NITROGEN AS A LIMITING NUTRIENT IN DANISH LAKES

Scientific Report from DCE – Danish Centre for Environment and Energy No. 703 2026

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Data sheet

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Abstract: This report explores the role of nitrogen for nutrient limitation in Danish lakes by quantifying and discussing potential nutrient thresholds, which split lakes in different ecological states, and probabilities for shifting ecological state by either reducing nitrogen, phosphorus, or both. Co-reduction of both nutrients seems favourable to achieve ecological state improvements.

Keywords: Lakes, nitrogen, phosphorus, nutrient limitation

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Preface

This report explores the role of nitrogen for nutrient limitation in Danish lakes by using summer-averaged water quality data and catchment characteristics. The conclusions drawn can inform future water management plans aiming at managing lakes that are either limited by both nitrogen and phosphorus, only by phosphorus and/or only by nitrogen. This report has been prepared at the request by the Danish Agency for Green Transition and Aquatic Environment (SGAV), which also had the opportunity to comment on a draft of the report. Further, results of the projects have been presented to SGAV.

As part of the project deliverables, SGAV has received the background data and calculations on which the report is based for each individual lake, as well as a visualization of these.

The report is an update of a previous version published by Søndergaard & Johansson (2024).

Sammenfatning

I nærværende projekt blev det undersøgt, hvilke danske søer der potentielt er begrænset af kvælstof (N), fosfor (P) eller begge næringsstoffer (såkaldt ”co-begrænsede”), ved at anvende gennemsnitlige sommerdata for vandkvalitet til at vurdere og diskutere, i hvilket omfang forvaltningstiltag kan forbedre deres økologiske tilstand. Udgangspunktet er den nuværende praksis, hvor søforvaltning hovedsageligt fokuserer på reduktion af P. Der findes dog tilfælde, hvor en yderligere reduktion af P ikke er realistisk, og hvor et alternativt fokus – reduktion af N – kan være en mulighed. Samtidig peger den videnskabelige konsensus på, at en reduktion af både N og P er den forvaltningsmæssige mest hensigtsmæssige tilgang til forbedring af søers tilstand.

Rapporten bygger på et omfattende datasæt bestående af langtidsovervågningsdata fra ODA, oplandskarakteristika (via CAMELS-DK-datasættet og arealanvendelsesdata) samt tilgængelig hydrologisk GIS-information til beregning af forbindelsen mellem søer. For at kvantificere næringsstofbegrænsning anvendtes fem klassifikationsmetoder og deres bias blev analyseret. Konklusionen var, at en klassifikation baseret på en støkiometrisk balanceret metode fungerer tilfredsstillende for danske søer, da den (a) kan indarbejde de danske søtypeklassifikationer, (b) kan anvende det fulde datasæt af total-N- og total-P, (c) ikke indeholder arbitrære tærskler og (d) giver en konservativ klassifikation, hvor de fleste søer er co-begrænsede efterfulgt af P-begrænsede – hvilket stemmer overens med nyere studier fra USA. Ved brug af den støkiometrisk balancerede metode sås, at P-begrænsede søer primært befinder sig i moderat til høj økologisk tilstand, mens N-begrænsede søer typisk er i moderat til dårlig tilstand. Desuden påvistes statistisk signifikante forskelle i vandkvalitet mellem søer klassificeret efter næringsstofbegrænsning og økologisk tilstand, hvilket betyder, at begge kriterier kan anvendes meningsfuldt i forvaltningen af søer. Den tidligere anvendte “vekselkurs” blev opdateret til et ”begrænsningsforhold” mellem N og P, men konklusionen var, at det i denne undersøgelse ikke gav yderligere forvaltningsmæssig indsigt.

Ved brug af maskinlæringsmetoder blev hele datasættet analyseret i forhold til næringsstofbegrænsning og økologisk tilstand. Brugen af metoden ”Conditional Inference Trees (CITs)” viste, at typen af næringsstofbegrænsning primært kan adskilles ved sigtdybden. Her var P-begrænsede søer typisk karakteriseret ved større sigtdybde, mens N-begrænsede søer generelt havde lavere sigtdybde og en højere andel af menneskelig arealanvendelse i oplandet. For den økologisk tilstand viste analyserne, at søer med dårligere tilstand (moderat til dårlig) havde højere TN-niveau end søer i god eller høj tilstand. Et TN-niveau omkring 1 mg L^{-1} syntes velegnet til at skelne mellem søer med dårligere og bedre økologisk tilstand. Søer i høj økologisk tilstand var desuden karakteriseret ved lave TP-koncentrationer. For dybe søer var sommer-TP dog den vigtigste parameter, hvilket viser, at fosforbegrænsning bliver mere afgørende med stigende dybde. Ordinale regressionsmodeller blev anvendt til teoretisk at belyse, hvilke reduktioner i TN og TP der øger sandsynligheden for, at en sø opnår god eller høj økologisk tilstand. Disse modeller pegede på de samme tærskler som CITs-analyserne: at søer har høj sandsynlighed for god/høj tilstand ved TN under 1-1,5 mg L^{-1} og samtidig TP under 0,1 mg L^{-1}. Ved hjælp af dobbelte maskinlæringsmetoder til påvisning af årsagssammenhænge fandt vi, at P-begrænsede søer har ca. 24 % større sandsynlighed for at være i god eller høj tilstand end N-begrænsede søer. Desuden viste analyserne, at co-begrænsede søer har størst signifikant sandsynlighed for at opnå de mest markante forbedringer i økologisk tilstand ved en reduktion af både TN og TP.

På baggrund af analyserne i denne rapport anbefales en tostrenget næringsstofindsats for danske søer – altså reduktion af både TP og TN. Resultaterne viste, at langt de fleste danske søer (77 %) omfattet af denne rapport er begrænset af både N og P, og at sandsynligheden for at forbedre deres økologiske tilstand er stor, hvis begge næringsstoffer begrænses. Generelt havde søer i dårligere økologisk tilstand højere TN-niveauer end søer i bedre tilstand, og søer i høj økologisk tilstand havde lave TP-niveauer. Forvaltningstiltag i særligt co-begrænsede søer bør derfor fokusere på både kvælstof- og fosforreduktion, hvor der dog tages søspecifikke hensyn i forhold til begrænsningstype, hydrologi og nuværende økologiske tilstand. Dette indebærer typisk reduktion af de eksterne tilførsler af N og P. Samme strategi gælder også for N- og P-begrænsede søer, selvom co-begrænsede søer ser ud til at reagere mest markant på yderligere næringsstofreduktion.

Summary

This project explored which lakes in Denmark are potentially nitrogen- (N), phosphorus- (P) or co-limited (i.e., by both nutrients) using averaged summer water quality data to infer and theorise to what extent management actions can improve their respective ecological states. The motivation stems from the current practice of focusing mostly on P reduction in lake management. However, there are cases where further P reduction is not feasible, and an alternative management angle, reduction of N, may be an option. Further, the scientific consensus is that dual nutrient reduction, targeting both N and P, is the preferable strategy for lake management.

An extensive dataset was curated consisting of long-term ODA monitoring data, catchment characteristics (through the CAMELS-DK dataset and land-use data) and available hydrological GIS information to derive connectivity metrics. To quantify nutrient limitation, five classification methods were applied, and their respective biases were analysed. The conclusion was that a nutrient limitation classification based on the stoichiometric balance method is a satisfactory choice for Danish lakes, as it (a) can incorporate the Danish lake type classifications, (b) can use complete total nitrogen and total phosphorus data, (c) has no arbitrary thresholds and (d) provides a conservative classification with most lakes being co-limited followed by P-limited, which agrees with recent studies from the US. Using the stoichiometric balance method, it was found that P-limited lakes are mostly in moderate to high ecological states, whereas most N-limited lakes are in moderate to bad states. We further highlight that lakes show statistically significant differences in water quality states when classified using different nutrient limitation methods depending on their ecological state. Therefore, both criteria, nutrient limitation and ecological state, are valid for use in lake management. The exchange ratio/"vekselkurs" from previous reports was updated to a limitation ratio, although we conclude that, for the current study, it did not provide further insights for management purposes.

Using machine learning, the full dataset in relation to nutrient limitation and ecological state was explored. Conditional inference trees, a machine learning method, highlighted that nutrient limitation types are primarily separated by Secchi depth. Here, P-limited lakes were mostly characterised by higher Secchi depths. N-limited lakes tended to have lower Secchi depths and a higher human land-use percentage in the catchment. Regarding ecological states, the inference trees underscored that lakes in poorer ecological conditions (moderate to bad) had higher TN (total nitrogen) than lakes in good to high states. Here, an approximate TN threshold of 1 mg L^{-1} seemed suitable for distinguishing lakes in poorer ecological conditions from lakes in better ecological conditions. Lakes in high ecological states were mostly characterised by low TP (total phosphorus) concentrations. However, for deep lakes, summer TP values were found to be the most important parameter, indicating that phosphorus control becomes increasingly important with depth. Ordinal regression models were used to theoretically elucidate the thresholds of TN and TP reduction at which lakes achieve a higher probability of being in good or high ecological condition. Here, the method quantified similar thresholds as those identified by the inference trees, with lakes converging toward a high probability for achieving good/high ecological status when TN falls below 1.5 to 1 mg L^{-1}, and TP is simultaneously below 0.1 mg L^{-1} (but ideally with TP

concentrations below 0.05-0.02 mg L^{-1}). Furthermore, we used double machine learning for causal inference. This analysis showed that P-limited lakes have a probability of about 24% of being in good or high ecological states compared to N-limited lakes. Further, the causal effects of reducing TN and TP on improving ecological status were examined. Double machine learning highlighted that co-limited lakes had the most statistically significant improvements in their probability of achieving a higher ecological state following reductions in both TN and TP.

