# Introduction

1. Enrichment of nitrogen (N) and phosphorus (P) are mainly responsible for eutrophication (Wetzel, 2001), one of the biggest, often anthropogenically-caused threats to freshwaters (Smith & Schindler, 2009) and biodiversity (Reid et al., 2019) across the globe.
   1. Eutrophication can have serious consequences on aquatic ecosystem health including decreased oxygen, formation of toxins, and changes in organismal communities (Camargo & Alonso, 2006).
   2. In the United States (US), an estimated $2.2 billion in annual losses because of eutrophication is likely an underestimate of the actual amount (Dodds et al., 2008)
      1. as determining economic value of freshwater is difficult with many important factors often excluded from these analyses (Keiser et al., 2019).
   3. Furthermore, many freshwaters across the US are generally at serious risk of or are already plagued by eutrophication (Dodds et al., 2008).
2. There is an ongoing debate about whether N or P is more important in causing eutrophication or which is the main limiting nutrient, and many studies focus solely on P or argue that P should be the top management priority (Carpenter, 2008; Ngatia & Taylor, 2019).
   1. Reasons supporting the P paradigm include factors like N-fixing bacteria that can increase N availability (Schindler et al., 2008), accumulation of P in soils and freshwater sediments (Bennett et al., 2001), potential for internal loading (Sun et al., 2022), past successful P reduction efforts (Foy, 2005), and geographic extent (e.g. focus on the northeast and Midwest US in (Liang et al., 2020).
   2. Furthermore, there is belief that P is primarily the limiting nutrient in freshwaters, while N is limiting in oceans (Correll, 1999), and that N-limitation in freshwaters is merely a result of P enrichment in eutrophic waters (Havens, 1995).
3. Despite the paradigm surrounding P, the importance of N is not unknown. In fact, some believe that most northern-hemisphere lakes were N-limited prior to an influx of N-deposition resulting in eutrophication and a shift toward P-limitation (Bergström & Jansson, 2006).
   1. Focusing on eutrophication management, P reduction alone is no longer an adequate solution. Rather, there is increased need for research on nutrient amounts, ratios, and N’s impact on eutrophication (Yao et al., 2018).
      1. Reductions in P pollution in large lakes may lead to accumulation of N (Finlay et al., 2013), negating the attempt to mitigate nutrient pollution.
      2. In the Western US, N-deposition can significantly alter ecosystems (Fenn et al., 2003).
      3. Projected precipitation patterns under a changing climate will only likely increase N loading into freshwaters worldwide (Sinha et al., 2017).
   2. Experiments have demonstrated how both nutrients together have an impact on productivity significantly more than additions of a single nutrient and how stoichiometrically imbalanced food can have detrimental impacts (Elser et al., 2011; Redoglio et al., 2022).
4. Because their cycles are coupled in the environment (Oviedo-Vargas et al., 2013), studying N and P in together terms of relative abundances may unfold large-scale patterns that would otherwise be unseen.
   1. Nutrient limitation can be defined using Liebig’s law of minimum, which states that organismal growth is limited by the resource or nutrient in lowest supply. And the relative abundance of these nutrients indicate the balance of the supply (Sterner & Elser, 2002).
      1. Limitation directly connects to eutrophication through nutrient drivers of primary productivity.
   2. Just as biological processes impact availability of nutrient supply and regulate global biogeochemical processes (Reiners, 1986), so too are the nutrient supply pools important as nutrient supply can limit biological function and growth (Sterner & Elser, 2002).
   3. Large-scale nutrient stoichiometry integrates biogeochemical processes like biotic dynamics, precipitation, geological weathering, and anthropogenic influences; and serves as the backdrop for many smaller-scale processes to occur (Sterner & Elser, 2002).
      1. Balancing nutrient stoichiometry may assist in eutrophication management, rather than focusing on a sole nutrient (Stutter et al., 2018).
5. In this study, we use US Environmental Protection Agency (EPA) National Lakes Assessment (NLA) data to evaluate differences in nutrient limitation across US lakes between survey years 2007 and 2017.
   1. This research is intended to support efforts to assess nutrient water quality and more effectively protect and restore waters from nutrient pollution.
   2. The NLA data are specifically designed to assess lakes across the US, rather than the individual lakes sampled, by using population weight estimates (USEPA, 2022b).
   3. Using these broad scale survey data, we aim to answer the following questions:
      1. Which nutrient correlates with eutrophication in lakes across ecoregions of the US?
      2. Which nutrient is limiting in lakes across ecoregions of the US?
      3. Where are shifts in nutrient limitation occurring?

# Methods

## NLA lakes and methods

NLA data from survey years 2007 and 2017 were used in the analyses (USEPA, 2010, 2022a). In 2007, 1156 lakes were surveyed, 95 were resampled in the same year and 124 were considered reference lakes. And in 2017, 1112 lakes were sampled, 97 were resampled in the same year, and 108 were considered reference lakes. 282 lakes were sampled in both survey years. In 2007, lakes greater than 4 ha were sampled. This changed in the later surveys and lakes with surface area > 1 ha and 1-m deep were included.

### Site selection

The EPA used a Generalized Random Tessellation Stratified survey design to randomly choose sampling sites (USEPA, 2022b). Stratification was based on Omernik level-3 aggregated ecoregions, state, and lake size. Discretizing the dataset into Omernik’s 9 aggregated ecoregions provides a qualitative understanding of spatial patterns and regional homogeneities (Omernik, 1987). Each lake is assigned a weight to indicate the number of lakes it represents with error. The NLA site weights are intended to broaden results to regional and national extents (USEPA, 2022b).

