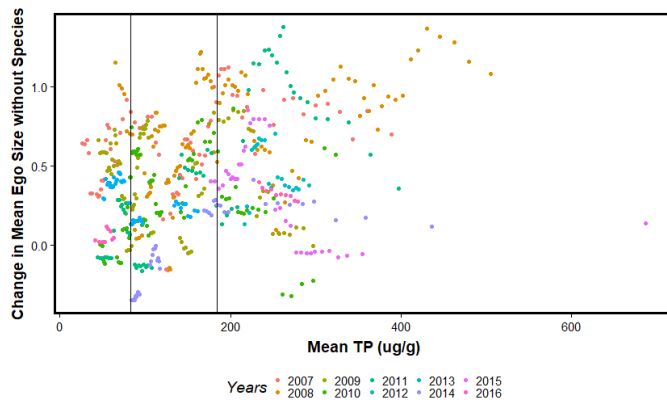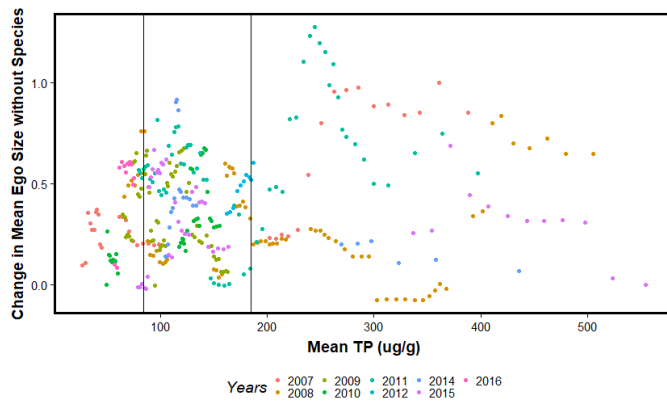
Figure B43. Plot of Effect of species removal on mean ego size in network along a mean Total Phosphorus gradient (µg/g) for taxon *Nitzschia palea* var. *tenuirostris*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: Differences

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	23.180	2.57558	38.612	< 2.2e-16 ***
TP	2	6.069	3.03442	45.491	< 2.2e-16 ***
Year:TP	12	8.772	0.73103	10.959	< 2.2e-16 ***
Residuals	527	35.153	0.06670		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
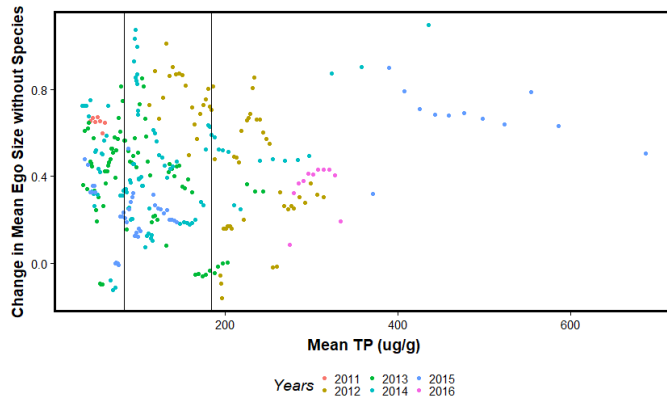
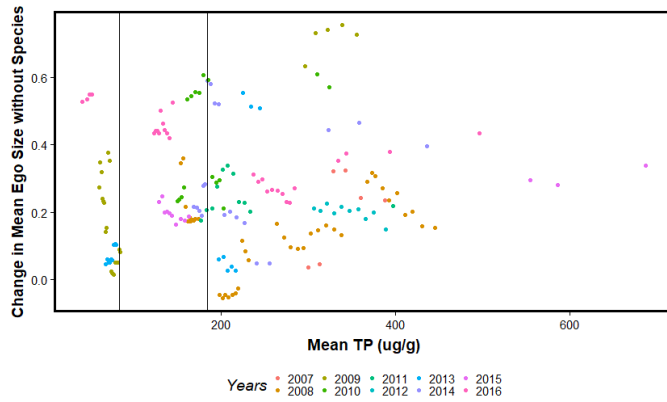Figure B44. Plot of Effect of species removal on mean ego size in network along a mean Total Phosphorus gradient (µg/g) for taxon *Pseudostaurosira brevistriata*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: Differences

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	5	1.3687	0.27375	5.7704	4.306e-05 ***
TP	2	0.0710	0.03550	0.7483	0.4741
Year:TP	5	4.5047	0.90095	18.9913	2.669e-16 ***
Residuals	285	13.5204	0.04744		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
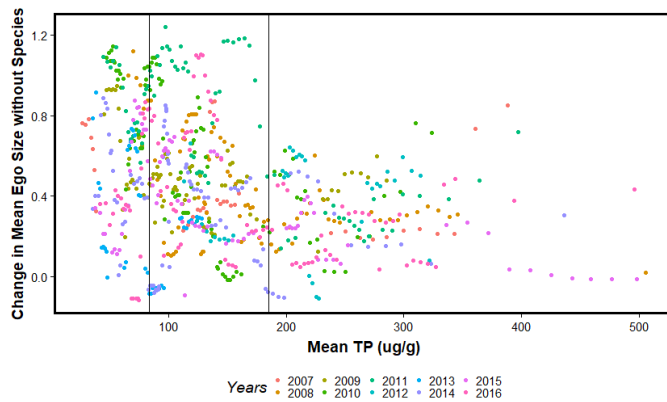
Figure B45. Plot of Effect of species removal on mean ego size in network along a mean Total Phosphorus gradient (µg/g) for taxon *Pinnularia microstauron*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: Differences

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	8	4.0663	0.50829	10.8899	1.248e-13 ***
TP	2	0.6473	0.32365	6.9341	0.001123 **
Year:TP	10	3.8440	0.38440	8.2357	6.298e-12 ***
Residuals	329	15.3561	0.04667		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
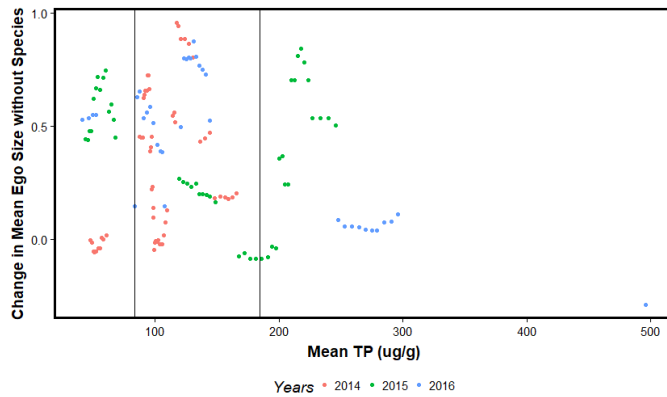
Figure B46. Plot of Effect of species removal on mean ego size in network along a mean Total Phosphorus gradient (µg/g) for taxon *Rhopalodia gibba*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: Differences

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	1.77746	0.197496	14.5073	< 2.2e-16 ***
TP	2	0.25912	0.129559	9.5169	0.0001229 ***
Year:TP	8	1.37732	0.172164	12.6466	4.495e-14 ***
Residuals	164	2.23262	0.013614		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

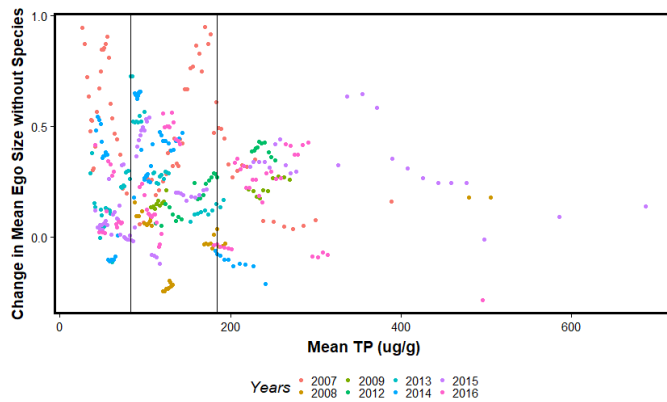Figure B47. Plot of Effect of species removal on mean ego size in network along a mean Total Phosphorus gradient (µg/g) for taxon *Sellaphora laevissima*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: Differences

|	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	12.048	1.3386	23.1457	< 2.2e-16 ***
TP	2	9.873	4.9366	85.3557	< 2.2e-16 ***
Year:TP	16	7.360	0.4600	7.9536	< 2.2e-16 ***
Residuals	679	39.270	0.0578		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

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Figure B48. Plot of Effect of species removal on mean ego size in network along a mean Total Phosphorus gradient (µg/g) for taxon *Sellaphora seminulum*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: Differences

|	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	2	0.2326	0.11631	2.0495	0.132911
TP	2	0.7989	0.39946	7.0385	0.001249 **
Year:TP	3	4.4692	1.48973	26.2493	2.321e-13 ***
Residuals	131	7.4347	0.05675		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Figure B49. Plot of Effect of species removal on mean ego size in network along a mean Total Phosphorus gradient (µg/g) for taxon *Sellaphora stroemii*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: Differences

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	7	6.7920	0.97029	29.2879	< 2.2e-16 ***
TP	2	0.3494	0.17469	5.2729	0.005508 **
Year:TP	11	4.3250	0.39318	11.8681	< 2.2e-16 ***
Residuals	383	12.6885	0.03313		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
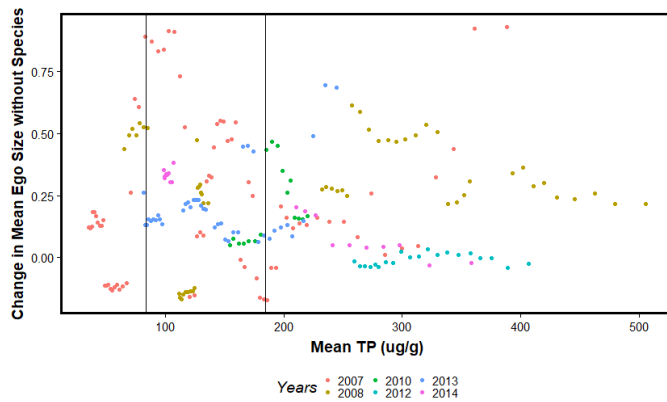
Figure B50. Plot of Effect of species removal on mean ego size in network along a mean Total Phosphorus gradient (µg/g) for taxon *Synedra filiformis* var. *exilis*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: Differences