Based on this study, a dual focus on managing nutrients for Danish lakes, targeting both TP and TN, is recommended. The results highlighted that most Danish lakes (77%), which were included in this study, are co-limited and have a significant probability of improving their ecological state by reducing both nutrients. Generally, lakes with poorer ecological state had higher TN concentrations than lakes in better ecological state. Lakes in high ecological states exhibited low TP concentrations. Management measures in especially co-limited lakes should focus on both nitrogen and phosphorus reduction, with lake-specific consideration depending on their limitation, hydrological regime or current ecological state. This implies reducing external loads of both TN and TP into the lake. The same management strategies are also valid for N- and P-limited lakes, although co-limited lakes seem to be more responsive to further nutrient management.

1 Background and objectives

1.1 Background

Current lake management strategy

Currently, the primary effort to improve the condition of Danish lakes is aimed at reducing the input of phosphorus (P), as phytoplankton – the primary producers in lake ecosystems – are primarily considered to be phosphorus-limited. Consequently, P availability is a crucial factor for many chemical and biological processes in lakes (Søndergaard, et al., 2007). However, recent research indicates that P is not the sole limiting factor in all lakes, and that nitrogen (N) also plays a significant role (Søndergaard & Johansson, 2024; Christensen, Søndergaard, Johansson, & Lauridsen, 2023). Therefore, current efforts aimed solely at reducing P inputs may not be the only or the optimal way to achieve good ecological status.

1.2 Objectives

The purpose of this project and report is to further elucidate the role of N in driving lake ecological conditions in Danish lakes, with the aim of determining to what extent N criteria can be established for targeted lakes to help achieve good ecological status. The project seeks to provide the best estimate of which lakes are likely to achieve target fulfilment by either phosphorus reduction, nitrogen reduction or a combination of both.

The project aims at proposing and discussing limiting values for N, exchange rates between phosphorus and nitrogen and other criteria that determine whether individual lakes can achieve target fulfilment. Furthermore, the project will clarify the methods and analyses required to identity the necessary nutrient limitation classification and criteria, as well as the efforts needed to achieve good ecological status. This leads to our basic research challenge: **What if P-only lake management is not the only optimal way to achieve good ecological state?** By elucidating the role of N in shaping lake ecological conditions and by discussing which N criteria/thresholds and efforts may be required to achieve good ecological state, this report aims to answer the following research questions:

1. Which method is optimal for quantifying nutrient limitation?
2. Do lakes, classified according to nutrient limitation and ecological state, exhibit distinct water quality characteristics?
3. Which criteria distinguish N-limited lakes from, for instance, P-limited lakes?
4. Are N-limited lakes suitable candidates for targeted nutrient reduction?

2 Methodology

2.1 Data

Collection of water chemistry data is included in the Danish national monitoring program for lakes, NOVANA. The monitoring data are stored in the database VanDa (vanda.miljoeportal.dk) and subsequently extracted from Overfladevandsdatabasen ODA (ODA.dk). Long-term water quality NOVANA monitoring data were accessed from the VanDA/ODAforAlle database. Summer averages for water quality conditions – chemical data (e.g. nitrate, total nitrogen, temperature, Chl-*a*, colour, alkalinity, etc.) and supplemental data (e.g. Secchi depth) – were calculated accounting for uneven and/or biased sampling intervals through linear interpolation. Dissolved inorganic nitrogen (DIN) was defined as the sum of nitrate ($N\text{-}NO_3$) and ammonium ($N\text{-}NH_4$). SRP was defined as equal to measured phosphate ($P\text{-}PO_4$) concentrations. Ecological states were defined based on measured summer average Chl-*a* concentrations in µg L^{-1} depending on lake type, as shown in Table 2.1 (Søndergaard, Johansson, E. Levi, Lauridsen, & Jeppesen, 2020; Søndergaard, Larsen, Johansson, Lauridsen, & Jeppesen, 2016):

Table 2.1. Ecological state based on Chl-*a* concentration (µg L-1) and associated lake type.

Lake types	High	Good	Moderate	Poor	Bad
1-8, 10, 12, 14	<7	<12	<27	<56	>56
9, 11, 13, 15	<11.7	<25	<56	<90	>90

To understand the effects of catchment-related processes on nutrient limitation in Danish lakes, we incorporated catchment-scale variables alongside in-lake physico-chemical variables. For integrating catchment characteristics, we used the CAMELS-DK dataset (Liu, et al., 2025). The CAMELS (Catchment Attributes and Meteorological Time Series for Large Samples) datasets are the outcome of a common framework for advancing hydrological and catchment analyses and have been developed for different regions in the world (Addor, Newman, Mizukami, & Clark, 2017; Coxon, et al., 2020; Höge, et al., 2023). CAMELS-DK was developed by researchers from GEUS and Aarhus University based on data from DMI, GEUS, VanDa, BASEMAP and open-source datasets such as CORINE landcover. The dataset included 304 gauged basins in Denmark along with 3026 ungauged catchments, covering a total of 3300 ID-15 Danish catchments.

Dynamic catchment attributes include climate variables from gridded daily datasets provided by the Danish Meteorological Institute (DMI), streamflow observations from the Danish surface water database (VanDa) and simulated data from the DK-model, such as streamflow and groundwater heads. The dataset also includes soil, geology, land-use, and topography information for each ID-15 catchment. In this project, we included only climate, hydrology and soil attributes in our analysis. A list of selected variables and their descriptions is given in Table 2.2.

Table 2.2. Overview of catchment and streamflow variables derived from the CAMELS-DK dataset, including precipitation, soil and hydrological indices used in the study. Further variables deemed important by inference trees from the other datasets are also included in the table (see section 3.4 (Conditional reference trees)).

Variable	Description
high_prec_freq	Frequency of high precipitation days (≥5 times mean daily precipitation) $d \cdot yr^{-1}$.
high_prec_dur	Average duration of high precipitation events (number of consecutive days ≥5 times means daily precipitation).
p_seasonality	Seasonality and timing of precipitation, where positive values indicate summer peaks, negative values indicate winter peaks, and values near zero reflect uniform precipitation throughout the year.
root_depth	Depth available for roots (m). Deep rainfall infiltration and well-drained conditions favour deeper root systems, enhanced percolation and reduced surface runoff, whereas poorly drained soils constrain roots to shallow depths, increasing soil saturation and runoff generation (Fan, Miguez-Macho, Jobbágy, Jackson, & Otero-Casal, 2017).
pct_organic	Organic carbon content of the soil (%).
pct_gravel	Coarse fragments in the soil (%).
tawc	Total available water content (mm) of the soil in the catchment.
high_Q_duration	Average duration of high-flow events (number of consecutive days > 9 times the median daily flow (d)).
low_Q_duration	Average duration of low-flow events (number of consecutive days < 0.2 times the mean daily flow (d)).
Q_var	Variance of streamflow ($mm^2 \cdot d^{-2}$).
BFI	Baseflow index (ratio of mean daily baseflow to mean daily streamflow).
QP_elasticity	Streamflow precipitation elasticity (sensitivity of streamflow to changes in precipitation at annual timescale using mean daily streamflow as reference).
t-mean	Annual air temperature (ºC).
Secchi	Secchi depth (m) derived from long-term water quality monitoring.
human_landuse	Combined land-use including agricultural areas, settlements, industry, roads, recreational urban areas.
summer_TN	Annual average summer TN concentration (mg L^{-1}).
summer_TP	Annual average summer TP concentration (mg L^{-1}).
Wtemp	Annual average summer water temperature (ºC).
Col	Water colour measured at Platinum-Cobalt colour scale.
Dybde_max	Maximum lake depth (m).
lake_area_m2	Lake area (m^2).

We used a 1000 m buffered region of each lake and intersected buffered lake polygons with the CAMELS-DK catchment datasets. For each overlapping polygon, the area of overlap was calculated, and weighted averages were used if the lake polygon intersected with more than one catchment. The resulting weighted means were used as catchment characteristics for each lake. To avoid multicollinearity among predictors, highly correlated variables were excluded from the analysis.

For GIS analysis of land-use and connectivity between Danish streams and lakes, we used stream data (Vandløbsmidte) from GeoDanmark/Klimadatastyrelsen and lake data from Miljøstyrelsen's MiljøGIS associated with the River Basin Management plans (vandområdeplanerne). Based on these GIS objects for streams and lakes, potential hydraulic pathways were identified. The distance to a lake's nearest neighbour was calculated as the minimum Euclidean distance (assuming connectivity via a stream and within a maximum distance of 10 km). The number of lake neighbours was categorised as 0, 1, 2, 3 or more than 3 within the threshold of 10 km.

In order to assess the effects of catchment/shoreline, we used the shoreline classification in Berthelsen et al. (2026). The shoreline is divided into two zones.

The shoreline zone: *The periodically wet part of the shore, characterised by fluctuating water levels and forming the transition between the lake and the surrounding land. In this study, the shoreline zone is defined as a 15-metre-wide strip on the land side adjacent to the littoral zone.*

The surrounding areas: *The terrestrial or semi-terrestrial area around the lake, defined here as a 100-metre-wide strip on the land side.*

These two classifications were chosen to facilitate the use of Miljøstyrelsen's existing 10x10m land-use maps when quantifying the surrounding areas for all lakes included in the River Basin Management plan. It would be ideal to also include the littoral zone – the submerged part of the shore – but no suitable data currently exist to support this. For the analysis, land-use types were divided into the following categories: undefined, buildings, industry, infrastructure (railway and airport), recreation (parks and paths), coast, agriculture (all types), raw material extraction, bare soil, forest, streams, dry natural types, wet natural types, sea and roads. Wet natural areas were the most common land-use type within 15 metres of Danish lakes. Land-use types of human origin accounted for only about one-quarter of the land-use types within this 15-metre zone. Wet natural areas still contribute the most to land-use types around lakes within the larger 100-metre radius, but here the proportion of human-origin land-use (especially agriculture) was considerably higher.