### Sampling and laboratory methods

Lakes were sampled during the summer month (May-September) of each survey year. In 2007 there were 9 sampling events in October and in 2017, there were 4 sampling events in October. The EPA used standardize sampling protocols in each survey year. Water was collected using an integrated sampler within the euphotic zone or up to 2m depth. Chlorophyll samples were stored in a dark 2L bottle and stored on ice until filtration with a 0.4 µm pore size polycarbonate filters. Nutrient samples were stored in 250 mL bottles on ice and sulfuric acid was added to stabilize samples at pH <2. More on these standardize sampling procedures can be found in the NLA field operations manuals (USEPA, 2007a, 2017a).

Samples were shipped overnight to approved laboratories and processed within 24 hours of receipt. Samples are analyzed for chlorophyll-a via extraction in 90% acetone followed by fluorometry. Total nutrients are analyzed via persulfate digestion then automated colorimetric analysis. Laboratory processing procedures must maintain quality assurance/control outlined by the EPA. More information about these processes and quality assurance can be found in the NLA laboratory operations manuals (USEPA, 2007b, 2017b).

### Trophic state determination

The NLA uses chlorophyll-a concentration as a proxy for trophic state, while acknowledging that trophic state is actually determined by a variety of characteristics including nutrients, climate, morphometry, etc. Oligotrophic lakes have chlorophyll-a concentrations ≤ 2 µg L-1. Mesotrophic lakes have concentrations greater > 2 µg L-1 and ≤ 7 µg L-1. Eutrophic lakes have concentrations > 7 µg L-1 and ≤ 30 µg L-1. And hypereutrophic lakes have concentrations > 30 µg L-1 (USEPA, 2022b).

## Data analyses

### Limitation calculation

Nutrient limitation for lakes can fall into three categories: P-limitation, N-limitation, or co-nutrient limitation. To calculate the potential limitation category, we used both a nutrient concentration threshold and a molar nutrient ratio in each ecoregion and survey year, since limitation is likely a function of both relative and absolute abundance (Guildford & Hecky, 2000). For nitrogen, we used the median between the 25th percentile dissolved inorganic nitrogen (DIN; nitrate plus ammonium) concentration of all the assessed lakes and the 75th percentile DIN concentration of reference lakes. For phosphorus, we used the median between the 25th percentile total phosphorus (TP) concentration of all the assessed lakes and the 75th percentile TP concentration of reference lakes. We used the median between these two methods because we have both a small set of reference lakes and a larger set of lakes to assess and this provided reasonable values for nutrient criteria (USEPA et al., 2000).

In addition to calculating the reference nutrient thresholds, we also used the mean of logged DIN:TP molar ratios in each ecoregion and year. Nutrient ratios have been extensively used to determine limitation (e.g., Downing & McCauley, 1992; Guildford & Hecky, 2000; Hellström, 1996; Ptacnik et al., 2010; Redfield, 1958; Rhee & Gotham, 1980) and DIN:TP may be more indicative of the bioavailable nutrient forms than TN:TP in lakes (Bergström, 2010). Once we had the concentration and ratio thresholds, we determined potential N-limitation occurred in lakes with TP values greater than the concentration threshold that also had log DIN:TP ratios below the average ratio. Potential P-limitation occurred in lakes with DIN values greater than the concentration threshold and log DIN:TP ratios above the average ratio. Lakes that did not meet any of these criteria were considered likely co-limited. There were 71 individual lakes in 2017 that did not include DIN data. There were no reference lakes in the Northern Plains in 2007. So, concentration thresholds were determined solely by the 25th percentile of all assessed lakes in that region in that year.

### Statistical analyses

All data analyses were performed in the R programming language (R Core Team, 2022) with heavy reliance on the tidyverse package suite for data wrangling and visualization (Wickham et al., 2019) and the sf package for creating maps (Pebesma, 2018). To assess whether N or P was a better predictor of trophic state, chlorophyll-a was used as a proxy for trophic state as the response variable. We used TN rather than DIN for these analyses because TN is more comparable to TP in their relationships with chlorophyll-a. Both the response and predictor variables were logged, and linear models were used. The r2 and AIC values were compared to determine which nutrient predicted chlorophyll-a better.

To assess shifts in limitation and trophic status, we used the change\_analysis function in the spsurvey package (Dumelle et al., 2022). Reference lakes observations from a second visits to sites were not included. These shifts were analyzed using the entire set of data representing lakes across the conterminous US (n = 1953) in addition to examining shifts in lakes that were sampled in both 2007 and 2017 (n = 464). The cat\_analysis function from the survey package was used to generate weighted percentages of lakes in each limitation category across the two survey years. Reference lakes and observations from second visits were not included (n = 1953).

# Results

### Nutrient stoichiometry

Between 2007 and 2017, DIN:TP stoichiometry showed a significant decrease across the full dataset (p <0.001). DIN:TP molar ratios were highest among P-limited lakes and lowest among N-limited lakes (p < 0.001). Grouped within limitation category, DIN:TP ratio decreases with increasing trophic state and ratios vary significantly between trophic states (all p < 0.01) with the following exceptions. In P-limited lakes, DIN:TP ratios do not differ between hypereutrophic and eutrophic lakes (p >0.05). In N-limited lakes, DIN:TP ratios do not differ between oligotrophic and mesotrophic lakes (p >0.05).

### Nutrient ability to predict trophic status

Both TN and TP were important in different ecoregions of the US, in fact, there was a clear divide in the data (Figure 1). In the western US, consisting of the Northern Plains, Southern Plains, Xeric, and Western Mountains ecoregions, the linear models using TN as a predictor of trophic state (chlorophyll-a as a proxy) were better based on the models’ higher r2 and lower AIC values. This was contrasted by the eastern US, consisting of Northern Appalachians, Southern Appalachians, Coastal Plains, Temperate Plains, and Upper Midwest ecoregions. In the eastern US, the linear models using TP as a predictor produced higher r2 and lower AIC values (Figure 2).