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	5	1.2251	0.24503	5.6116	7.027e-05 ***
TP	2	0.1406	0.07030	1.6101	0.2023
Year:TP	6	2.3823	0.39705	9.0932	7.464e-09 ***
Residuals	212	9.2569	0.04366		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
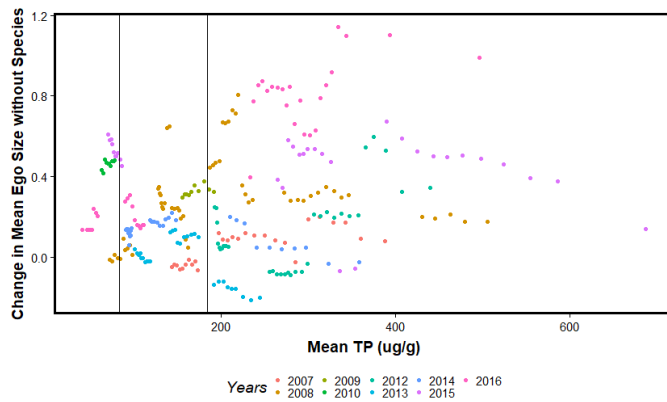
Figure B51. Plot of Effect of species removal on mean ego size in network along a mean Total Phosphorus gradient (µg/g) for taxon *Ulnaria delicatissima*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: Differences

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	8	9.4955	1.18693	65.548	< 2.2e-16 ***
TP	2	1.1984	0.59918	33.090	1.828e-13 ***
Year:TP	8	3.8509	0.48137	26.583	< 2.2e-16 ***
Residuals	248	4.4907	0.01811		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Appendix C: Species Frequency Relative to Network Mean TP– Plots and ANOVA

tables
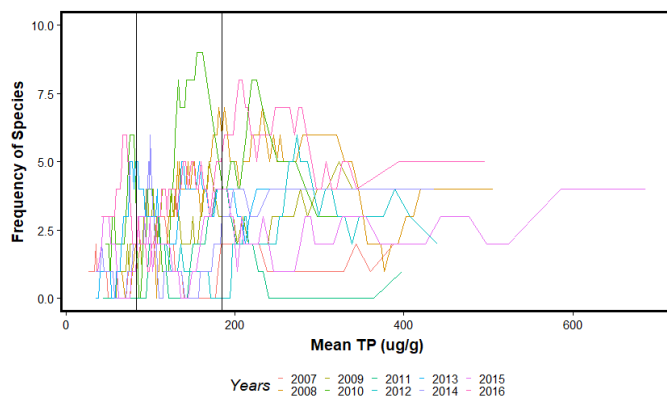
Figure C1. Plot of species frequency in network along a mean Total Phosphorus gradient (µg/g) for taxon *Achnantidium minutissimum* var. *gracillima*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: ACMINGRA

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	1255.32	139.480	75.4274	< 2.2e-16 ***
TP	2	375.61	187.807	101.5619	< 2.2e-16 ***
Year:TP	17	166.40	9.788	5.2934	2.706e-11 ***
Residuals	888	1642.08	1.849		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

119
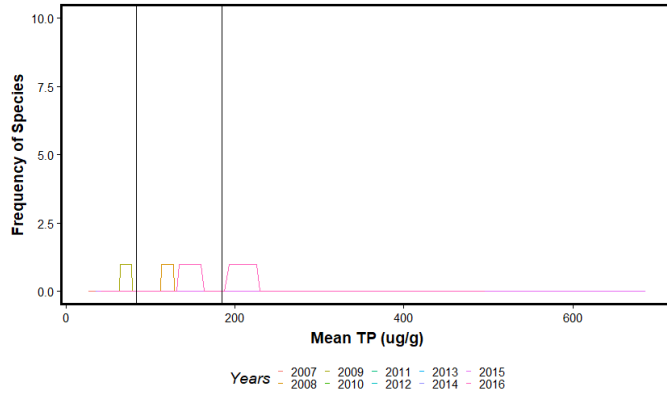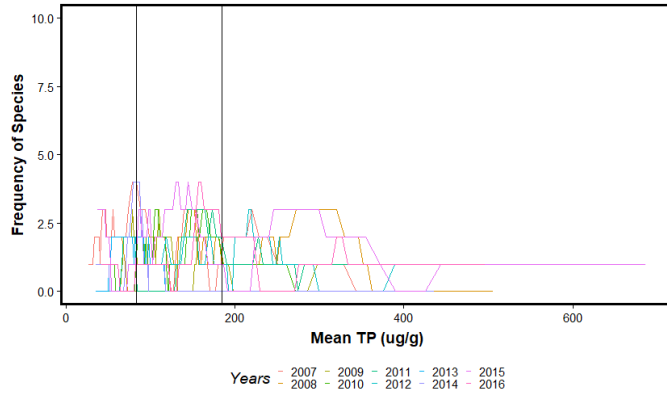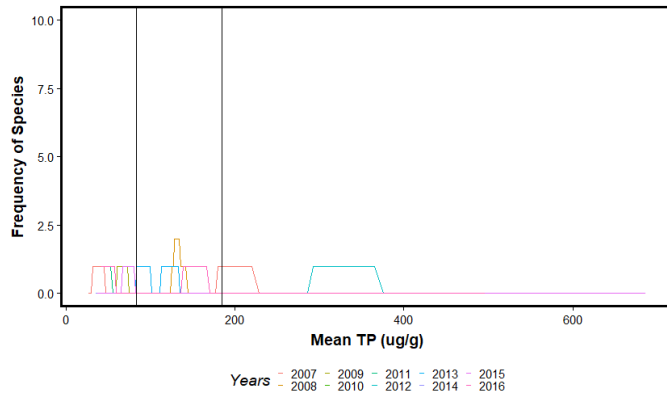
Figure C2. Plot of species frequency in network along a mean Total Phosphorus gradient (µg/g) for taxon *Amphora ovalis*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: AMOVAOVA

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	4.2869	0.47632	14.6799	<2e-16 ***
TP	2	0.0820	0.04099	1.2633	0.2832
Year:TP	17	5.0735	0.29844	9.1978	<2e-16 ***
Residuals	888	28.8129	0.03245		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
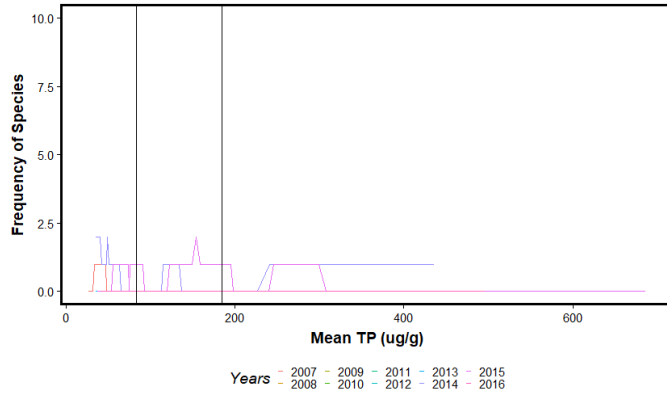
Figure C3. Plot of species frequency in network along a mean Total Phosphorus gradient (µg/g) for taxon *Amphora sulcata*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: AMSULSUL

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	85.06	9.4515	11.5629	< 2.2e-16 ***
TP	2	27.79	13.8941	16.9980	5.697e-08 ***
Year:TP	17	118.33	6.9608	8.5158	< 2.2e-16 ***
Residuals	888	725.85	0.8174		

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Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Figure C4. Plot of species frequency in network along a mean Total Phosphorus gradient (µg/g) for taxon *Aulacoseira* cf. *distans*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: AUCFDIST

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	6.760	0.75113	7.7915	4.185e-11 ***
TP	2	2.576	1.28778	13.3582	1.924e-06 ***
Year:TP	17	15.863	0.93313	9.6795	< 2.2e-16 ***
Residuals	888	85.606	0.09640		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

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Figure C5. Plot of species frequency in network along a mean Total Phosphorus gradient (µg/g) for taxon *Aulacoseira* cf. *granulata*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: AUCFGRAN

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	30.092	3.3436	55.421	< 2.2e-16 ***
TP	2	2.652	1.3258	21.976	4.836e-10 ***
Year:TP	17	11.994	0.7055	11.694	< 2.2e-16 ***
Residuals	888	53.574	0.0603		


Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
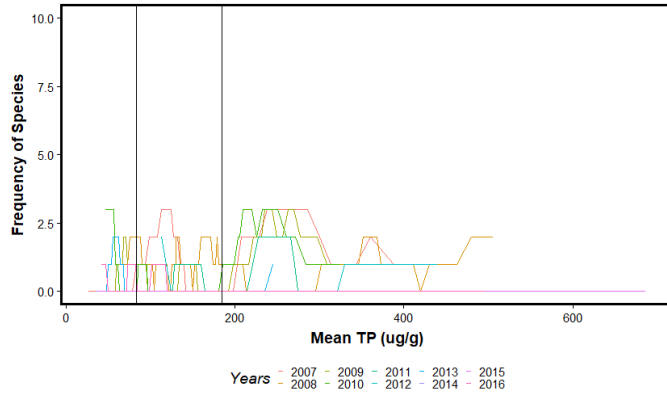
Figure C6. Plot of species frequency in network along a mean Total Phosphorus gradient (µg/g) for taxon *Brachysira brebissonii*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: BRBREBRE