2.2 Classifying nutrient limitation

Multiple methodologies exist for quantifying nutrient limitation types for lake ecosystems, each differing in underlying assumptions, data needs and ease of computation. What they share is their focus on which nutrient limits algal growth (Redfield, 1958). To highlight the importance of selecting a nutrient limitation methodology and acknowledging its specific assumptions and limitations, we used five specific approaches. For a general method inter-comparison, see Table 2.3.

Table 2.3. Comparison of nutrient classification methods based on their variables, assumptions, strengths and weaknesses.

Method	Variables	Assumption	Strength	Weakness
Nutrient ratios (dissolved and total)	SRP, DIN, TP, TN	Fixed ratios can identify limitations based on Redfield's (1958) molar ratio.	Straightforward calculation. Most data available for total nutrient ratios.	Assumption weakens for varying algal groups and at saturating conditions.
Chl-*a* to nutrient ratio	TP, DIN, Chl-*a*	A high ratio indicates that the nutrient is in demand (limited).	Straightforward calculation.	No objective thresholds, low ratio may have multiple ecological causes, less data available for DIN, no co-limitation.
Supportive capacity	SRP, DIN	Theoretical approach based on the amount of energy needed to produce biomass (Liebig's Law of Minimum).	Straightforward calculation.	Empirical equations derived by Reynolds & Maberly (2002), limited data on SRP and DIN, no co-limitation.
Stoichiometric balance	TP, TN, Chl-*a*	Nutrient-deficient lakes have greater Chl-*a* yield per nutrient; all TP and TN are assumed to be bioavailable.	Most data available, calculations are based on quantile regressions without empirical thresholds. Danish lake types can be incorporated in the regression	Strong assumption that TP and TN are bioavailable.

Specific descriptions of each methodology follow in the below sub-sections.

Nutrient ratios

Based on Redfield's insight that the molar ratio of marine algae composition of C:N:P is approximately 106:16:1 (Redfield, 1958), molar ratios of N to P are often considered a key metric for assessing nutrient limitation (Maberly, Pitt, Davies, & Carvalho, 2020). Summer average concentrations of DIN and SRP were used for the dissolved nutrient ratios. Molar concentrations of DIN and TN as well as SRP and TP were first derived using the molar masses of N and P, respectively. We relied on literature thresholds (Maberly, Pitt, Davies, & Carvalho, 2020) for dissolved N:P ratios:

$$\frac{DIN_{molar}}{SRP_{molar}} \begin{cases} < 10 & \rightarrow N\ limitation \\ 10 \leq \frac{DIN_{molar}}{SRP_{molar}} \leq 20 & \rightarrow Co\ limitation \\ > 20 & \rightarrow P\ limitation \end{cases} \quad (1)$$

Thresholds for total ratios were defined based on the findings for Danish brackish lakes (Christensen, Søndergaard, Johansson, & Lauridsen, 2023):

$$\frac{TN_{molar}}{TP_{molar}} \begin{cases} < 8 & \rightarrow N\ limitation \\ 8 \leq \frac{TN_{molar}}{TP_{molar}} \leq 22 & \rightarrow Co\ limitation \\ > 22 & \rightarrow P\ limitation \end{cases} \quad (2)$$

The thresholds are very close to ones used for the continental US (McCullough, Sun, Hanly, & Soranno, 2024).

Chlorophyll-*a* ratios

Following Maberly et al. (2020), summer averages of Chl-*a*, TP and DIN were used to calculate chlorophyll *a* to nutrient ratios. Arbitrary thresholds for limitations were defined for $Chl-a/_{DIN} > 0.042$ mg Chl-*a* mg^{-1} DIN^{-1} as N-limited and $Chl-a/_{TP} > 0.3$ mg Chl-*a* mg^{-1} TP^{-1} as P-limited.

Supportive capacity

For each potentially limiting variable, the supportive capacity – defined as the theoretical amount of algal biomass that can be produced by the variable – was calculated following Reynolds & Maberly (2002). The variable producing the smallest yield or gain in algal biomass (expressed as Chl-*a*) is considered the most likely to be limiting. The theoretical stoichiometric yield (Reynolds & Maberly, 2002) of phytoplankton biomass per nitrogen (expressed as DIN) was assumed to be:

$$\Upsilon_{DIN} = 0.11\ DIN \quad (3)$$

For the Chl-*a*: phosphorus yield (expressed as SRP), the simplification according to Maberly et al. (2020) was used:

$$\Upsilon_{SRP} = 6.32\ SRP^{0.585} \quad (4)$$

with the underlying assumption that all SRP is bioavailable phosphorus.

Stoichiometric balance

As stoichiometric ratios of nutrients are still imperfect indicators of limitation (due to their varying range across lake types, assumptions about bioavailability of nutrients for algae and other ecological factors), we implemented the sample-by-sample phytoplankton-nutrient stoichiometric imbalance methodology following Moon et al. (2021), with a recent implementation for the continental US by Rock & Collins (2024). This approach is based on quantile regressions under the assumption that P-deficient samples provide a greater Chl-a yield per unit P, and vice versa for N.

For this purpose, lake-years were grouped according to their Danish lake type and subsequently ranked from low to high TP and TN, respectively. The ranked data were used to calculate a moving 95th percentile Chl-a and a 50th percentile of TP or TN concentrations. This procedure aims at estimating the "high yield" Chl-a response (95th quantile) associated with a mid-range nutrient level (50th percentile). Paired percentile values were log-transformed and regressed to derive a linear equation between the 95th quantile Chl-a and the 50th quantile nutrient concentration ("high yield lines"), i.e., of the form: $log_{10}Chla_{95} = m(log_{10}TP_{50}) + n$ and $log_{10}Chla_{95} = m(log_{10}TN_{50}) + n$, where m is the linear slope and n the intercept to the y-axis. Using these "high yield lines", the nutrient-based yields, ϒ, were calculated:

$$\Upsilon_{TP} = {Chla_{observed}(TP)} \Big/ {Chla_{95th}(TP)} \qquad (5)$$

$$\Upsilon_{TN} = {Chla_{observed}(TN)} \Big/ {Chla_{95th}(TN)} \qquad (6)$$

This provides the "high yield relationship", which normalises both nutrient concentrations to the same base scale and allows predicting that $\Upsilon_{TP} > \Upsilon_{TN}$ is potentially deficient in P, and vice versa for N.

To quantify if a lake-year is either P- or N-limited, a "tipping N/P ratio" was calculated. Tipping points of N/P were defined as $\Upsilon_{TP} = \Upsilon_{TN}$. For this purpose, we used the described "high yield lines". We assumed a hypothetical gradient of possible TP_{50} concentrations from 1 to 5000 µg TP/L representing low and high concentrations, respectively, although the upper end is unrealistic in Danish lakes. Corresponding concentrations of $Chla_{95}$ were calculated depending on the low or high TP_{50} value. Next, the equations were re-arranged to calculate the corresponding TN_{50} concentration. The corresponding N/P tipping points were then calculated as the ratios of the derived TN_{50} to the low to high TP_{50} values and regressed in the form of $log_{10} {N}/{P} = m(log_{10}TP_{50}) + n$. Finally, the N/P tipping point regressions were used to derive an N/P tipping point for each lake-year sample based on its TP concentration. The discrepancy, δ, of the lake-year actual molar TN:TP ratio, TN_{molar}/TP_{molar}, to its derived N/P tipping point, $({N}/{P})$ was used to estimate nutrient limitation:

$$\delta = TN_{molar}/TP_{molar} \, ({N}/{P})^{-1} \qquad (7)$$

$$\delta \begin{cases} \delta < 0.5 & \rightarrow N - limitation \\ 0.5 \leq \delta \leq 2 & \rightarrow Co - limitation \\ \delta > 2 & \rightarrow P - limitation \end{cases} \qquad (8)$$

We note that values of δ are arbitrarily.

2.3 Statistical and machine learning approaches

The project involves three connected analysis steps – method selection, ecological mapping and criteria – that all rely on the same data inputs, namely the ODA database, CAMELS, GIS data, derived hydromorphological characteristics and shoreline indices (see flow chart in Fig. 2.1 illustrating the methodologies).

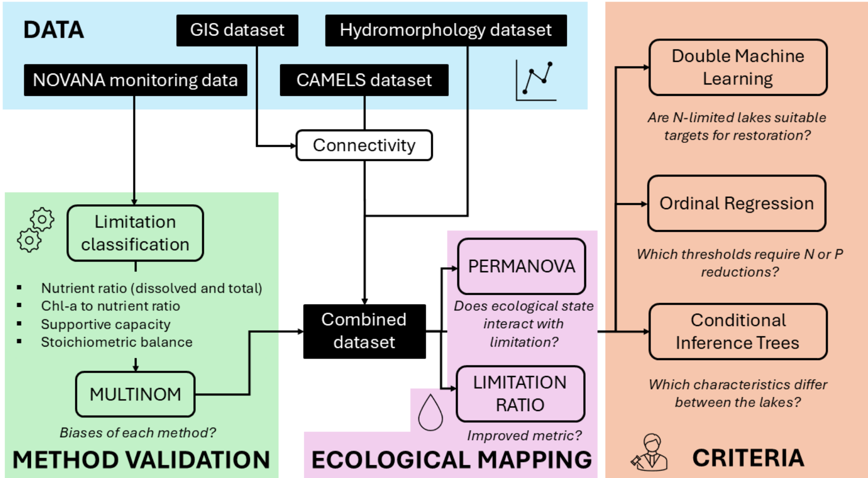


Figure 2.1. Sketch of the methodological workflow of this research report for discussing the role of nitrogen as a limiting nutrient in Danish lakes. Black squares indicate datasets; rounded text boxes indicate methods.