### Limitation and trophic state

There were 718 observations of P-limited lakes, 1034 N-limited lakes, and 649 co-nutrient limited lakes across the entire dataset based on the criteria used (Table 1, Figure 3). The 75th percentile nutrient concentrations from the total assessed lakes dataset were not statistically different from the 25th percentile nutrient concentrations from the reference lakes (p = 0.135 and p = 0.159, for TP and DIN, respectively). The proportion of lakes in each limitation status varies across ecoregions and survey years (Figure 4). Across the conterminous US, in 2007, 21% of lakes were co-nutrient limited, which increased to 32.3% in 2017. N-limited lakes decreased from 49.3% in 2007 to 45.9% in 2017. And in 2007, 29.7% of lakes were P-limited vs in 2017, 21.8% of lakes were P-limited.

In the dataset, there were 368 oligotrophic lakes (Oligo.), 737 mesotrophic lakes (Meso.), 753 eutrophic lakes (Eutro.), and 513 hypereutrophic lakes (Hyper.). Across the conterminous US, oligotrophic lakes decreased from 12.8% in 2007 to 9.2% in 2017. Mesotrophic lakes also decreased from 36.6% in 2007 to 22.7% in 2017. Eutrophic and hypereutrophic lakes both showed an increase between 2007-2017 of 30.3%-43.8% and 20.3%-24.3%. These patterns varied across ecoregions (Figure 5).

### Shifts in nutrient limitation

#### Lakes sampled in both 2007 and 2017

At the national scale among lakes sampled in both surveys (dotted lines in Figure 6a), there was a significant increase in co-nutrient limited lakes (17.3%) and a significant decrease in N-limited lakes (-18.4%). P-limited lakes showed an insignificant increase. There is significant variation from the national pattern when the data are segregated into the nine aggregated ecoregions (Figure 6b). In the Northern Appalachians, lakes shifted from co-nutrient and N-limitation (-13.4% and -20.8%, respectively) toward P-limitation (34.1%). Lakes in the Temperate Plains shifted from N-limited (-35.9%) toward both co-limited (14%) and P-limited (21.9%). The Upper Midwest lakes shifted from being N-limited (-41%) and P-limited (-24.3%) toward co-nutrient limitation (65.3%). In the Northern Plains, lakes became more co-nutrient limited (42%) and less P-limited (-28%), while showing an insignificant decrease in N-limited lakes. The Southern Plains showed the opposite pattern with a decrease in co-nutrient limitation (-16.8%), increase in N-limitation (11.1%), and non-significant increase in P-limited lakes. Western Mountains lakes shifted from P-limited (-19.5%) to co-nutrient limited (9.3%) with an insignificant increase in N-limited lakes. In the Southern Appalachians, Coastal Plains, and Xeric ecoregions, no shifts were significant.

#### Full population of lakes

When extrapolated to the entire population of lakes across the US (solid lines in Figure 6a), co-nutrient limited lakes increase significantly (11.3%), P-limited lakes decrease significantly (-7.9%), and N-limited lakes show an insignificant decrease; indicating a difference in N vs P-limited lake shifts when assessing the full population rather than the resampled population only. The trends of the resampled lakes were generally representative of the full population in the ecoregions, though magnitudes differed and there were a few stark differences (solid lines in Figure 6b). There was a change sign of the significant shift in co-nutrient limited lakes in the Northern Appalachians so that among the full population assessment, there was a 16% increase. N-limited lakes in the Southern Appalachians and Western Mountains changed signs in the full population assessment compared to the resampled lakes assessment, but the shifts remained insignificant. Shifts in P-limited lakes in the Northern Appalachians, Temperate Plains, Northern Plains, and Western Mountains were insignificant in the full population assessment, compared to the resampled population where the shifts were positive, positive, negative, and negative, respectively. Finally, in the Xeric ecoregion, N-limited lakes increase significantly (16.5%) in the full population assessment.

### Shifts in trophic status at the national scale

#### Lakes sampled in both 2007 and 2017

At the national scale, resampled lakes became less hypereutrophic (-8.3%) and more mesotrophic (9%), while showing no significant change in oligotrophic and eutrophic lakes. Co-nutrient limited lakes followed the overall national pattern, with these lakes becoming less hypereutrophic (-10.4%) and more mesotrophic (14%) and there was not significant change in oligotrophic or eutrophic co-limited lakes. N-limited lakes only had a significant increase in shifting toward hypereutrophy (13.7%). P-limited lakes showed now significant change in mesotrophic state, but decreased in oligotrophic (-12.9%) and hypereutrophic (-23.9%) states and increased in eutrophic state (35%) (Figure 7).

#### Full population of lakes

The resampled lakes results differed significantly from the full population estimate. When considering all the lakes in the NLA survey years 2007 and 2017, lakes became less oligotrophic (-3.7%) and mesotrophic (-13.9%) and more eutrophic (13.5%) and hypereutrophic (4%). Co-nutrient limited lakes shifted from mesotrophic (-13.2%) and hypereutrophic (-4%) toward eutrophic (21%) status with no significant change in oligotrophic status. N-limited lakes followed the national pattern, becoming less oligotrophic (-3.8%) and mesotrophic (-19.6%) and more eutrophic (7.9%) and hypereutrophic (15.6%). P-limited lakes showed a significant decrease in oligotrophic lakes (-5.8%) and a significant increase in eutrophic lakes (14.6%) with no significant changes in mesotrophic or hypereutrophic status lakes (Figure 7).