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	123.476	13.7195	41.995	< 2.2e-16 ***
TP	2	15.535	7.7673	23.776	8.736e-11 ***
Year:TP	17	115.780	6.8106	20.847	< 2.2e-16 ***
Residuals	888	290.101	0.3267		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
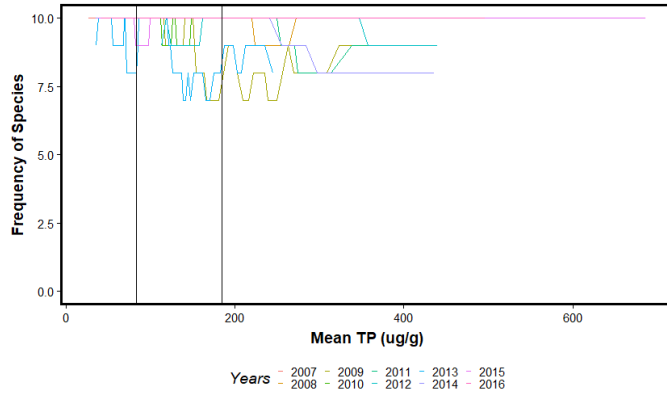
Figure C7. Plot of species frequency in network along a mean Total Phosphorus gradient (µg/g) for taxon *Brachysira microcephala*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: BRMICMIC

|	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	100.708	11.1898	50.910	< 2.2e-16 ***
TP	2	16.355	8.1775	37.205	3.044e-16 ***
Year:TP	17	58.954	3.4679	15.778	< 2.2e-16 ***
Residuals	888	195.180	0.2198		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
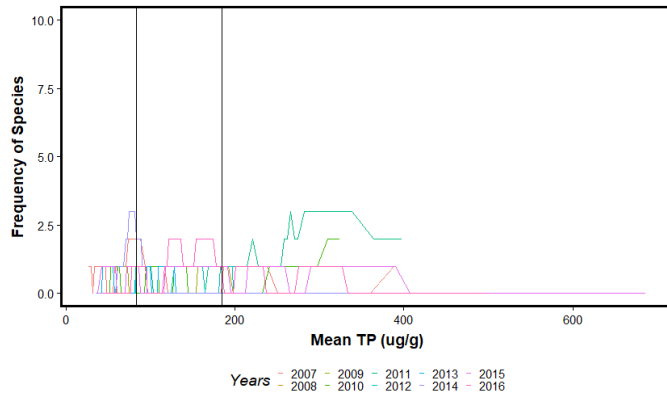
Figure C8. Plot of species frequency in network along a mean Total Phosphorus gradient (µg/g) for taxon *Caponea caribbea*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: CPCARCAR

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	44.555	4.9505	18.5238	<2e-16 ***
TP	2	0.024	0.0120	0.0451	0.9559
Year:TP	17	72.630	4.2723	15.9860	<2e-16 ***
Residuals	888	237.321	0.2673		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
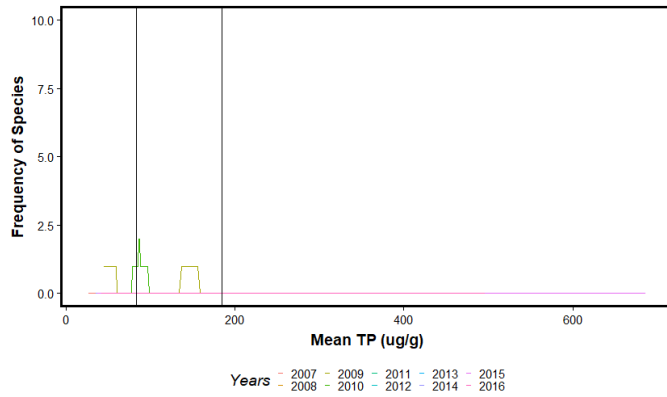
Figure C9. Plot of species frequency in network along a mean Total Phosphorus gradient (µg/g) for taxon *Craticula accomodiformis*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: CRACCACC

|	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	6.258	0.69538	18.6005	< 2.2e-16 ***
TP	2	0.472	0.23580	6.3074	0.001905 **
Year:TP	17	2.328	0.13691	3.6622	8.122e-07 ***
Residuals	888	33.198	0.03738		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
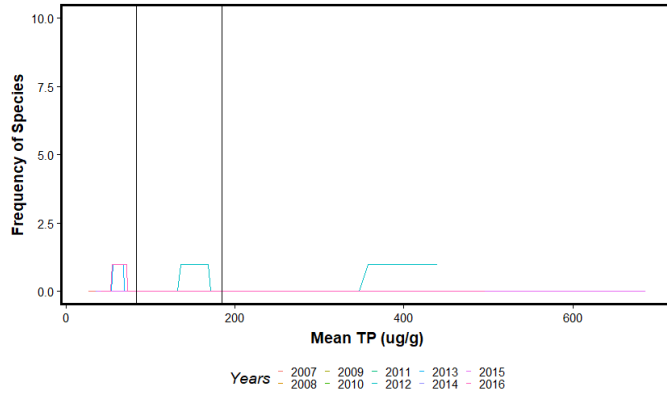
Figure C10. Plot of species frequency in network along a mean Total Phosphorus gradient (µg/g) for taxon *Cyclotella iris*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: CYIRIIRI

|	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	4.3288	0.48098	18.168	< 2.2e-16 ***
TP	2	1.3488	0.67438	25.474	1.748e-11 ***
Year:TP	17	5.4007	0.31769	12.000	< 2.2e-16 ***
Residuals	888	23.5084	0.02647		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
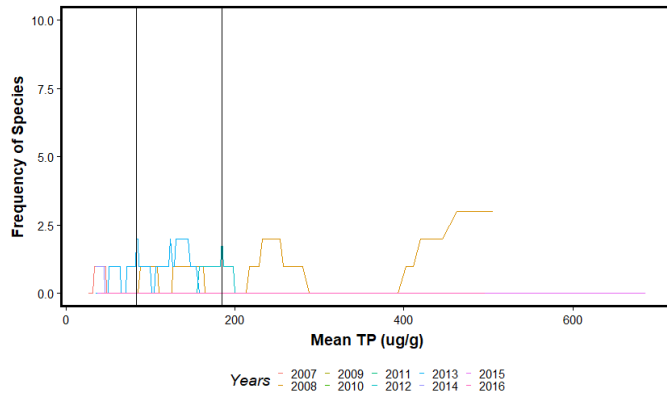
Figure C11. Plot of species frequency in network along a mean Total Phosphorus gradient (µg/g) for taxon *Diploneis puella*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: DIPUEPUE

|	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	64.420	7.1578	58.1636	<2e-16 ***
TP	2	0.424	0.2119	1.7219	0.1793
Year:TP	17	17.256	1.0151	8.2483	<2e-16 ***
Residuals	888	109.280	0.1231		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
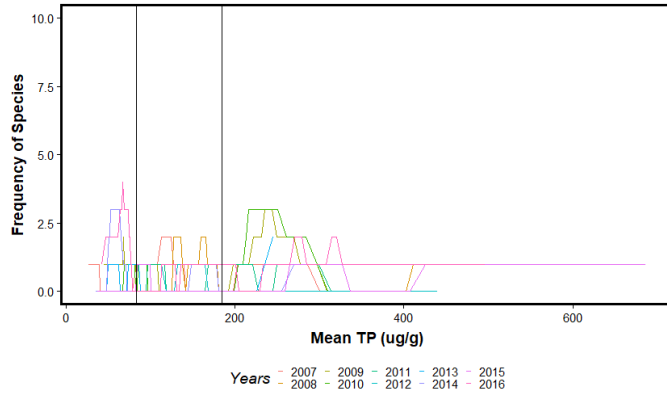
Figure C12. Plot of species frequency in network along a mean Total Phosphorus gradient (µg/g) for taxon *Encyonopsis* egsp01. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: ECEGSP01

|	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	51.466	5.7184	16.897	< 2.2e-16 ***
TP	2	12.989	6.4945	19.190	6.934e-09 ***
Year:TP	17	108.039	6.3552	18.779	< 2.2e-16 ***
Residuals	888	300.527	0.3384		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

130
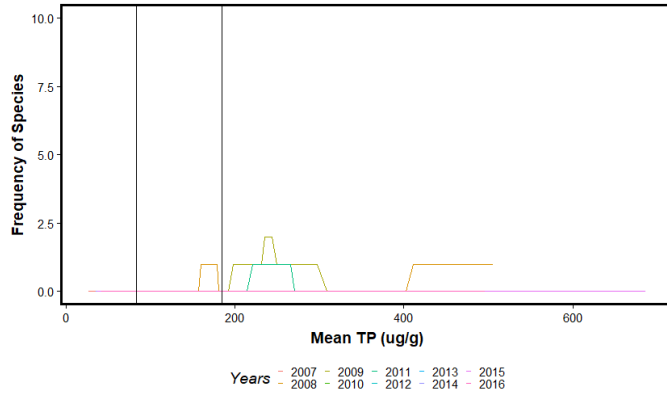
Figure C13. Plot of species frequency in network along a mean Total Phosphorus gradient (µg/g) for taxon *Encyonopsis floridana*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: ECFLOFLO

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	5.7606	0.64006	20.002	< 2.2e-16 ***
TP	2	2.7114	1.35570	42.365	< 2.2e-16 ***
Year:TP	17	13.7031	0.80606	25.189	< 2.2e-16 ***
Residuals	888	28.4160	0.03200		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
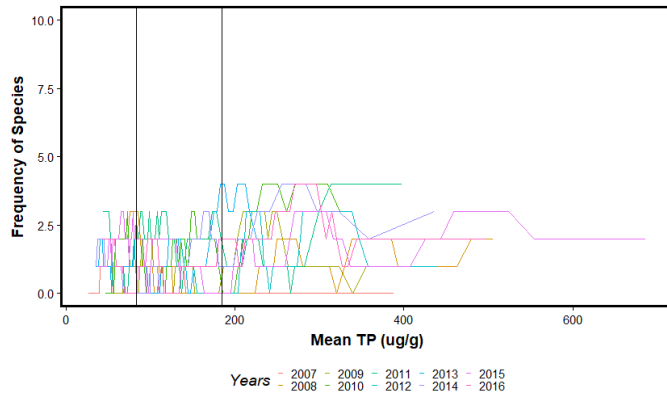
Figure C14. Plot of species frequency in network along a mean Total Phosphorus gradient (µg/g) for taxon *Encyonopsis subminuta*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: ECSUBSUB