Nutrient limitation

To support the Water Framework Directive by applying nitrogen reduction to brackish Danish lakes, the use of molar N to P ratios has been suggested (Christensen, Søndergaard, Johansson, & Lauridsen, 2023). A TN:TP threshold of 13.3 was identified as the breaking point between dominantly P- and N-limited lakes, respectively. As this intermediate TN:TP ratio varied between 8 and 22, brackish lakes with a ratio below 8 were classified as N-limited, indicating that further N reduction could improve their ecological state (Christensen, Søndergaard, Johansson, & Lauridsen, 2023). Nonetheless, the authors still recommend that the main management focus should be placed on a combined reduction of N and P (Christensen, Søndergaard, Johansson, & Lauridsen, 2023).

Based on this analysis, the seasonal N:P patterns of all Danish lakes in the River Basin Management plans were investigated (Søndergaard & Johansson, 2024). Here, low molar N:P ratios during the summer months for a majority of lakes indicated a potential management angle to improve ecological conditions. Especially, focusing on N reduction in shallow lakes was identified as a potentially sound management strategy (Søndergaard, Lauridsen, Johansson, & Jeppesen, 2017). Shallow lakes are also characterised by dynamic mixing patterns (Søndergaard, Nielsen, Johansson, & Davidson, 2023), convoluting their water quality dynamics, which can potentially make sound management more challenging. But long-term mesocosm studies support that N reduction is a feasible management strategy (Jeppesen, et al., 2025). In addition, the importance of dual nutrient control – both P and N – in Danish lakes was recently underscored by analyses of long-term stoichiometric data (Graeber, et al., 2024).

The importance of nutrient-specific lake management and restoration measures is recognised by the scientific community (Paerl, et al., 2016). Especially, a shift from an exclusive focus on only P reduction toward the reduction of both N and P has been highlighted in several studies (Abell, Özkundakci, & Hamilton, 2010; Maberly, King, Dent, Jones, & Gibson, 2002; Maberly, Pitt, Davies, & Carvalho, 2020; Paerl, et al., 2024). This is consistent with recent studies showing that nutrient limitation is dynamic in both space and time across lakes in the continental US (Rock & Collins, 2024), where the majority of lakes are classified as co-limited (43%) compared to P- (41%) and N-limited (16%) systems (McCullough, Sun, Hanly, & Soranno, 2024). In addition to overall nutrient reduction, targeted N reduction can also significantly reduce algae biomass in P-rich (and therefore N-limited lakes), as recently quantified in aquatic mesocosm experiments (Scott, Taylor, Andersen, Hoke, & Kelly, 2025). Globally, the potential importance of N removal in watersheds has been highlighted by an upscaling study demonstrating that such nutrient reductions could accelerate a lake's transition to improved water quality by more than 70% (Yan, et al., 2025). Nonetheless, the effectiveness of N-only reduction depends strongly on the lake and may, for instance, be low in tropical lakes (Fadum & Hall, 2023).

Method selection

Method selection aims to identify and discuss a suitable method for classifying Danish lakes as P-, N- or co-limited by comparing the five nutrient limitation classification approaches, discussing their shortcomings and providing recommendations for which method to use. Multinomial log-linear models (MULTINOM) were fitted to the data using the `nnet` R-package (Venables & Ripley, 2002) to predict the limitation classification based on the chosen limitation method and associated water quality characteristics. The resulting prediction probabilities were averaged to highlight which categories each method is biased toward.

Ecological mapping

Ecological mapping explores if lake water quality characteristics differ between limitation types and their ecological states. Using the limitation classifications derived in the first step, a two-way permutational MANOVA (PERMANOVA) was conducted using the `vegan` R-package (Oksanen, et al., 2025) to explore if scaled, multivariate water quality data differ among lake-years based on nutrient limitation and ecological state (the latter defined using Chl-*a* -based ecological states) by analysing and partitioning sums of squares using dissimilarities. The data were scaled due to different units and ranges across variables. Euclidean distance was chosen as dissimilarity measure.

Limitation ratio

We based our metric, the limitation ratio, which highlights TN to TP limitation, on the exchange ratio concept ("vekselkurs") (Christensen, Søndergaard, Johansson, & Lauridsen, 2023). The limitation ratio combines limitation status and nutrient stability into a single indicator representing the exchange rate or coupling between N and P dynamics. Here, limitation ratios for TP and TN were calculated for each lake using the VanDa/ODA dataset. For this purpose, nutrient limitation thresholds were first quantified based on a half-saturation coefficient, *K*. Previously, coefficients of 0.0062 mg/L DIP and 0.048 mg/L DIN for P and N, respectively, were used (Christensen, Søndergaard, Johansson, & Lauridsen, 2023). TP and TN follow a hyperbolic relationship and plateau at about 0.5 µmol TP L^{-1} and 30 µmol TN L^{-1} (Guildford & Hecky, 2000). These values were used as half-saturation coefficients (K_X) for TP and TN. Thresholds, L, were quantified for a given nutrient X as:

$$L_X = 1 - {[X]}/{([X] + K_X)} = {K_X}/{[X] + K_X} \qquad (9)$$

Basically, the lower the concentration, the larger its threshold becomes. For each lake, these thresholds were normalised and hence weighted based on the TP and TN data to derive $\widehat{L_X}$. To account for varying ranges, we scaled L_{TP} and L_{TN} between 0 to 1 as $L_{X,s}$, respectively:

$$\widehat{L_X} = {L_{X,s}}/{(L_{TP,s} + L_{TN,s})} \qquad (10)$$

We then related each $\widehat{L_X}$ to the observed Chl-a concentrations to identify conditions in a lake under which a limiting nutrient contributed more to a change in algae biomass:

$$L_{Chl-X} = {\sum \widehat{L_X\,[Chla]}}/{\sum[Chla]} \qquad (11)$$

To derive the limitation ratio, ζ, the Chl-a -normalised thresholds of TP and TN were multiplied with the ratio of TP to TN variability across all lakes:

$$\zeta = \widehat{L_{Chl-TN}}/\widehat{L_{Chl-TP}}\; \sigma_{TP}/\sigma_{TN} \qquad (12)$$

where σ is the standard deviation of the respective nutrient. The limitation ratio can therefore be expressed as ≈ (relative TN limitation strength depending on algae biomass) × (relative TP to TN variability).

The derived limitation ratio, ζ, qualitatively quantifies the relative strength of N- versus P-limitation. If the range as well as half-saturation coefficients of both nutrients, P and N, were identical, values >1 indicate stronger N-limitation during high Chl-a conditions, while values <1 indicate stronger P-limitation during high Chl-a conditions.

ζ is an index that ranks the relative potential for N- vs. P-limitation based on the chosen half-saturation coefficients and the observed variability. It is not a strict mass conversion nor a direct measure of biological limitation without experimental validation. In this report, we explore if the limitation ratio is a useful metric to quantify between-nutrient limitation classifications. For this purpose, we compared the limitation ratio to the commonly used molar ratio of TN to TP.

Criteria

The final step of the analysis discusses if N-limitation is a valid management criterion and explores the underlying factors determining ecological status across systems exhibiting different nutrient limitations.

Conditional inference trees

To understand the factors (e.g. multivariate variables or covariates) and to what extent N-limited lakes differ from P-limited lakes, we constructed conditional inference trees (or decision trees) using the `partykit` R-package (Hothorn, Seibold, & Zeileis, 2015) to infer potential thresholds. First, decision trees were applied to the full multivariate dataset with limitation classification as the target variable ("which thresholds differentiate limitation types?"). Subsequently, separate decision trees were run for each limitation class (P-, N- or co-limited) with ecological state as the target ("which thresholds differentiate ecological states within each limitation type?"). Finally, the ecological state analysis based on limitation was re-run after filtering the dataset into shallow and deep lakes. We calculated overall accuracy and Kappa value (the latter quantifying agreement between predictions and observations beyond what would be expected by random chance) to assess the performance of the decision tree.

Ordinal logistic regression

To determine the concentration threshold at which either TP or TN should be reduced to improve ecological state, we fitted ordinal regression models using the `ordinal` R-package (Christensen R. , 2023) based on the results from conditional inference trees, including both average summer TN and TP concentrations, water temperature, maximum lake depth, total human land-use in the catchment, BFI (ratio of long-term baseflow to streamflow and root depth (linked to the catchment's soil water infiltration capacity). Data were filtered to include all data up to the 95^{th} percentile of TN and TP. "Ordinal" refers to the use of categories (categorical data, i.e., ecological states) as targets. By applying cumulative link models from the `ordinal` R-package (Christensen R. , 2023), we estimated the probability of data belonging to a specific category based on multivariate predictors. We fitted this cumulative link model to the lake's ecological state, grouped as either "high and good" or "moderate to bad". To analyse how an increase or decrease of either TP or TN affects the probability of a lake being in good and/or high condition, we synthetically changed the nutrient concentrations across a gradient from its minimum to maximum observed values. The other covariates were either set to their average values, while TN and TP were set to their concentrations at the 5, 10, 25, 50, 75, 90 and 95% quantiles. Applying bootstrap sampling, we estimated the probability across this gradient for a lake to be in good and/or high condition. To evaluate the synthetic effect of changing both nutrients, we simultaneously ran gradients (minimum to maximum observed concentrations) in bootstrapped cumulative link models.

Double machine learning

We framed the question whether P-, N- or co-limited lakes are valid and/or suitable targets for nutrient management as a conditional transition problem, aiming to explore if different variables drive an improvement from a moderate or worse to a good or better ecological state under co-limitation, P- or N-limitation. To explore this, we first removed redundant and/or highly correlated variables from the dataset. The data were then filtered to include only two limitation classifications for comparison as outcomes. Ecological states were categorised as either "good or high" or "moderate to bad", and rows with missing data were removed. We then applied the double/debiased machine learning framework for interactive regression models using the `DoubleML` R-package (Bach, Kurz, Chernozhukov, Spindler, & Klaassen, 2024; Chernozhukov, et al., 2024), with limitation (P- or N-limited) as the treatment and ecological state ("good or high" or "moderate to bad") as the outcome. In simplified terms, double machine learning estimates causal parameters for multivariate datasets by first modelling the effects of the outcome and the treatment separately and then regressing both against each other.