# Discussion

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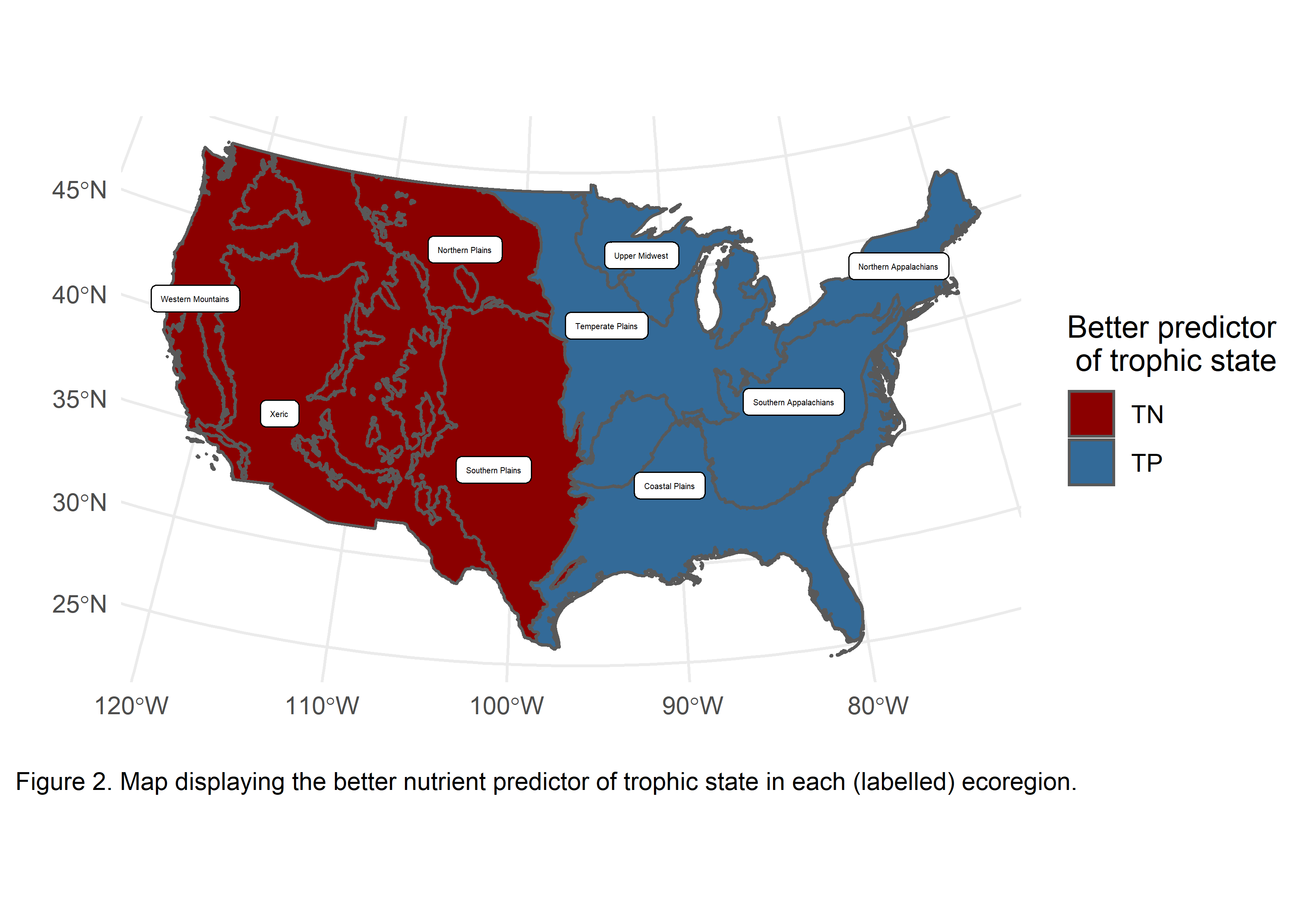
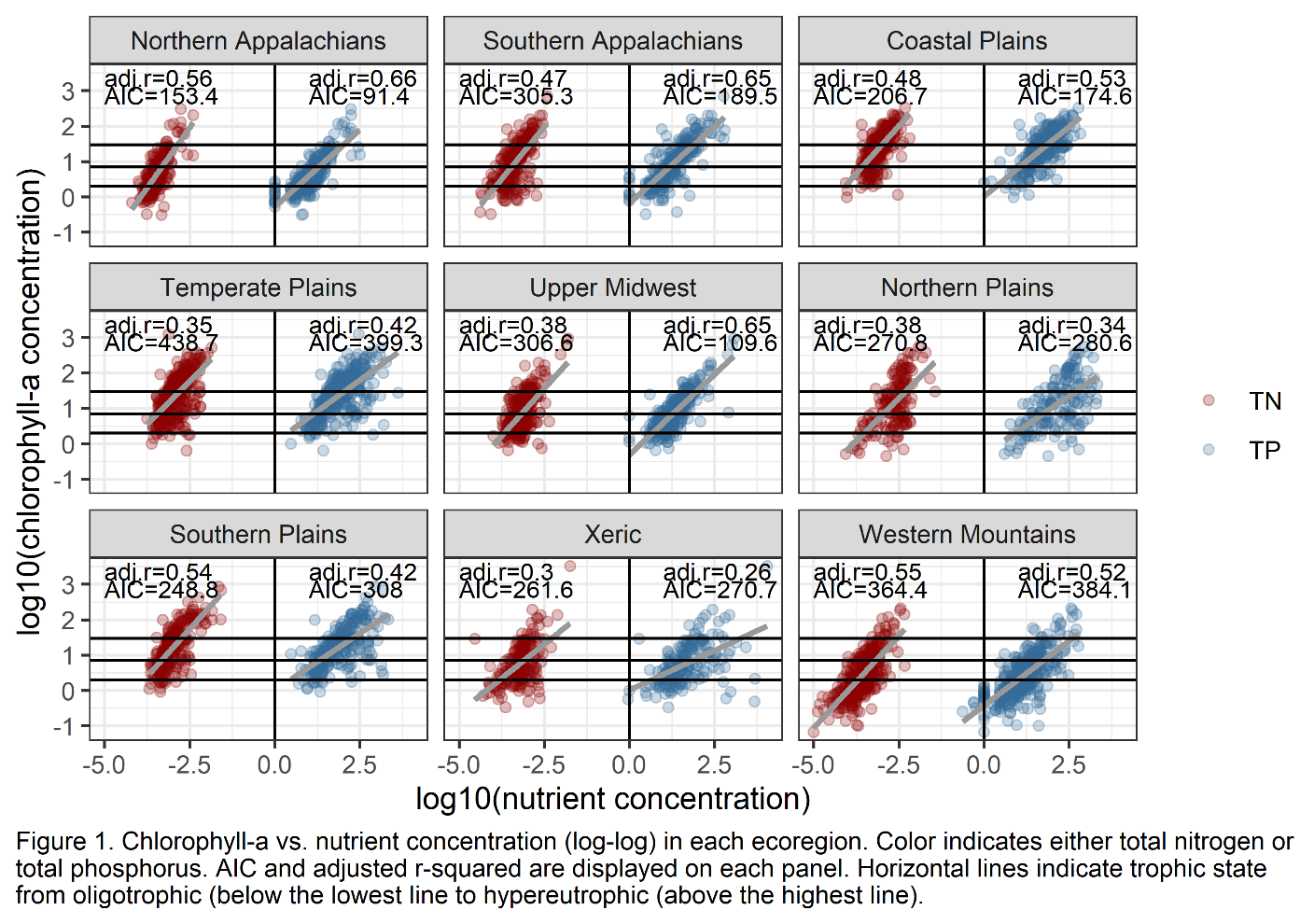