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	146.24	16.249	22.416	< 2.2e-16 ***
TP	2	102.80	51.400	70.906	< 2.2e-16 ***
Year:TP	17	126.52	7.442	10.266	< 2.2e-16 ***
Residuals	888	643.71	0.725		

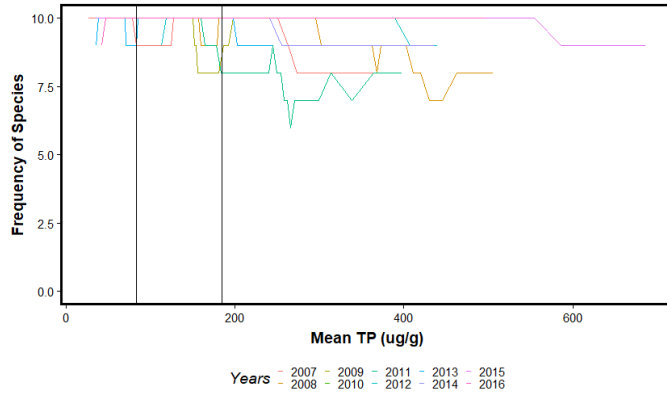Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
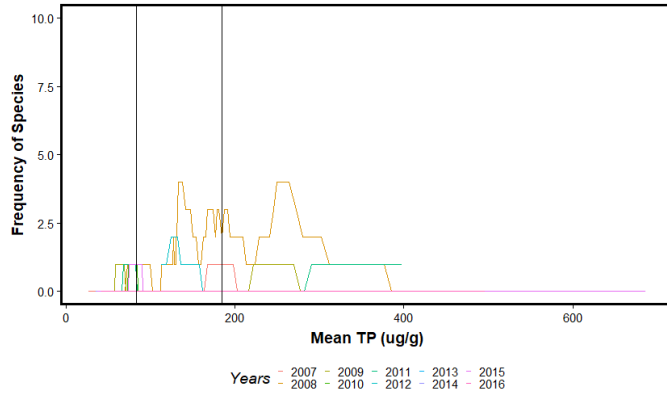
Figure C15. Plot of species frequency in network along a mean Total Phosphorus gradient (µg/g) for taxon *Encyonema* cf. *evergladianum*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: ENCFEVER

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	198.944	22.1049	104.4734	< 2.2e-16 ***
TP	2	1.237	0.6184	2.9229	0.0543
Year:TP	17	23.594	1.3879	6.5595	7.023e-15 ***
Residuals	888	187.887	0.2116		

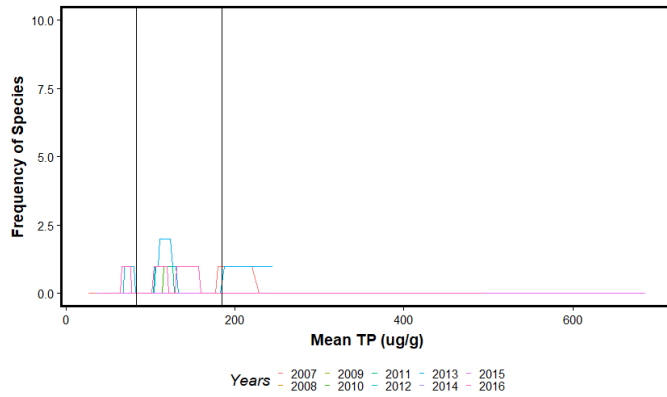Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

133

Figure C16. Plot of species frequency in network along a mean Total Phosphorus gradient (µg/g) for taxon *Encyonema evergladianum*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: ENEVEEVE

|	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	49.516	5.5017	36.892	< 2.2e-16 ***
TP	2	28.292	14.1462	94.858	< 2.2e-16 ***
Year:TP	17	82.245	4.8379	32.441	< 2.2e-16 ***
Residuals	888	132.427	0.1491		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

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Figure C17. Plot of species frequency in network along a mean Total Phosphorus gradient (µg/g) for taxon *Encyonema* ftsp04. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: ENFTSP04

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	20.570	2.28561	29.3411	< 2.2e-16 ***
TP	2	1.553	0.77627	9.9652	5.248e-05 ***
Year:TP	17	11.799	0.69404	8.9097	< 2.2e-16 ***
Residuals	888	69.173	0.07790		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

135
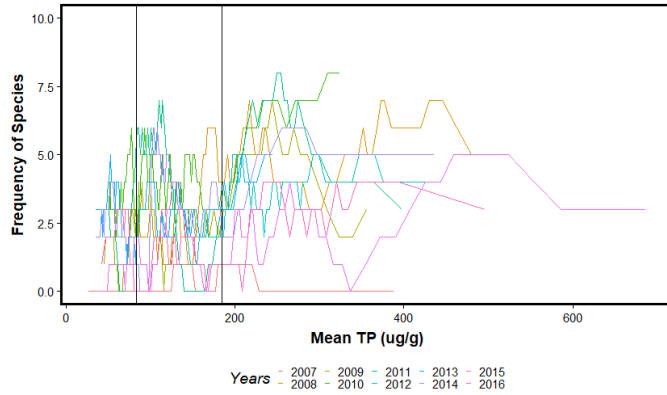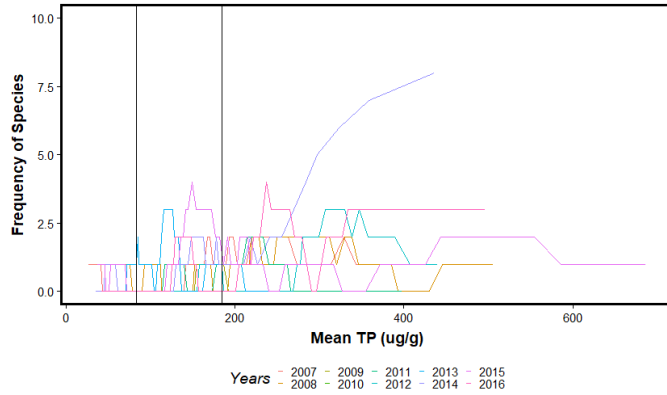
Figure C18. Plot of species frequency in network along a mean Total Phosphorus gradient (µg/g) for taxon *Encyonema silesiacum* var. *elegans*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: ENSILELE

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	1099.01	122.112	85.2147	< 2.2e-16 ***
TP	2	536.81	268.405	187.3032	< 2.2e-16 ***
Year:TP	17	130.89	7.700	5.3731	1.615e-11 ***
Residuals	888	1272.50	1.433		

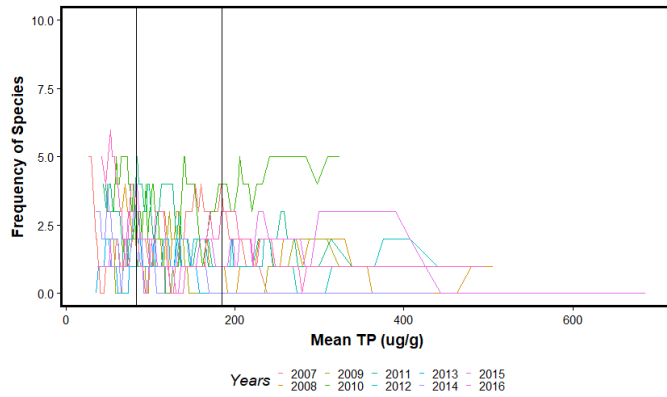Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

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Figure C19. Plot of species frequency in network along a mean Total Phosphorus gradient (µg/g) for taxon *Encyonema silesiacum*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: ENSILSIL

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	93.05	10.339	19.875	< 2.2e-16 ***
TP	2	87.97	43.987	84.558	< 2.2e-16 ***
Year:TP	17	108.87	6.404	12.311	< 2.2e-16 ***
Residuals	888	461.93	0.520		

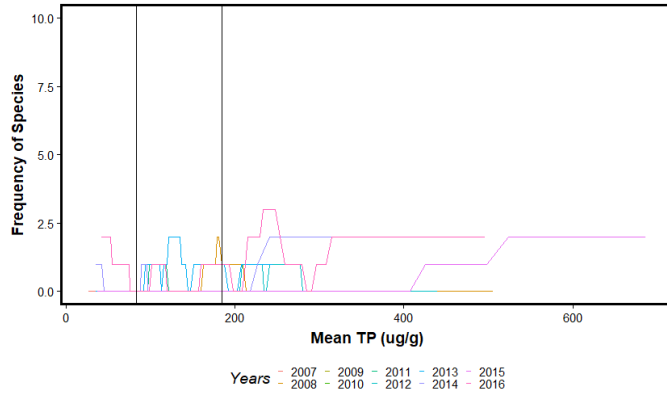---

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Figure C20. Plot of species frequency in network along a mean Total Phosphorus gradient (µg/g) for taxon *Encyonema* sjsp03. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: ENSJSP03