We applied double machine learning experimentally, as its use in ecology is rare but common in economics. It is used when data are limited and include multiple confounding variables, which is also common in environmental datasets and in the current study. We did not apply double machine learning to explore the synergistic effects of TN and TP reduction. Furthermore, double machine learning assumes a causal DAG (directed acyclic graph). Therefore, causal covariates should be present in the dataset, which we cannot fully ensure as potentially important covariates – such as groundwater inflow – are absent from the current analysis.

By applying double machine learning (using partially linear regression), we estimated the average treatment effect in the form "If changing from N- to P-limitation, how much higher would the probability be to reach a better state?" To verify the robustness of double machine learning in quantifying the average treatment effect, we evaluated combinations of learners (random forest, gradient boosting, logistic regression), random seeds and several cross-fitting folds (2, 3, 5, 7, 10). Furthermore, we applied double machine learning to infer the estimated probability of improving the ecological state (target) by changing TP or TN concentrations (treatment) directly in the lake depending on each limitation classification (P-, N- or co-limited). The same robustness checks as above were applied. Among the tested machine learning algorithms, only the random forest classifier provided stable and consistent results across all cross-fitting folds and was therefore used for further analysis. Average treatment effects (probability of being in a better ecological state by changing one unit of either TN or TP) were then multiplied by 10% of mean TN or TP concentrations of lakes in either P-, N- or co-limited states. This approach mimicked the probability of improving ecological states by modifying the current TN or TP concentration by 10% of its mean value. However, we note that this is only a linear approximation assuming that the treatment is linear. Therefore, these values are not causal estimates, but approximations intended to highlight a potential indicator for management actions.

3 Results

This chapter presents and discusses the results of the individual analyses. The chapter begins by evaluating 5 alternative methods for classifying lakes based on their nutrient limitation, after which the most suitable method is selected (*Section 3.1*). Subsequently, the relationship between ecological status and the resulting classifications of N-, P- and co-limited lakes from the chosen method is examined. It is further explored whether there is a significant relationship to water quality characteristics based on nutrient limitation (*Section 3.2*).

It is further explored how the modified limitation ratio compares to nutrient ratios (*Section 3.3*) to highlight if the limitation ratio can provide additional insights for potential management purposes.

To further explore the relationship between N-, P-, and co-limited lakes, ecological state and water quality characteristics, different machine learning methods were used (*Section 3.4*). In *Section "Conditional inference trees"*, conditional inference trees are used to determine the most important parameters for explaining the ecological states and nutrient limitation classes, and exploratively quantifying thresholds of water quality characteristics. The analysis is done separately for all lakes, deep lakes and shallow lakes according to the three different limitation types.

After this, ordinal regression analysis is used to identify how changes in nutrient concentrations could improve the ecological status of Danish lakes. This method aims at quantifying the probability that a certain nutrient reduction can lead to a change in ecological state (*Section 3.4 "Ordinal regression"*). To approximate the general probability whether nutrient limitation types (P-, N-, co-limited) occur in specific ecological status, we applied a double machine learning approach (*Section 3.4 "Double machine learning"*). This final method is also used to provide linear approximations of the probability of achieving a better ecological state by decreasing either TN or TP depending on the limitation type.

3.1 Nutrient limitation classification

The five nutrient limitation methods (stoichiometric balance, dissolved nutrient ratio, total nutrient ratio, Chl-*a* to nutrient ratio, and supportive capacity) differed in clustering of lake-year combinations. Further, only three of these (stoichiometric balance, dissolved nutrient ratio and total nutrient ratio) provided a co-limitation category. The methods are biased toward specific categories (Fig. 3.1), which is further highlighted by the multinomial analysis (here given with averaged predictions in percentage):

- Stoichiometry (Stoich): mostly co-limitation (69%), sometimes P-limitation (22%)
- Dissolved nutrient ratios (Nutrient): balanced between N-limitation (54%) and P-limitation (32%), low chance of co-limitation (14%)
- Total nutrient ratios (TNutrient): mostly P-limitation (68%), sometimes co-limitation (27%)
- Chl-*a* to nutrient ratio (Chla): mostly P-limitation (71%), rarely N-limitation (28%)

- Supportive capacity (Carrying): mostly N-limitation (79%), sometimes P-limitation (20%)

Conceptually, similar methods such as dissolved and total nutrient ratios provided nearly opposite classifications: dissolved ratios mostly classified lakes as N-limited, whereas total nutrient ratios classified 68% of the lakes as P-limited. Similar to the total nutrient ratio method, the Chl-*a* to nutrient ratio method classified most lakes as P-limited (77%), whereas supportive capacity clustered most lakes as N-limited. Only the stoichiometric balance method classified most lakes as co-limited (69%), followed by P-limited at 22%.

Although there were clear differences in nutrient limitation classifications between the methods, certain patterns stayed consistent (Fig. 3.1). Most lakes defined as P-limited by the stoichiometric balance remained consistently P-limited across the other four methods. Only a small subset was identified as N-limited by dissolved nutrient ratio, Chl-*a* ratio and supportive capacity. Furthermore, only a marginal number of these P-limited lakes was classified as co-limited by the dissolved nutrient ratio method. Lakes classified as N-limited by the stoichiometric balance method generally remained N-limited, although the total nutrient ratio diverged by defining about half of them as co-limited. Lakes defined as co-limited by the stoichiometric balance method were classified variably by the other four methods as either P-, N- or co-limited.

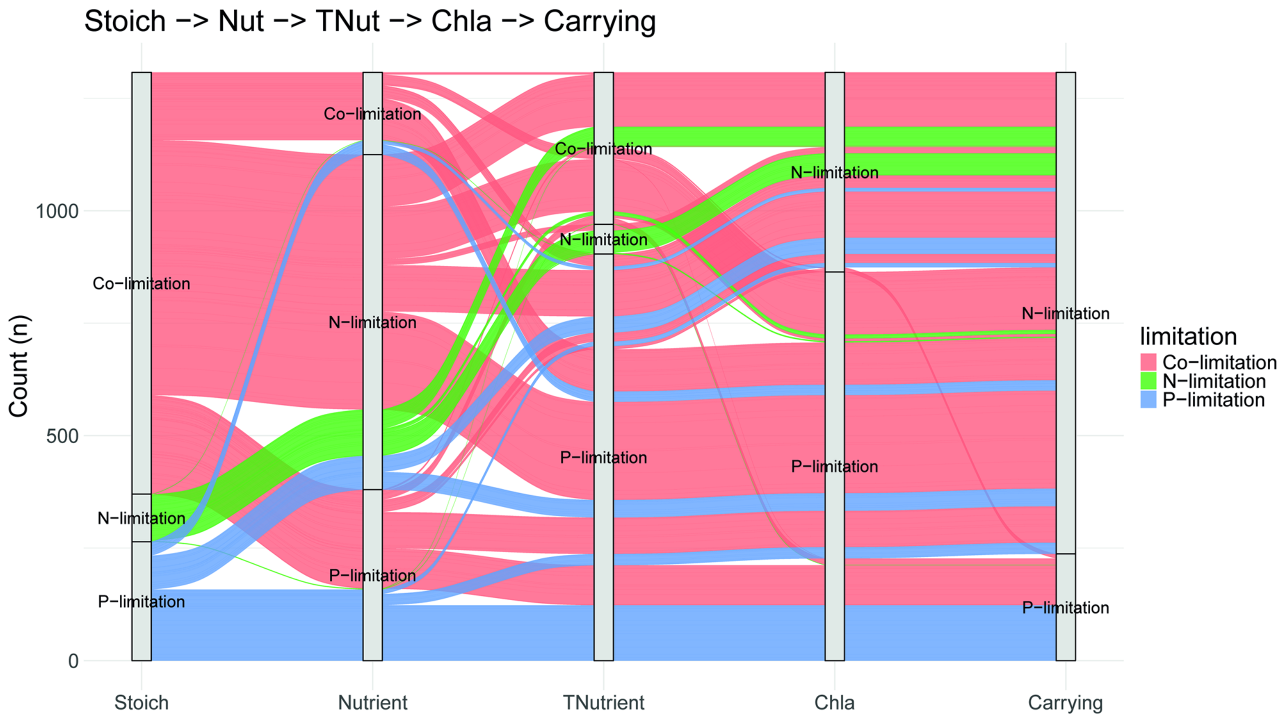


Figure 3.1. Flow chart showing nutrient classification methods after removing missing data. Each lake-year was classified as either P-, N- or co-limited. Flow originates from the stoichiometric balance method: Stoich – stoichiometric balance, Nutrient – dissolved nutrient ratio, TNutrient – total nutrient ratio, Chla – Chl-*a* to nutrient ratio, carrying – supportive capacity, represented by the five grey columns in the figure. The three colours indicate P-limitation (blue), N-limitation (green) and co-limitation (red).

The amount of missing data (due to absence of DIN or SRP) heavily biased the dissolved nutrient ratio, Chl-*a* to nutrient ratio and supportive capacity methods (Fig. 3.2), as they all had a substantial number of lake-years that were not classified at all (up to 50% for nutrient ratio and supportive capacity).