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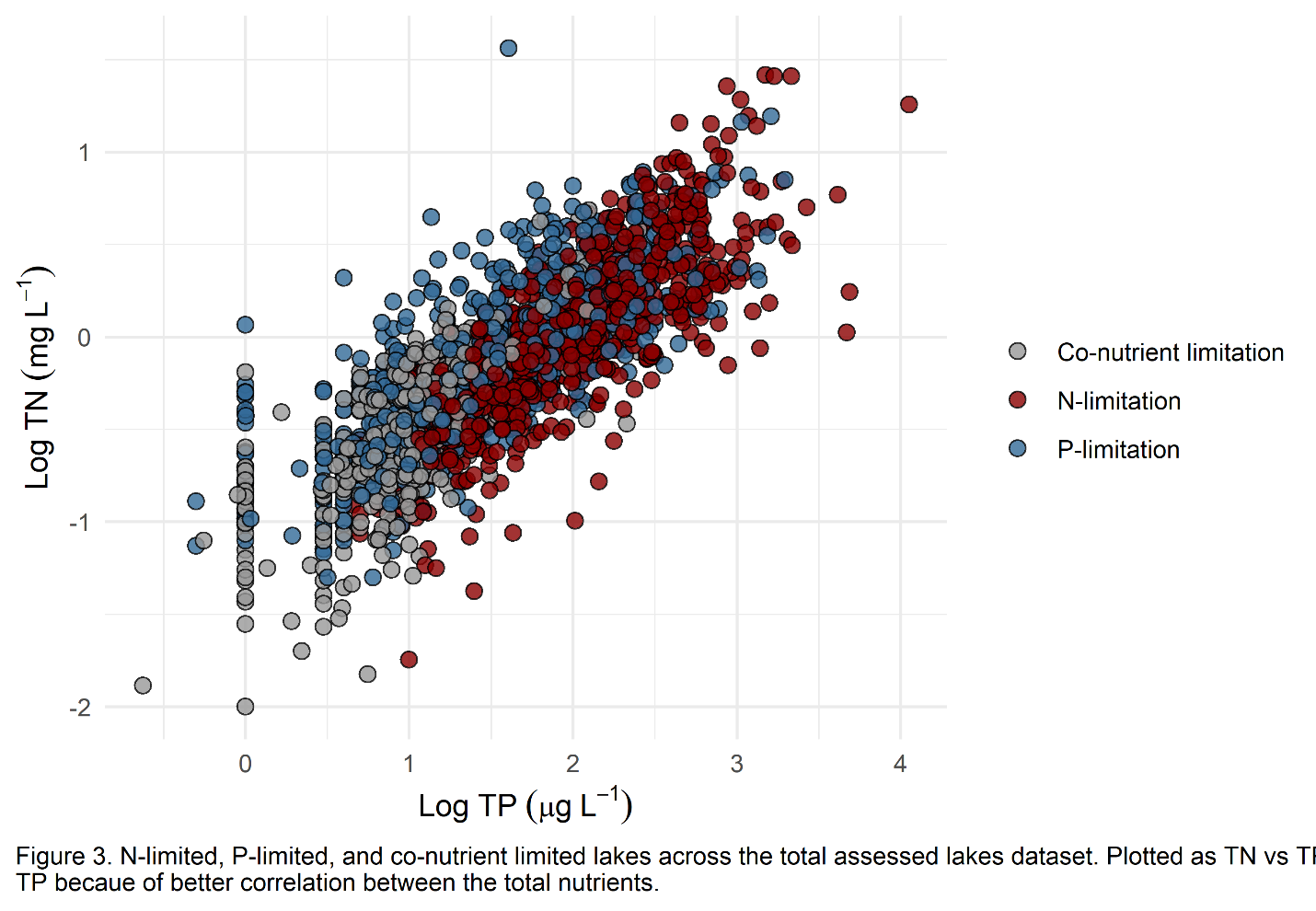
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