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	415.18	46.131	53.772	< 2.2e-16 ***
TP	2	81.54	40.770	47.523	< 2.2e-16 ***
Year:TP	17	198.42	11.672	13.605	< 2.2e-16 ***
Residuals	888	761.82	0.858		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
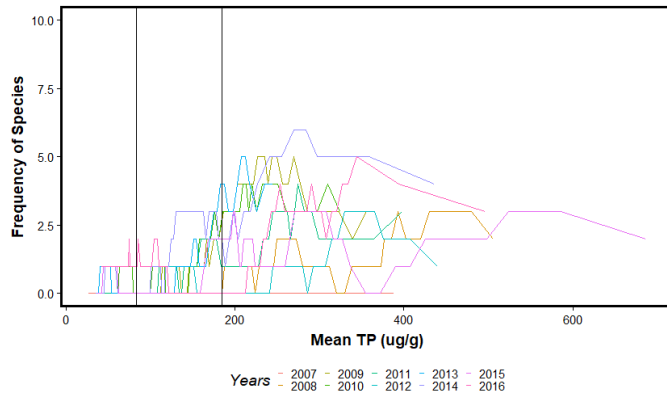
Figure C21. Plot of species frequency in network along a mean Total Phosphorus gradient (µg/g) for taxon *Eunotia* egsp01. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: EUEGSP01

|	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	68.399	7.5999	45.697	< 2.2e-16 ***
TP	2	11.813	5.9067	35.516	1.45e-15 ***
Year:TP	17	43.416	2.5539	15.356	< 2.2e-16 ***
Residuals	888	147.683	0.1663		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Figure C22. Plot of species frequency in network along a mean Total Phosphorus gradient (µg/g) for taxon *Eunotia flexuosa*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: EUFLEFLE

|	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	115.73	12.859	22.166	< 2.2e-16 ***
TP	2	587.47	293.735	506.337	< 2.2e-16 ***
Year:TP	17	218.31	12.842	22.137	< 2.2e-16 ***
Residuals	888	515.15	0.580		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
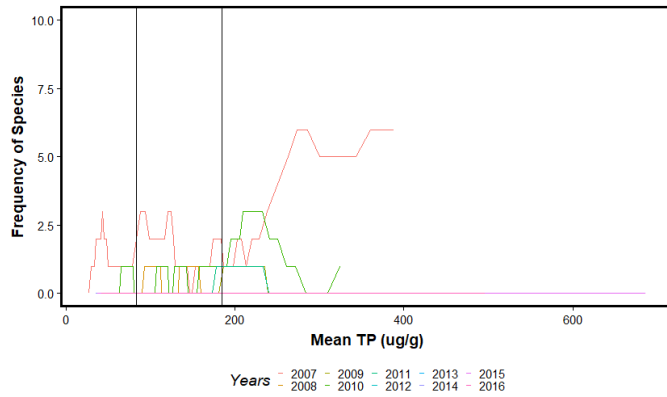
Figure C23. Plot of species frequency in network along a mean Total Phosphorus gradient (µg/g) for taxon *Eunotia formica*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: EUFORFOR

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	283.616	31.5129	161.298	< 2.2e-16 ***
TP	2	18.464	9.2319	47.253	< 2.2e-16 ***
Year:TP	17	76.427	4.4957	23.011	< 2.2e-16 ***
Residuals	888	173.489	0.1954		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
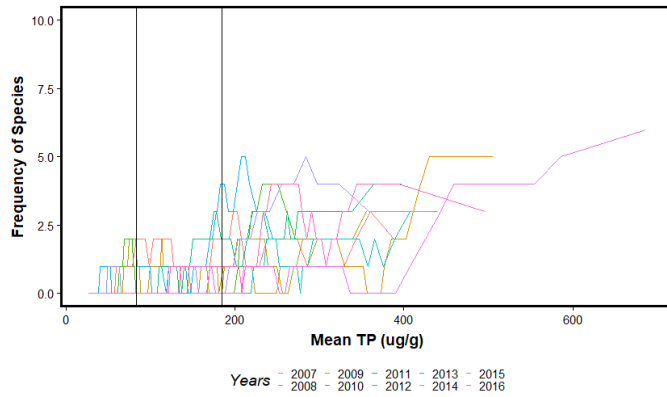
Figure C24. Plot of species frequency in network along a mean Total Phosphorus gradient (µg/g) for taxon *Eunotiea naegelii*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: EUNAENAE

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	36.84	4.094	6.2179	1.529e-08 ***
TP	2	406.16	203.082	308.4497	< 2.2e-16 ***
Year:TP	17	105.72	6.219	9.4451	< 2.2e-16 ***
Residuals	888	584.66	0.658		

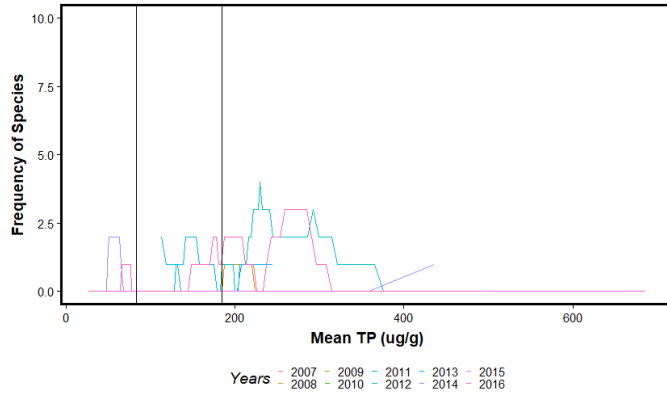---

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Figure C25. Plot of species frequency in network along a mean Total Phosphorus gradient (µg/g) for taxon *Fragilaria* cf. *tenera*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: FACFTENE

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	116.076	12.8973	68.217	< 2.2e-16 ***
TP	2	13.155	6.5775	34.790	2.841e-15 ***
Year:TP	17	48.408	2.8475	15.061	< 2.2e-16 ***
Residuals	888	167.886	0.1891		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

143
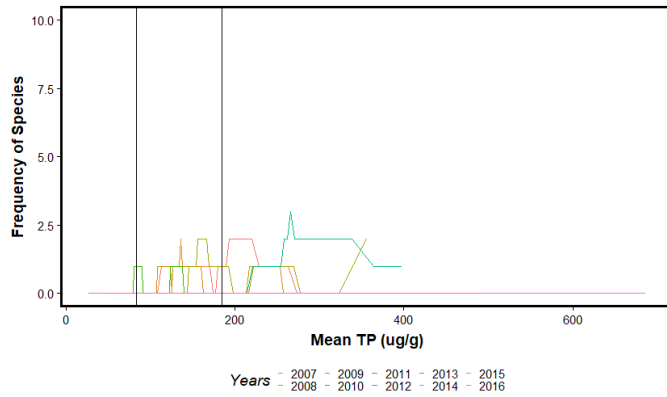
Figure C26. Plot of species frequency in network along a mean Total Phosphorus gradient (µg/g) for taxon *Fragilaria* ftsp16. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: FAFTSP16

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	33.912	3.7680	33.101	< 2.2e-16 ***
TP	2	8.571	4.2854	37.647	< 2.2e-16 ***
Year:TP	17	40.461	2.3801	20.909	< 2.2e-16 ***
Residuals	888	101.082	0.1138		

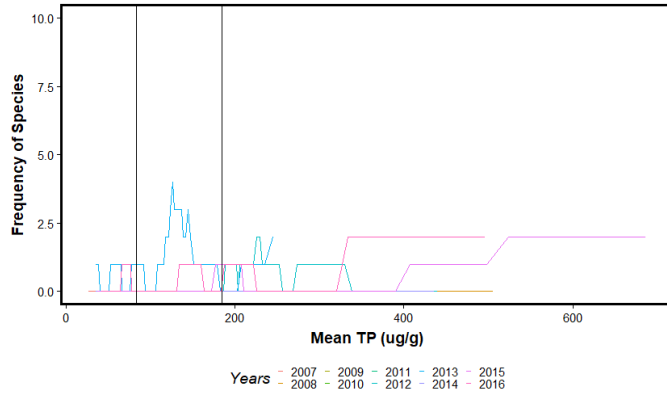
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

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Figure C27. Plot of species frequency in network along a mean Total Phosphorus gradient (µg/g) for taxon *Fragilariforma virescens* var. *capitata*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: FFVIRCAP

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	80.479	8.9422	60.3826	< 2.2e-16 ***
TP	2	4.731	2.3653	15.9719	1.532e-07 ***
Year:TP	17	19.162	1.1272	7.6114	< 2.2e-16 ***
Residuals	888	131.506	0.1481		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
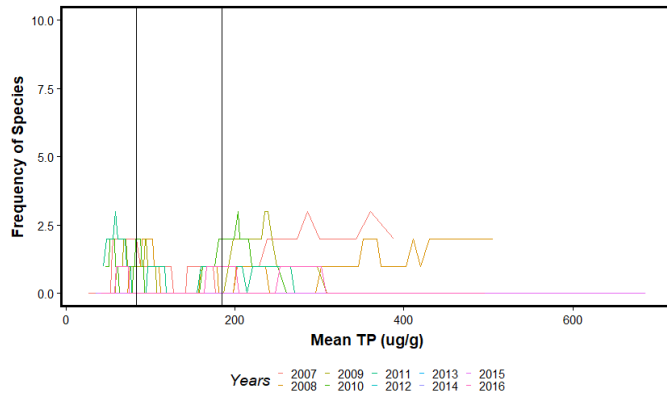
Figure C28. Plot of species frequency in network along a mean Total Phosphorus gradient (µg/g) for taxon *Frustulia crassinervia*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: FRCRACRA

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	112.616	12.5129	44.3500	< 2.2e-16 ***
TP	2	19.702	9.8510	34.9150	2.531e-15 ***
Year:TP	17	31.260	1.8388	6.5175	9.252e-15 ***
Residuals	888	250.541	0.2821		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

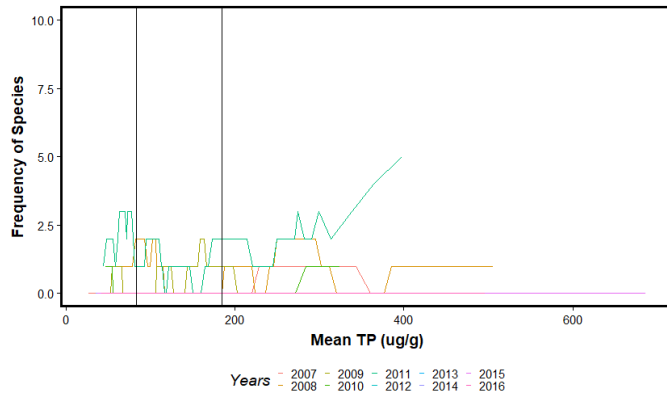Figure C29. Plot of species frequency in network along a mean Total Phosphorus gradient (µg/g) for taxon *Gomphonema affine*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: GOAFFAFF