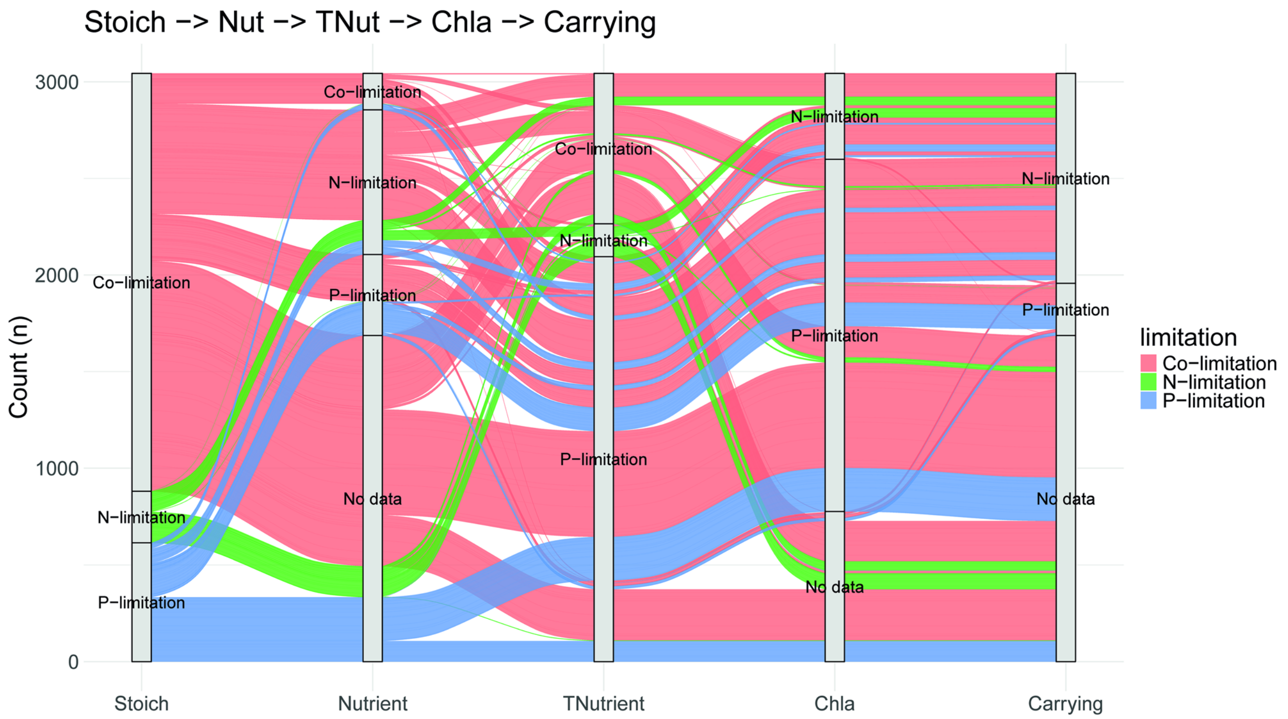


Figure 3.2. Flow showing nutrient classification methods including missing data. Each lake-year was classified as either NA (not available), P-, N- or co-limited. Flow originates from the stoichiometric balance method: Stoich – stoichiometric balance, Nutrient – dissolved nutrient ratio, TNutrient – total nutrient ratio, Chla – Chl-*a* to nutrient ratio, carrying – supportive capacity, represented by the five grey columns in the figure. The three colours indicate P-limitation (blue), N-limitation (green) and co-limitation (red).

Although the methods differed, general trends in how P-, N- and co-limitation clustered along TP and Chl-*a* concentrations were similar across nutrient limitation classification methods (Fig. 3.3). Data sparsity for dissolved nutrients like DIN and SRP (expressed as NA values) severely limited the interpretations for the dissolved nutrient ratio, Chl-*a* to nutrient ratio and supportive capacity. These methods were biased toward overpredicting N-limitation for dissolved nutrient ratio and supportive capacity, and potentially overpredicting P-limitation for the Chl-*a* to nutrient ratio method. The general patterns between stoichiometric balance and total nutrient ratio agreed, but the stoichiometric method seemed to classify a wider range of lakes as co-limited.

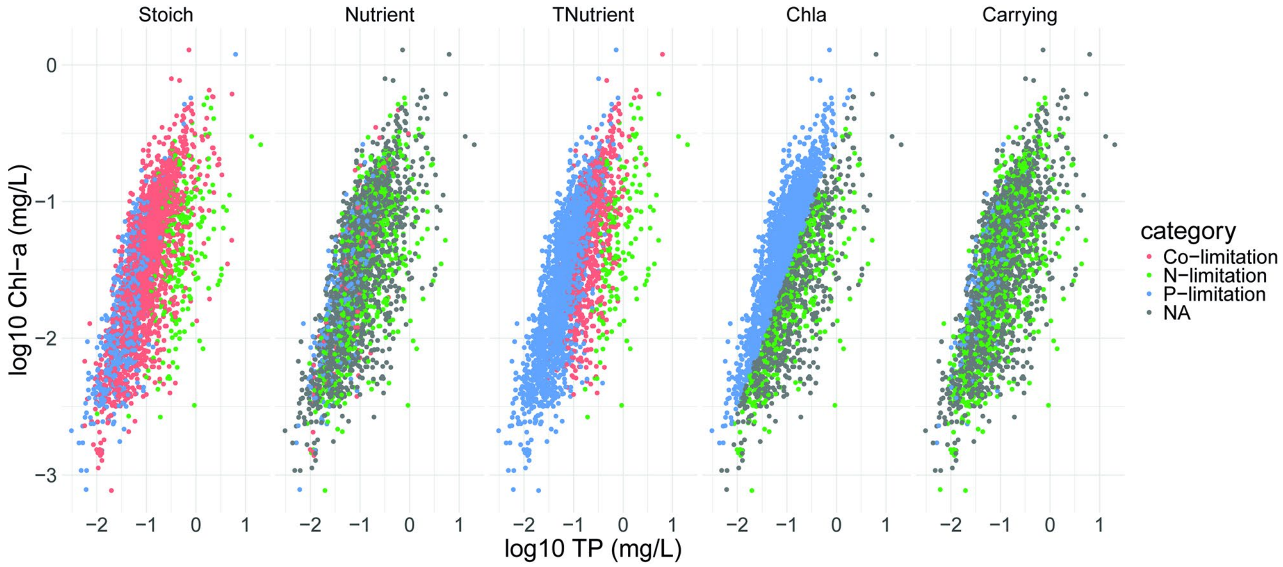


Figure 3.3. Log10-transformed TP concentrations against log10-transformed Chl-*a* concentrations for each nutrient classification method. Lake-years in red indicate co-limitation, green indicate N-limitation, blue indicate P-limitation, and grey represent missing values (NA). Stoich – stoichiometric balance, Nutrient – dissolved nutrient ratio, TNutrient – total nutrient ratio, Chla – Chl-*a* to nutrient ratio, Carrying – supportive capacity.

Neglecting the seemingly biased methods – biased due to a lack of data – we further compared the total nutrient ratio with the stoichiometric balance approach (Fig. 3.4). For the limitation ratio, differences between the two methods regarding ecological state (expressed through Chl-*a* thresholds) and nutrient limitation type were statistically significant, although with stronger differences for co- and P-limitation and weaker differences for N-limitation, where distributions overlapped slightly. For the Chl-*a*, method differences were mostly statistically insignificant, except for co-limited lakes. Lakes classified as P-limited were characterised by statistically significant differences between the two methods across ecological states for TP, TN and the molar ratio of TN to TP. Similarly, co-limited lakes mostly exhibited statistically significant differences for TP, TN and the molar TN: TP ratio. For N-limited lakes, differences were statistically significant for TP and the molar TN:TP ratio, but less so for TN. Overall, across ecological states, the stoichiometric balance method classified lakes as having slightly higher limitation ratios and lower TP as well as TN concentrations (P-limited lakes being the exception for TN) than the total nutrient ratio method.