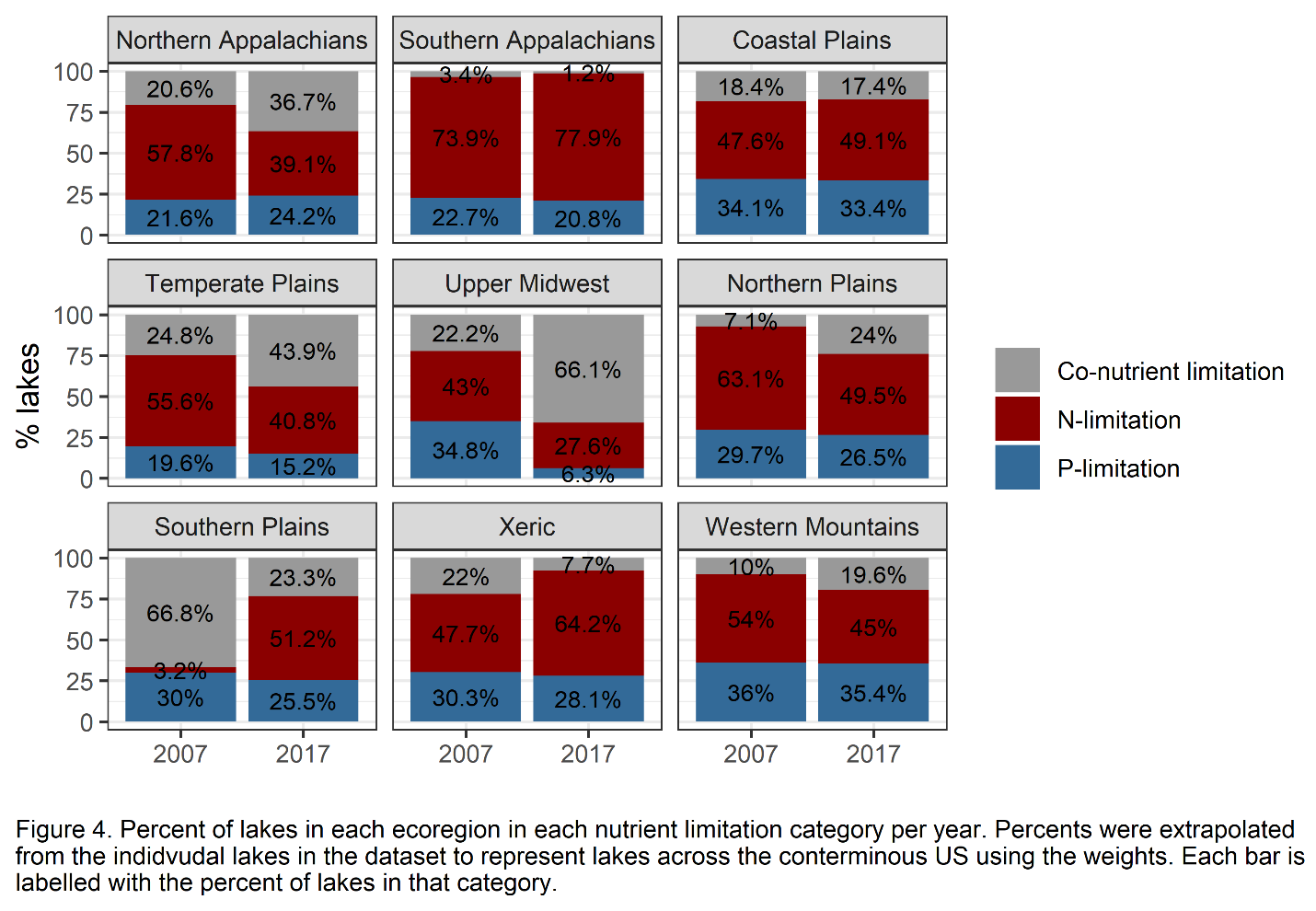
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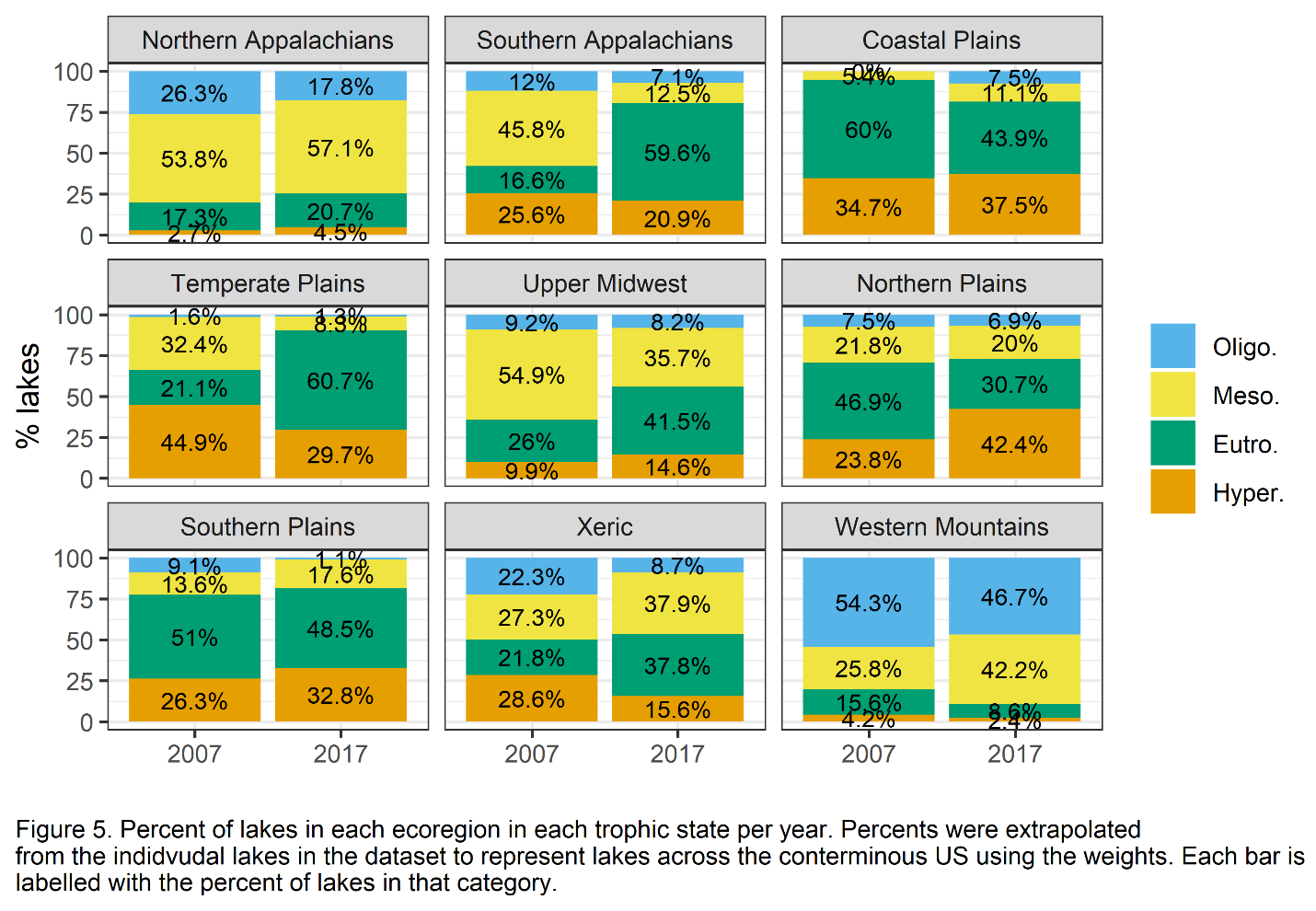