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	220.008	24.4454	159.887	< 2.2e-16 ***
TP	2	5.153	2.5763	16.851	6.565e-08 ***
Year:TP	17	27.490	1.6171	10.577	< 2.2e-16 ***
Residuals	888	135.767	0.1529		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
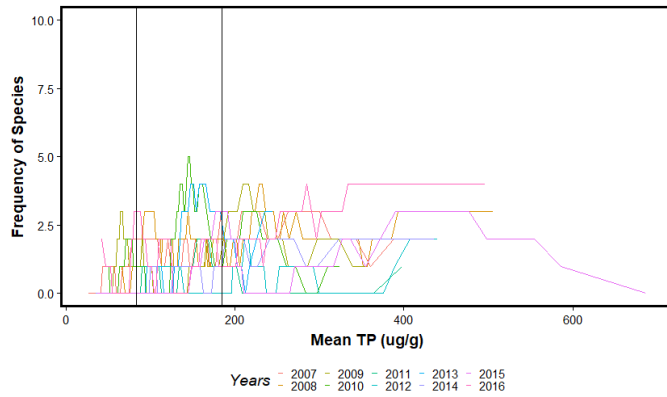
Figure C30. Plot of species frequency in network along a mean Total Phosphorus gradient (µg/g) for taxon *Gomphonema* cf. *vibrioides*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: GOCFVIBR

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	234.33	26.037	37.2771	< 2.2e-16 ***
TP	2	184.17	92.087	131.8413	< 2.2e-16 ***
Year:TP	17	92.38	5.434	7.7798	< 2.2e-16 ***
Residuals	888	620.24	0.698		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
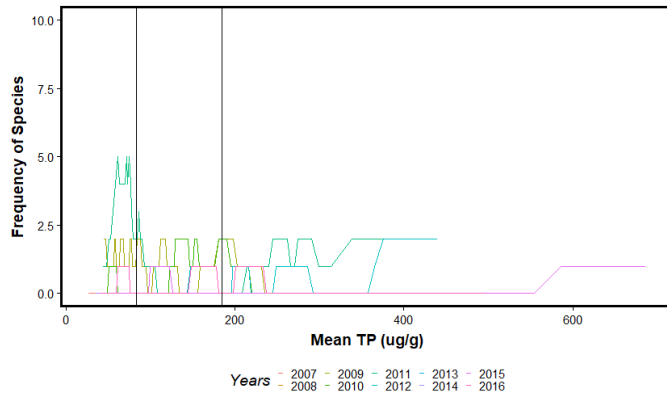
Figure C31. Plot of species frequency in network along a mean Total Phosphorus gradient (µg/g) for taxon *Gomphonema gracile*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: GOGRAGRA

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	174.883	19.4315	80.129	< 2.2e-16 ***
TP	2	10.322	5.1609	21.282	9.375e-10 ***
Year:TP	17	97.391	5.7289	23.624	< 2.2e-16 ***
Residuals	888	215.341	0.2425		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

149
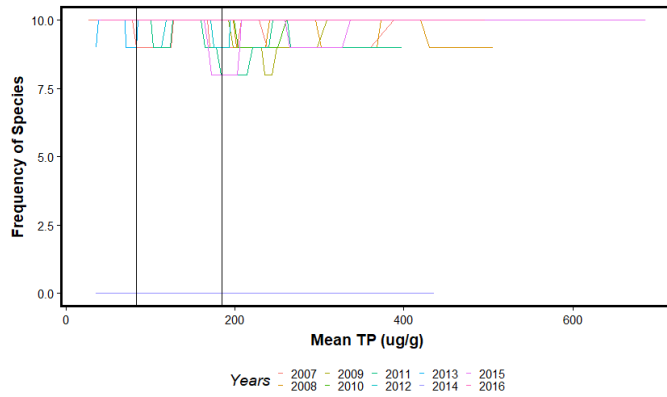
Figure C32. Plot of species frequency in network along a mean Total Phosphorus gradient (µg/g) for taxon *Mastogloia calcarea*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: MACALCAL

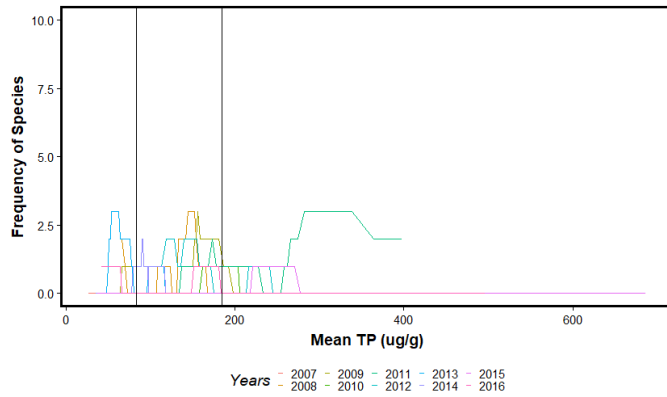|	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	8544.5	949.38	7767.381	< 2.2e-16 ***
TP	2	23.0	11.49	93.976	< 2.2e-16 ***
Year:TP	17	26.3	1.55	12.665	< 2.2e-16 ***
Residuals	888	108.5	0.12		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Figure C33. Plot of species frequency in network along a mean Total Phosphorus gradient (µg/g) for taxon *Mastogloia lanceolata*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: MALANLAN

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	42.486	4.7207	16.605	< 2.2e-16 ***
TP	2	5.991	2.9954	10.537	3.003e-05 ***
Year:TP	17	93.960	5.5270	19.442	< 2.2e-16 ***
Residuals	888	252.445	0.2843		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
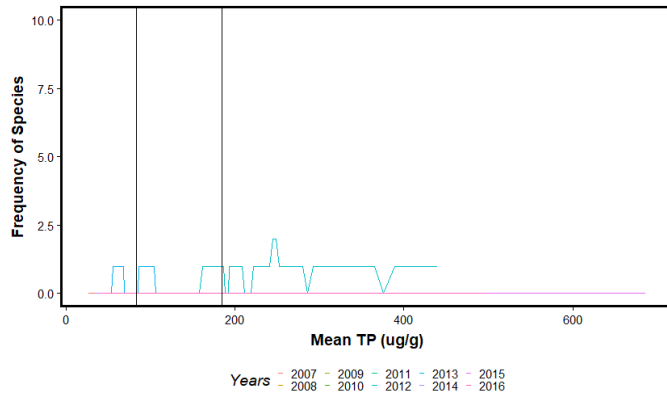
Figure C34. Plot of species frequency in network along a mean Total Phosphorus gradient (µg/g) for taxon *Navicula densilineolata*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: NADENDEN

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	37.908	4.2120	125.4085	< 2.2e-16 ***
TP	2	0.164	0.0820	2.4415	0.08762
Year:TP	17	3.291	0.1936	5.7644	1.272e-12 ***
Residuals	888	29.825	0.0336		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

152
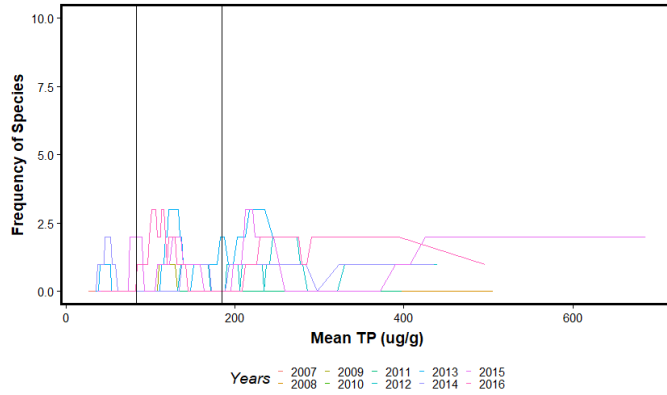
Figure C35. Plot of species frequency in network along a mean Total Phosphorus gradient (µg/g) for taxon *Navicula radiosafallax*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: NARAFRAF

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	152.715	16.9684	56.928	< 2.2e-16 ***
TP	2	21.813	10.9066	36.592	5.364e-16 ***
Year:TP	17	59.691	3.5112	11.780	< 2.2e-16 ***
Residuals	888	264.682	0.2981		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

153
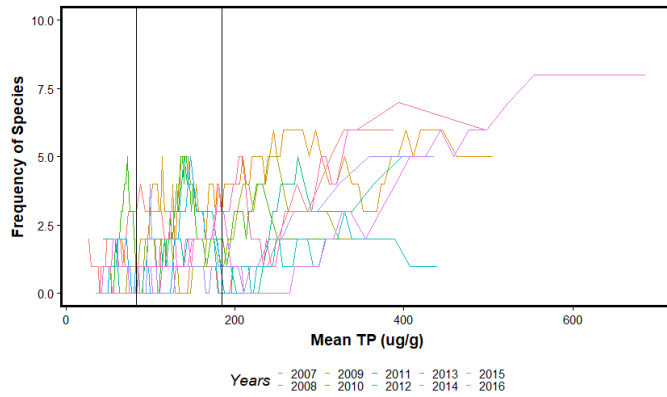
Figure C36. Plot of species frequency in network along a mean Total Phosphorus gradient (µg/g) for taxon *Nitzschia amphibia*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: NIAMPAMP

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	598.46	66.495	45.7820	< 2.2e-16 ***
TP	2	327.28	163.642	112.6677	< 2.2e-16 ***
Year:TP	17	222.88	13.110	9.0266	< 2.2e-16 ***
Residuals	888	1289.76	1.452		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