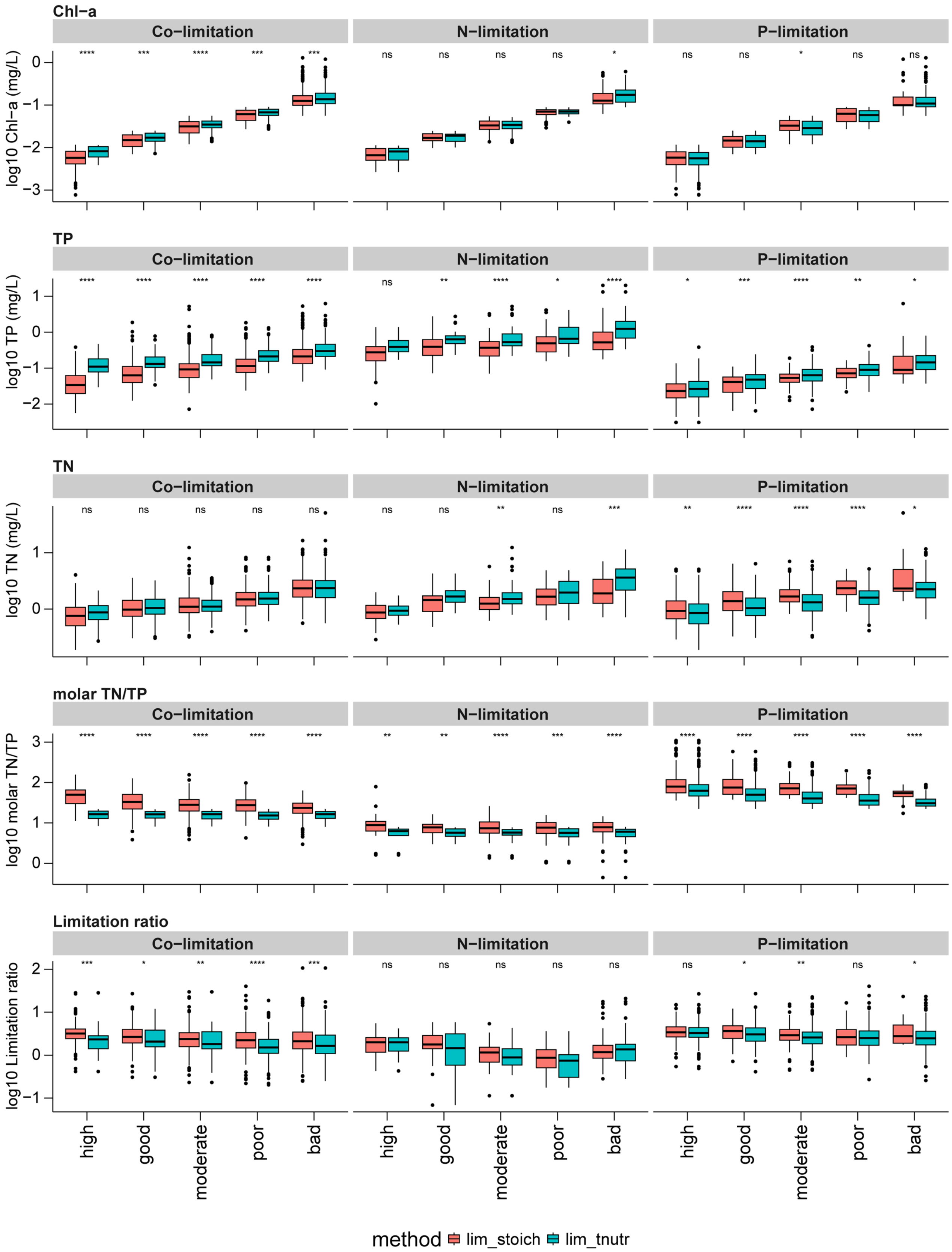


Figure 3.4. Box-whisker plots of variables grouped relative to ecological state (Chl-*a* thresholds) and coloured according to nutrient limitation method (stoichiometric balance, lim_stoich (red), total nutrient ratio, lim_tnutr (blue)). Plots are facetted by limitation classification: co-limited, N-limited and P-limited. From top to bottom: Chl-*a*, TP, TP, TN:TP molar ratio, limitation ratio. Statistical significance is indicated by varying degrees of * quantified by Wilcoxon test: *** p<0.001, ** p>0.01, *p>0.05.

We chose to proceed with the nutrient limitation classification based on the stoichiometric balance method (Moon, Scott, & Johnson, 2021), as this approach (i) does not over-favour any of the extreme limitation categories (P- or N-limitation), (ii) mostly agrees with the other methods regarding P- and N-limitation (Fig. 3.1), (iii) can utilise all data (as it is based on TP and TN), (iv) has similar Chl-*a* distributions as the total nutrient ratio method and (v) allows classification using the Danish lake types[1]. **Therefore, this more conservative method was our preferred choice as it agrees with other continental studies (Rock and Collins, 2024, McCullough, et al., 2024) that highlight most continental US lakes as co-limited.** Nonetheless, we note that the results of the subsequent analyses could differ depending on the chosen nutrient limitation classification.

3.2 Mapping stoichiometric balance limitation onto ecological state

Applying the stoichiometric balance method for nutrient limitation classification, we found that most P-limited lakes reached high to moderate ecological state (based on Chl-*a* concentrations), whereas most N limited lakes were in moderate to bad condition (Fig. 3.5). Most lakes in our dataset belonged to lake types 9 (1261 lake-years), 10 (570 lake-years), 13 (340 lake-years), 11 (316 lake-years) and 5 (217 lake-years). The majority of lake types 9 and 10 were typically shallow and deep freshwater lakes with high alkalinity and low colour.

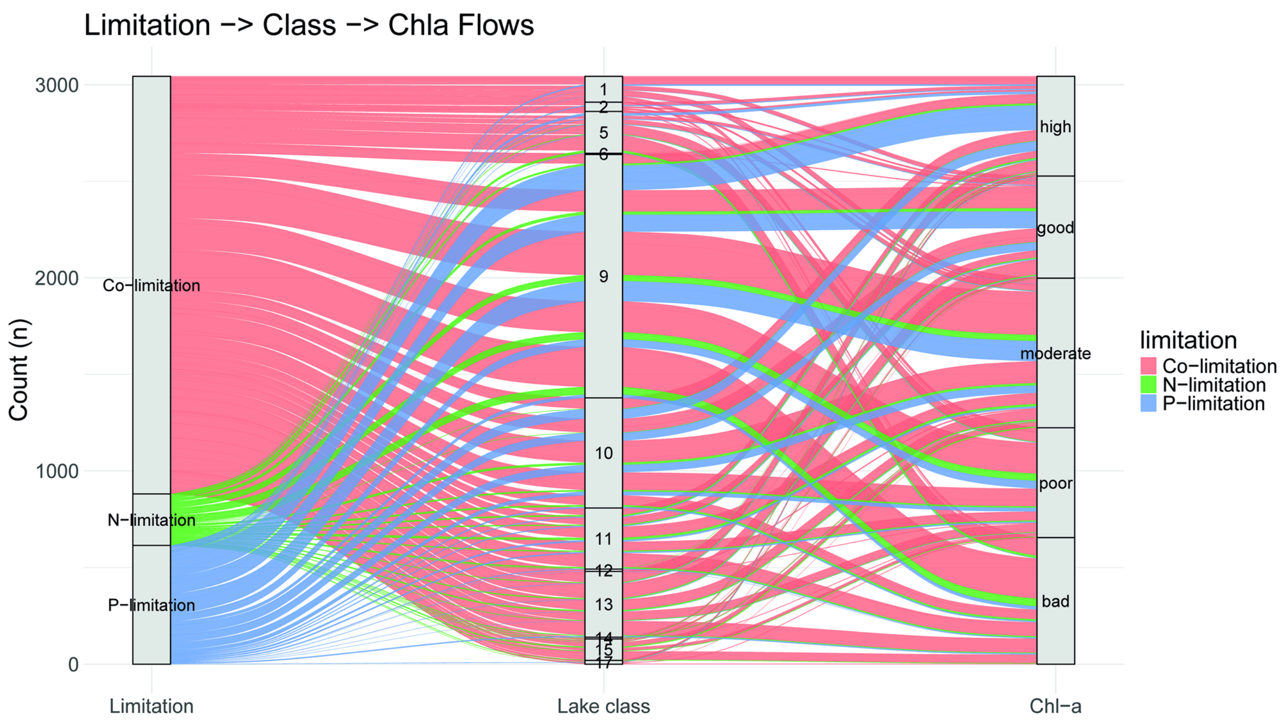


Figure 3.5. Flow chart showing nutrient limitation classification by the stoichiometric balance method (left grey column), Danish lake type (central grey column) and corresponding ecological state based on Chl-*a* (right grey column). Red represents co-limited lake-years, green represents N-limited lake-years, and blue represents P-limited ones.

[1] All lake types and, therefore, all data can be used with the total nutrient ratio method. However, the method cannot incorporate Danish lake types into its classification. The total nutrient ratio method uses fixed ratios of TN to TP as thresholds across all lakes. In contrast, the stoichiometric method can regress tipping point ratios for each lake type, allowing it to build lake type-specific regressions instead of relying on fixed threshold values.

For the dominant lake types 9 and 10, 56% and 51% of the N-limited lakes were characterised by either poor or bad conditions, respectively (Fig. 3.6). In contrast, only 14 to 16% of the P-limited lakes in types 9 and 10 were in poor or bad conditions. The reverse held true for N-limited lakes of types 9 and 10, where lakes in good or high ecological states represented only 18% and 12%, respectively. Again, in contrast, in P-limited lakes of types 9 and 10, 56% and 58%, respectively, achieved good or high conditions. For moderate conditions, N- and P-limited lakes of either type 9 or 10 had similar numbers. Regarding co-limitation, ecological states followed patterns similar to N-limited lakes in type 9 and to P-limited lakes in type 10.

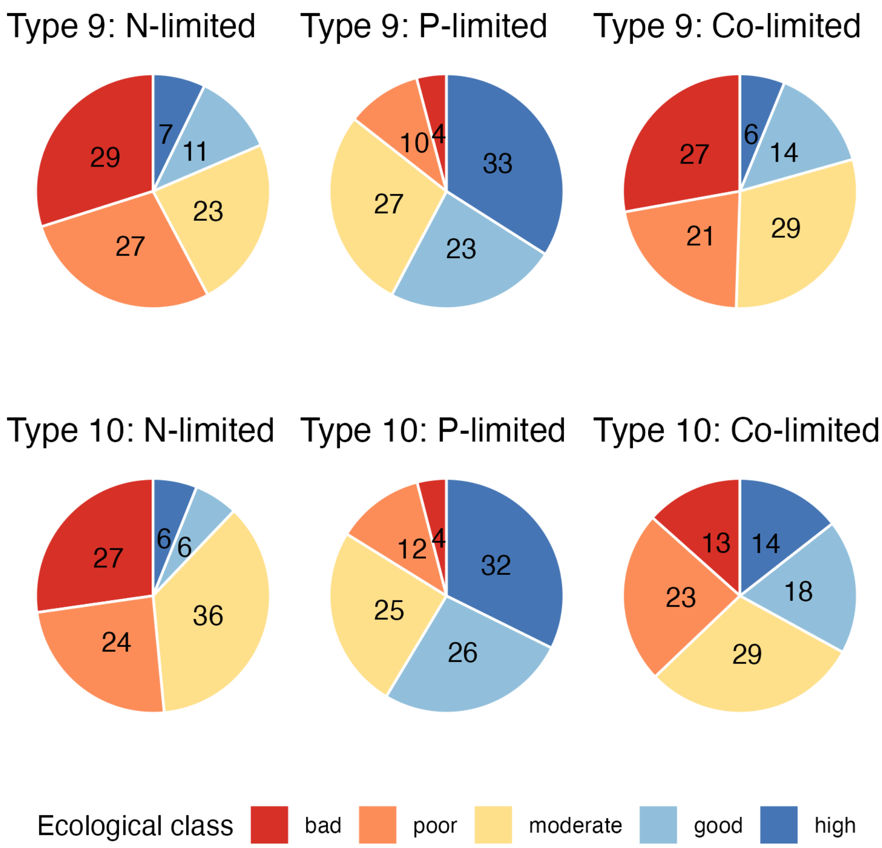


Figure 3.6. Percentages (in pie-diagrams) of lake-years in lake types 9 and 10 classified by ecological state ranging from high (blue) to poor (red) for lake-years classified as N-, P- or co-limited.