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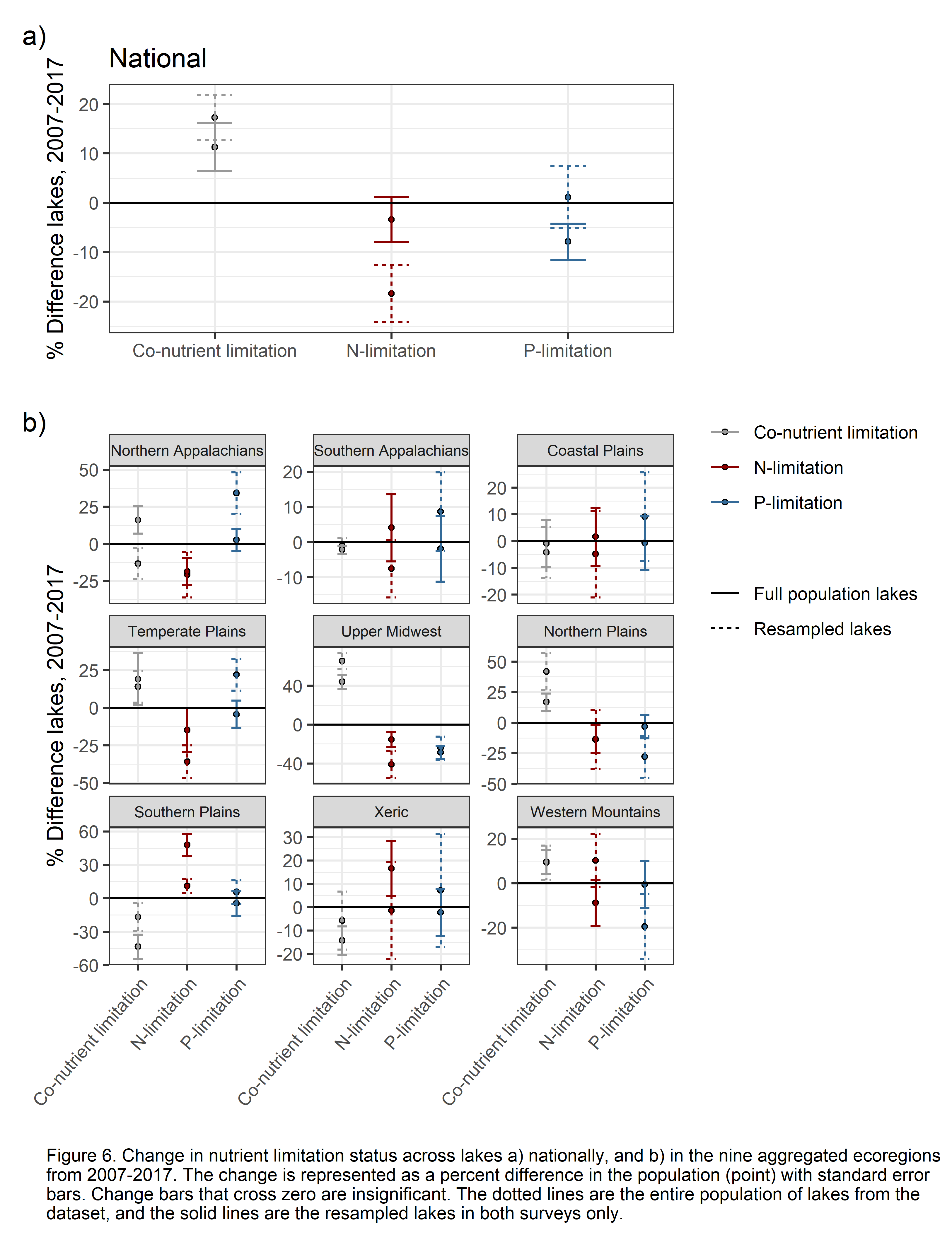
# Figures and Tables