154

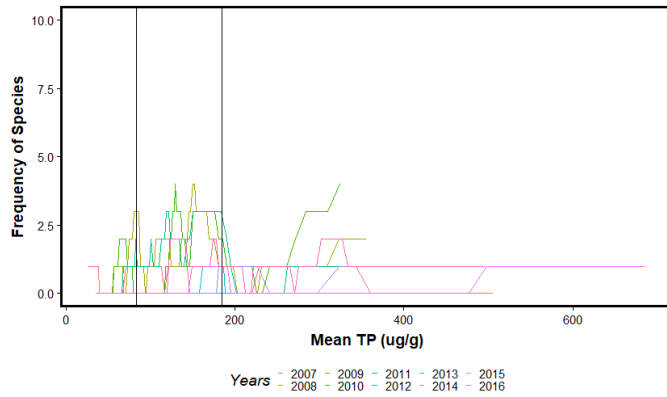Figure C37. Plot of species frequency in network along a mean Total Phosphorus gradient (µg/g) for taxon *Nitzschia* cf. *semirobusta*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: NICFSEMI

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	245.23	27.2483	75.7340	< 2.2e-16 ***
TP	2	29.08	14.5419	40.4179	< 2.2e-16 ***
Year:TP	17	58.29	3.4286	9.5296	< 2.2e-16 ***
Residuals	888	319.49	0.3598		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
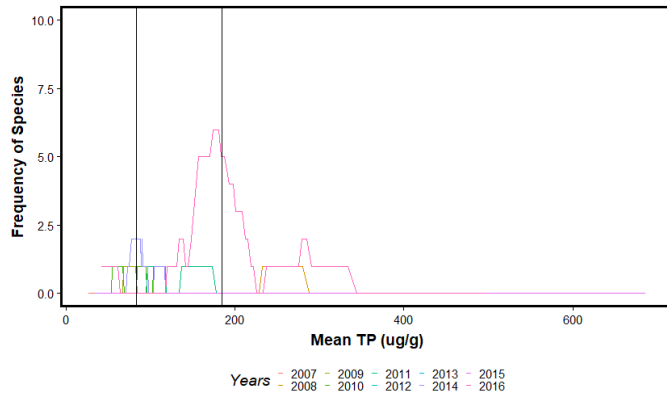
Figure C38. Plot of species frequency in network along a mean Total Phosphorus gradient (µg/g) for taxon *Nitzschia* ftsp16. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: NIFTSP16

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	135.67	15.0741	42.1465	< 2.2e-16 ***
TP	2	4.57	2.2845	6.3872	0.001761 **
Year:TP	17	42.39	2.4936	6.9720	4.681e-16 ***
Residuals	888	317.60	0.3577		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

156

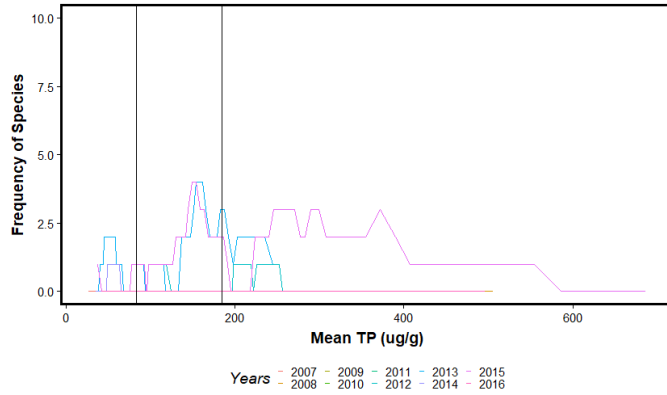Figure C39. Plot of species frequency in network along a mean Total Phosphorus gradient (µg/g) for taxon *Nitzschia intermedia*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: NIINTINT

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	210.843	23.4270	113.695	< 2.2e-16 ***
TP	2	5.240	2.6199	12.715	3.595e-06 ***
Year:TP	17	37.225	2.1897	10.627	< 2.2e-16 ***
Residuals	888	182.973	0.2061		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
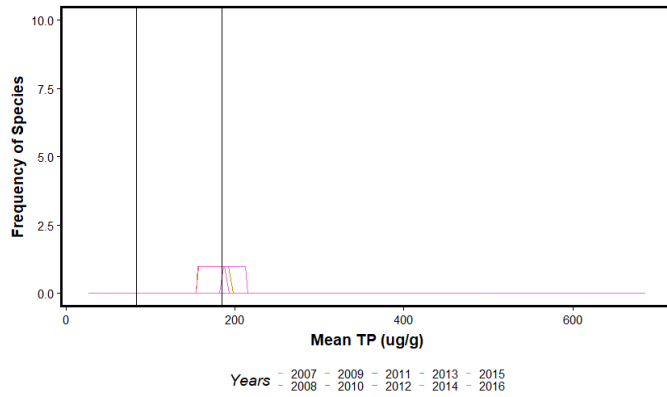
Figure C40. Plot of species frequency in network along a mean Total Phosphorus gradient (µg/g) for taxon *Nitzschia microcephala*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: NIMICMIC

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	2.0199	0.22444	8.2212	8.284e-12 ***
TP	2	0.3658	0.18291	6.7000	0.001294 **
Year:TP	17	2.3906	0.14062	5.1511	6.783e-11 ***
Residuals	888	24.2422	0.02730		

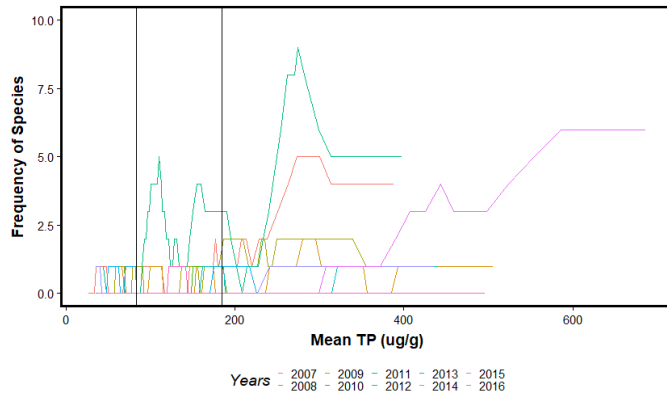
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

158

Figure C41. Plot of species frequency in network along a mean Total Phosphorus gradient (µg/g) for taxon *Nitzschia nana*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: NINANNAN

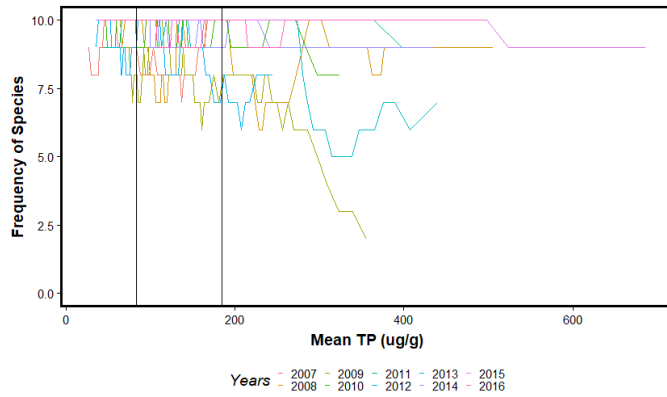|	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	398.11	44.235	74.121	< 2.2e-16 ***
TP	2	125.90	62.951	105.483	< 2.2e-16 ***
Year:TP	17	238.40	14.023	23.498	< 2.2e-16 ***
Residuals	888	529.95	0.597		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Figure C42. Plot of species frequency in network along a mean Total Phosphorus gradient (µg/g) for taxon *Nitzschia palea* var. *debilis*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: NIPALDEB

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	292.39	32.488	47.705	< 2.2e-16 ***
TP	2	45.38	22.690	33.318	1.116e-14 ***
Year:TP	17	157.65	9.273	13.617	< 2.2e-16 ***
Residuals	888	604.75	0.681		

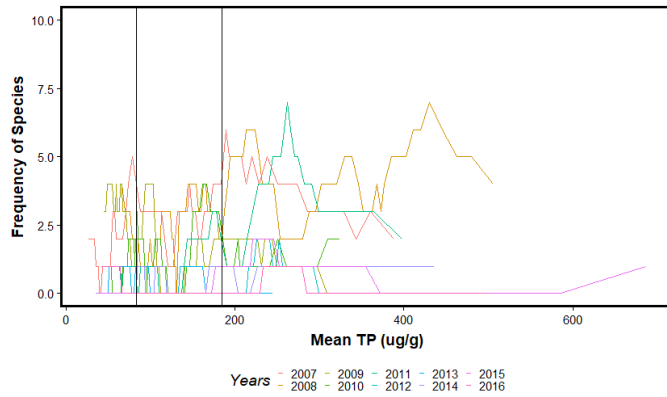
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

160

Figure C43. Plot of species frequency in network along a mean Total Phosphorus gradient (µg/g) for taxon *Nitschia palea* var. *tenuirostris*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: NIPALTEN

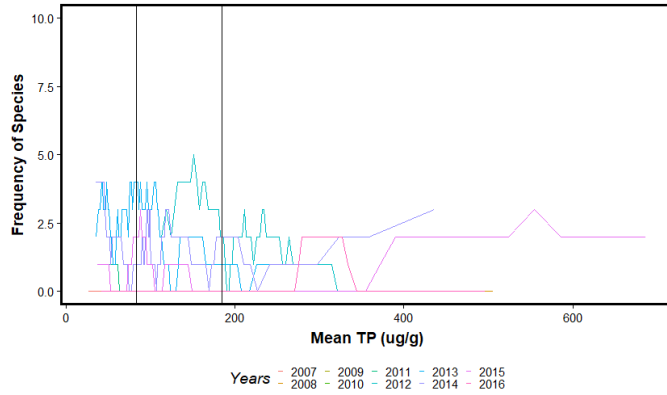|	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	1003.99	111.555	154.560	< 2.2e-16 ***
TP	2	111.06	55.528	76.935	< 2.2e-16 ***
Year:TP	17	226.64	13.332	18.471	< 2.2e-16 ***
Residuals	888	640.92	0.722		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Figure C44. Plot of species frequency in network along a mean Total Phosphorus gradient (µg/g) for taxon *Pseudostaurosira brevistriata*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: PEBREBRE