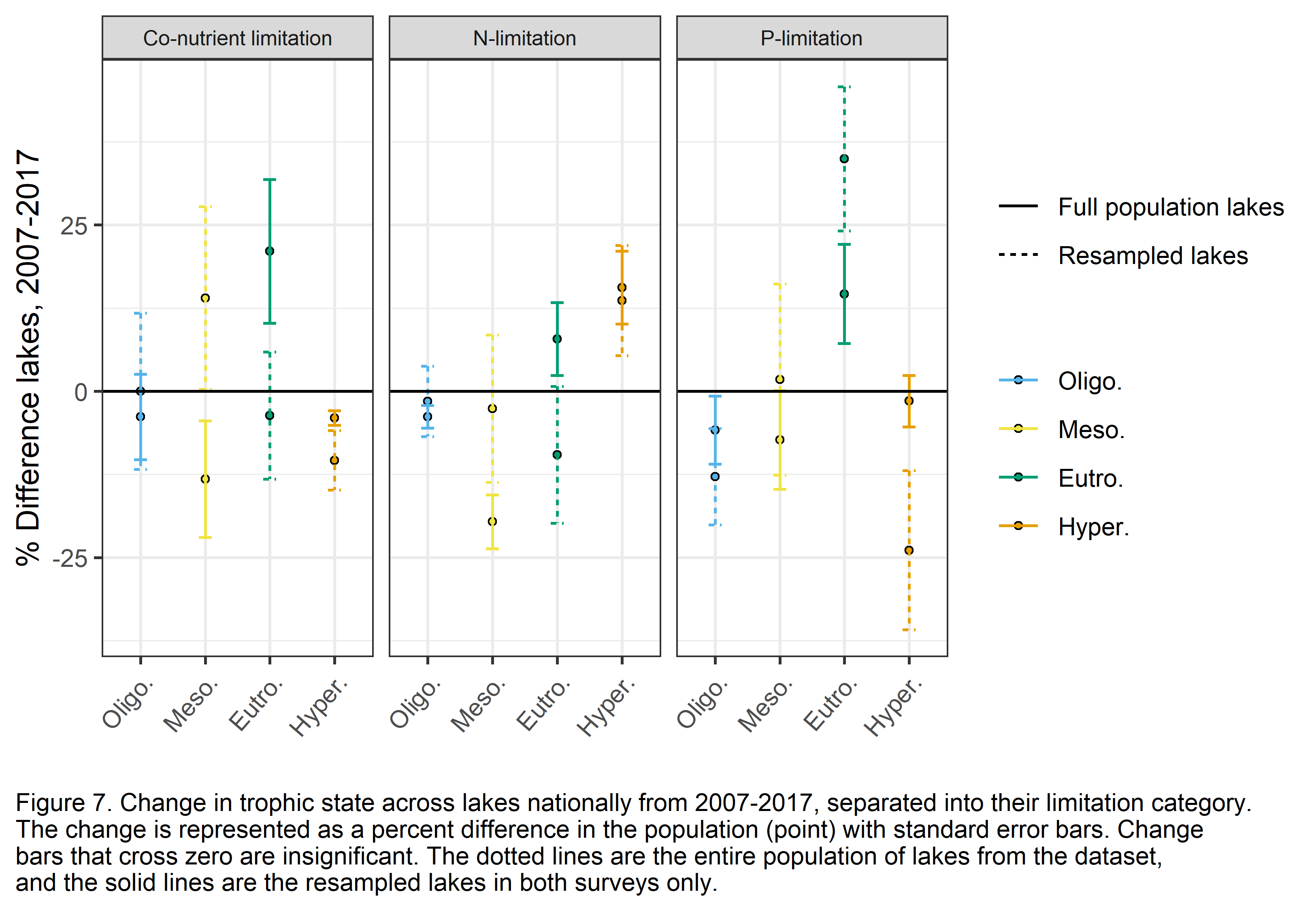


Table 1. Nutrient molar ratios and concentration thresholds used in limitation calculations.

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|  | **Average DIN:TP molar ratio** | | **Average TN:TP molar ratio** | | **25th Percentile DIN mg L-1 from all lakes** | | **75th Percentile DIN mg L-1 from reference lakes** | | **DIN mg L-1 concentration threshold** | | **25th Percentile TP µg L-1 from all lakes** | | **75th Percentile TP µg L-1 from reference lakes** | | **TP µg L-1 concentration threshold** | |
|  | **2007** | **2017** | **2007** | **2017** | **2007** | **2017** | **2007** | **2017** | **2007** | **2017** | **2007** | **2017** | **2007** | **2017** | **2007** | **2017** |
| **Northern Appalachians** | 21.54 | 3.99 | 108.49 | 62.62 | 0.01 | 0.01 | 0.02 | 0.01 | 0.02 | 0.01 | 4.75 | 8.16 | 11.00 | 13.66 | 7.88 | 10.91 |
| **Southern Appalachians** | 38.07 | 14.14 | 96.36 | 61.84 | 0.01 | 0.01 | 0.02 | 0.01 | 0.02 | 0.01 | 7.38 | 9.94 | 10.00 | 16.37 | 8.69 | 13.15 |
| **Coastal Plains** | 4.42 | 5.90 | 43.78 | 46.17 | 0.02 | 0.01 | 0.02 | 0.01 | 0.02 | 0.01 | 28.25 | 24.61 | 36.00 | 24.73 | 32.13 | 24.67 |
| **Temperate Plains** | 6.34 | 6.67 | 46.14 | 46.36 | 0.02 | 0.02 | 0.14 | 0.03 | 0.08 | 0.02 | 33.75 | 35.15 | 80.50 | 49.75 | 57.13 | 42.45 |
| **Upper Midwest** | 17.81 | 13.83 | 132.50 | 88.94 | 0.02 | 0.01 | 0.04 | 0.64 | 0.03 | 0.32 | 8.00 | 13.37 | 9.50 | 33.35 | 8.75 | 23.36 |
| **Northern Plains** | 4.43 | 23.89 | 48.86 | 67.02 | 0.04 | 0.02 | NA | 0.04 | 0.04 | 0.03 | 61.00 | 48.22 | NA | 275.62 | 61.00 | 161.92 |
| **Southern Plains** | 3.61 | 1.99 | 41.37 | 37.89 | 0.02 | 0.01 | 0.06 | 0.06 | 0.04 | 0.04 | 24.00 | 25.89 | 533.75 | 55.12 | 278.88 | 40.50 |
| **Xeric** | 29.05 | 6.45 | 78.38 | 43.61 | 0.02 | 0.01 | 0.02 | 0.02 | 0.02 | 0.01 | 15.00 | 19.10 | 48.25 | 21.51 | 31.63 | 20.30 |
| **Western Mountains** | 15.16 | 4.38 | 71.70 | 44.22 | 0.01 | 0.01 | 0.01 | 0.02 | 0.01 | 0.01 | 4.00 | 9.60 | 4.00 | 14.07 | 4.00 | 11.83 |