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	622.38	69.154	193.533	< 2.2e-16 ***
TP	2	13.19	6.594	18.455	1.403e-08 ***
Year:TP	17	102.09	6.005	16.806	< 2.2e-16 ***
Residuals	888	317.30	0.357		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

162
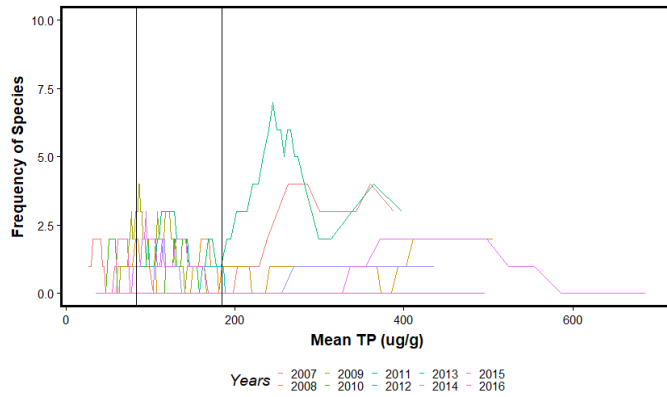
Figure C45. Plot of species frequency in network along a mean Total Phosphorus gradient (µg/g) for taxon *Pinnularia microstauron*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: PIMICMIC

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	275.81	30.6460	67.531	< 2.2e-16 ***
TP	2	10.37	5.1864	11.428	1.257e-05 ***
Year:TP	17	306.35	18.0207	39.710	< 2.2e-16 ***
Residuals	888	402.98	0.4538		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Analysis of Variance Table
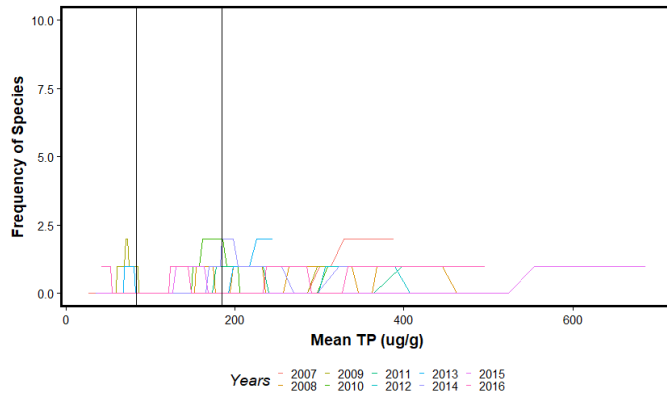
Figure C46. Plot of species frequency in network along a mean Total Phosphorus gradient (µg/g) for taxon *Rhopalodia gibba*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: RHGIBGIB

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	5.995	0.6661	4.065	3.977e-05 ***
TP	2	16.020	8.0100	48.884	< 2.2e-16 ***
Year:TP	17	28.540	1.6788	10.246	< 2.2e-16 ***
Residuals	888	145.506	0.1639		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

164

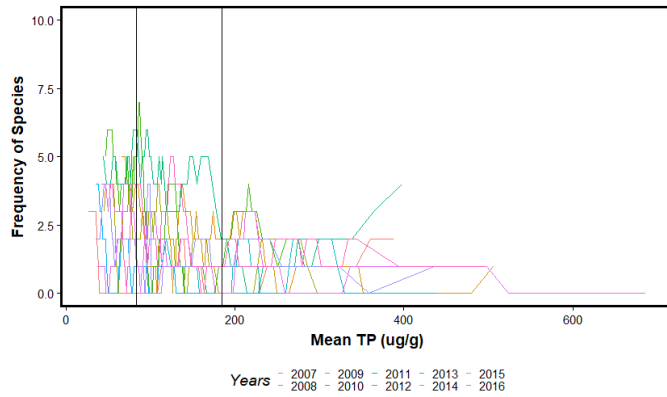Figure C47. Plot of species frequency in network along a mean Total Phosphorus gradient (µg/g) for taxon *Sellaphora laevissima*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: SELAELAE

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	662.28	73.587	65.0805	< 2.2e-16 ***
TP	2	287.82	143.909	127.2743	< 2.2e-16 ***
Year:TP	17	188.83	11.107	9.8235	< 2.2e-16 ***
Residuals	888	1004.06	1.131		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
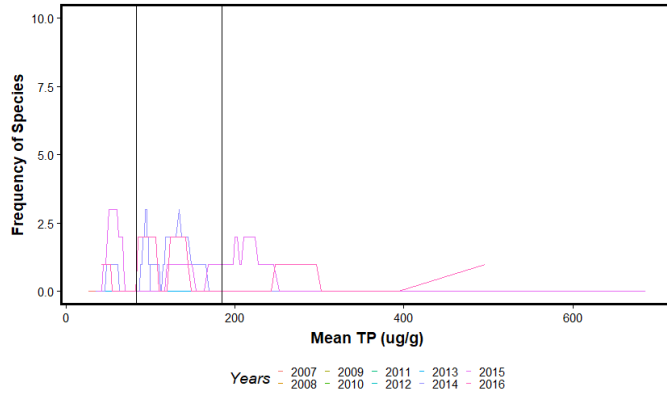
Figure C48. Plot of species frequency in network along a mean Total Phosphorus gradient (µg/g) for taxon *Sellaphora seminulum*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: SESEMSEM

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	96.300	10.7000	55.982	< 2.2e-16 ***
TP	2	4.781	2.3905	12.507	4.399e-06 ***
Year:TP	17	40.366	2.3745	12.423	< 2.2e-16 ***
Residuals	888	169.725	0.1911		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
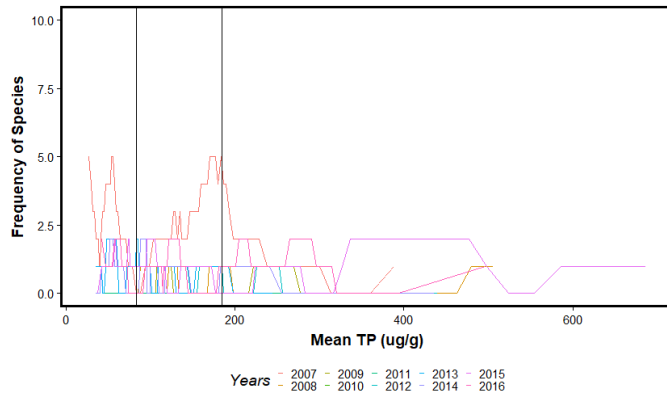
Figure C49. Plot of species frequency in network along a mean Total Phosphorus gradient (µg/g) for taxon *Sellaphora stroemii*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: SESTRSTR

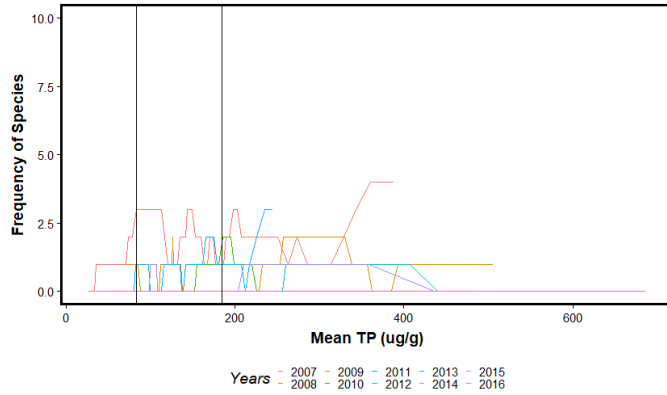|	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	381.00	42.333	111.6033	< 2.2e-16 ***
TP	2	9.55	4.774	12.5854	4.076e-06 ***
Year:TP	17	39.87	2.345	6.1825	8.297e-14 ***
Residuals	888	336.83	0.379		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Figure C50. Plot of species frequency in network along a mean Total Phosphorus gradient (µg/g) for taxon *Synedra filiformis* var. *exilis*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: SYFILEXI

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	194.024	21.5582	129.742	< 2.2e-16 ***
TP	2	20.022	10.0109	60.248	< 2.2e-16 ***
Year:TP	17	34.943	2.0555	12.370	< 2.2e-16 ***
Residuals	888	147.552	0.1662		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

168
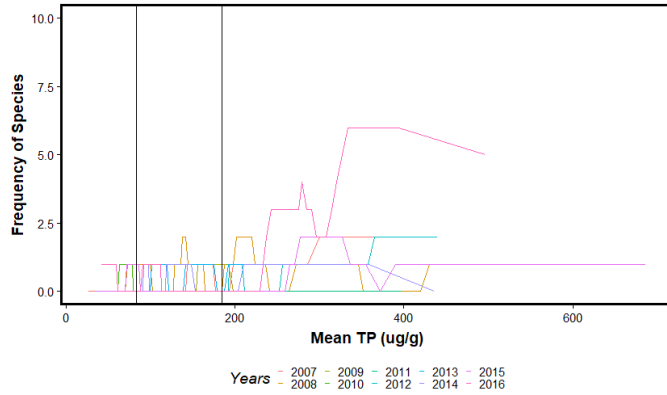
Figure C51. Plot of species frequency in network along a mean Total Phosphorus gradient (µg/g) for taxon *Ulnaria delicatissima*. Vertical lines indicate thresholds at 83 and 195 µg/g. Two-way ANOVA with replication follows immediately.

Analysis of Variance Table

Response: ULDELDEL

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Year	9	65.579	7.2865	22.108	< 2.2e-16 ***
TP	2	55.189	27.5944	83.725	< 2.2e-16 ***
Year:TP	17	78.256	4.6033	13.967	< 2.2e-16 ***
Residuals	888	292.668	0.3296		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

169