As nutrient limitation classification (P-, N- or co-limited) seemed to profoundly affect the ecological state, we explored if the underlying multivariate characteristics of water quality data differed statistically significantly. For this purpose, we used a two-way PERMANOVA on scaled water quality data (only from VanDa) to test differences among lake-years based on nutrient limitation, their ecological state and their interaction. The analysis highlighted statistically significant multivariate differences in lake-years according to nutrient limitation and ecological state, with a model R^2 of 29%, $p<0.001$ and a residual R^2 of 70%.

The analysis indicated that N-limited lakes were generally characterised by poorer ecological conditions compared to P-limited lakes. However, the number of lake-years in moderate condition was similar between N- and P-limited lakes. Therefore, lake management strategies should consider whether a lake in moderate condition can be more effectively improved depending on whether it is N- or P-limited. Furthermore, the water quality characteristics of lake-years differed depending on both nutrient limitation classification and ecological state, highlighting that this method is suitable for informing management recommendations.

3.3 Limitation ratio

To explore if the modified limitation ratio can provide additional information about a lake's nutrient limitation, did we compare the distributions of limitation ratios classified by their ecological state facetted across nutrient limitation to the distributions of the most commonly used method: molar TN:TP ratio. To assess statistical differences, we used the Wilcoxon test. Statistically, the differences between the molar nutrient ratio and the limitation ratios within each ecological grouping were very similar (Fig. 3.7), indicating that both methods are useful for distinguishing between ecological states. However, for P-limitation, the differences between ecological states in molar nutrient ratios were all statistically significant in contrast to the limitation ratio. Nonetheless, additional benefits of using the modified limitation ratio for further analyses, instead of e.g., the nutrient ratio, were not apparent for this project.

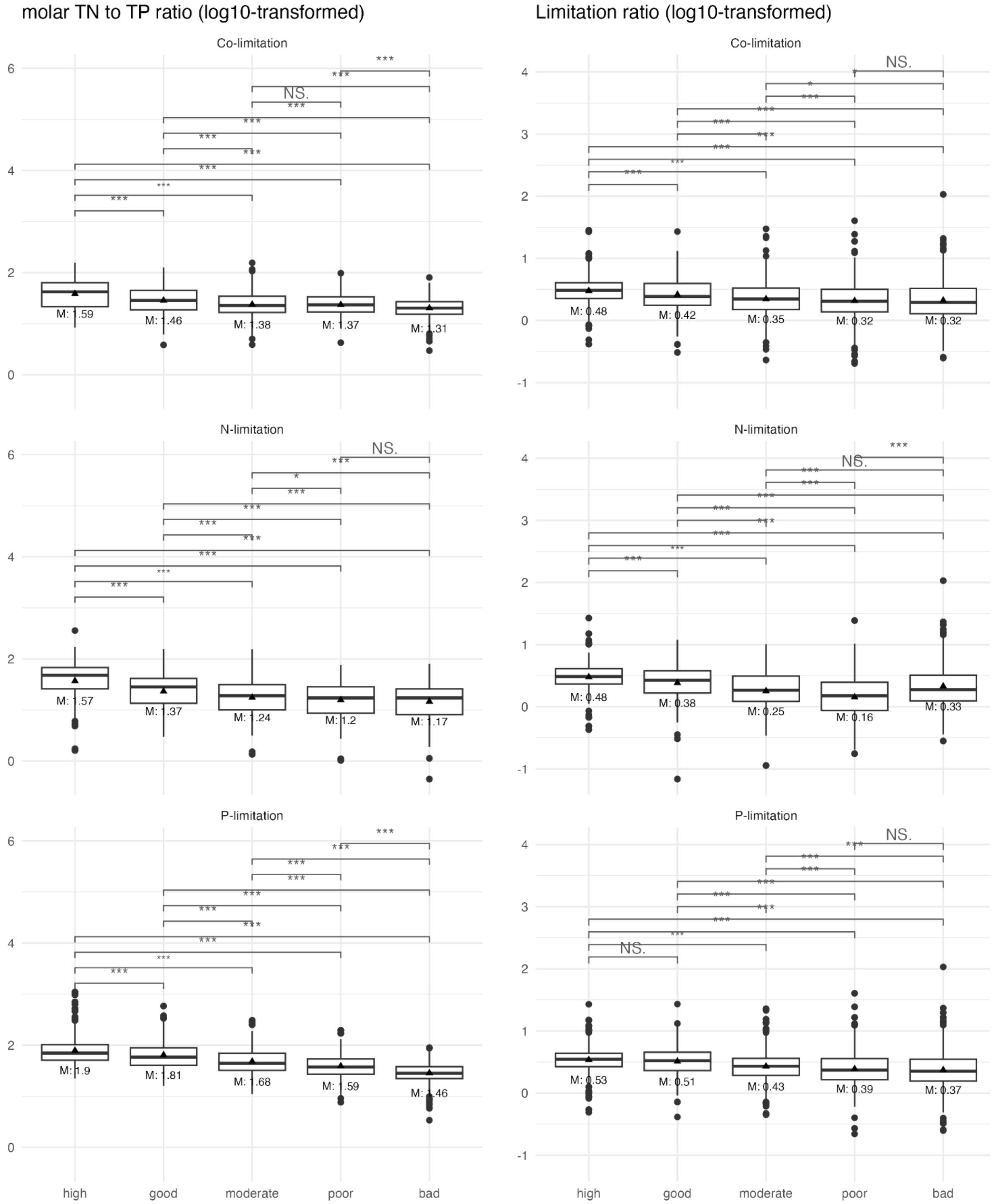


Figure 3.7. Boxplots of molar TN: TP ratios and limitation ratios (modified “Vekselkurs”) for ecological states, facetted by nutrient classification. Statistical significance is indicated by varying degrees of * quantified by Wilcoxon test: *** p<0.001, ** p>0.01, *p>0.05.

3.4 Management criteria quantified by machine learning

After merging the water quality datasets, 676 lakes (77%) were classified as co-limited, 58 as N-limited (6%) and 141 as P-limited (16%). The analyses below incorporated additional datasets, potentially reducing the number of lake-years available. For an overview of statistical core values, see Table 3.1.

Table 3.1. Statistical values of lake characteristics grouped by limitation type. 676 lakes (77%) were classified as co-limited, 58 as N-limited (6%) and 141 as P-limited (16%).

	TN (mg/L)				TP (mg/L)				Chl-*a* (mg/L)			
	Min.	*Median*	*Mean*	*Max.*	*Min.*	*Median*	*Mean*	*Max.*	*Min.*	*Median*	*Mean*	*Max.*
Co-limited lakes	0.18	1.33	1.67	16.42	0.005	0.10	0.17	5.34	0.0007	0.03	0.06	1.28
N-limited lakes	0.28	1.44	1.76	6.99	0.010	0.40	0.75	20.18	0.0026	0.04	0.07	0.57
P-limited lakes	0.28	1.43	1.80	50.77	0.003	0.04	0.06	6.29	0.0007	0.01	0.02	1.19
All lakes	0.18	1.36	1.71	50.77	0.003	0.08	0.20	20.18	0.0007	0.03	0.05	1.28
	Mean depth (m)				Secchi depth (m)				Area (ha)			
	Min.	*Median*	*Mean*	*Max.*	*Min.*	*Median*	*Mean*	*Max.*	*Min.*	*Median*	*Mean*	*Max.*
Co-limited lakes	0.05	1.30	2.07	13.40	0.11	0.89	1.28	8.57	1	13.8	97.19	3955.2
N-limited lakes	0.07	1.09	1.69	16.24	0.14	0.79	1.05	5.84	1	14.5	52.35	1737.7
P-limited lakes	0.05	1.69	2.86	15.09	0.08	1.38	1.87	8.55	1	21.5	86.12	1612.2
All lakes	0.05	1.35	2.20	16.24	0.08	0.98	1.38	8.57	1	14.7	90.89	3955.2

Conditional inference trees

To explore the factors controlling nutrient limitation patterns and ecological status in Danish lakes, we applied conditional inference tree (CIT) analyses across nutrient limitation types (P-, N- and co-limited) as well as ecological states, split into lake morphologies (shallow vs. deep). This approach allowed us to identify the key physicochemical and catchment-level thresholds that best distinguish limitation regimes and ecological conditions based on Chl-*a* metrics. The CIT method is particularly suited for uncovering hierarchical relationships and nonlinear effects among predictors, providing interpretable decision rules that illustrate how combinations of nutrient concentrations, water transparency and catchment attributes influence lake functioning. Consequently, we focused on feature importance, splitting thresholds and how data partition into distinct regimes. Therefore, metrics such as AUC (area under the ROC curve), which evaluate prediction accuracy, are of less importance in this context.

Limitation classification

We used conditional inference trees to determine thresholds for predictor variables explaining nutrient limitation classes. The conditional inference tree identified Secchi depth as the primary variable for separating lake nutrient limitation types. At lower Secchi depths, human land-use (including agricultural areas, settlements, industry, roads, recreational urban areas) further separated the groups. In contrast, at higher Secchi depth levels, mean temperature and duration of low flow periods contributed most to classification. Lakes with greater Secchi depth and higher low-flow duration periods were mostly P-limited (Fig. 3.8). We assessed the robustness of the tree using accuracy and Cohen's kappa (κ). Overall, the accuracy of the tree was 0.711, which is slightly above the majority-class baseline (0.708), while $\kappa = 0.064$, indicating minimal improvement over chance. This pattern is due to the dominance of co-limited lakes, which biases predictions toward the majority class. To assess structural stability, we also examined seed sensitivity using 50 different seeds. The root split was Secchi depth in 100% (50/50) of seeds, and key split variables recurred across seeds with consistent thresholds. Overall, these results indicate that the tree's splits and thresholds are reliable for interpretation, even though the overall predictive accuracy is constrained by class imbalance.

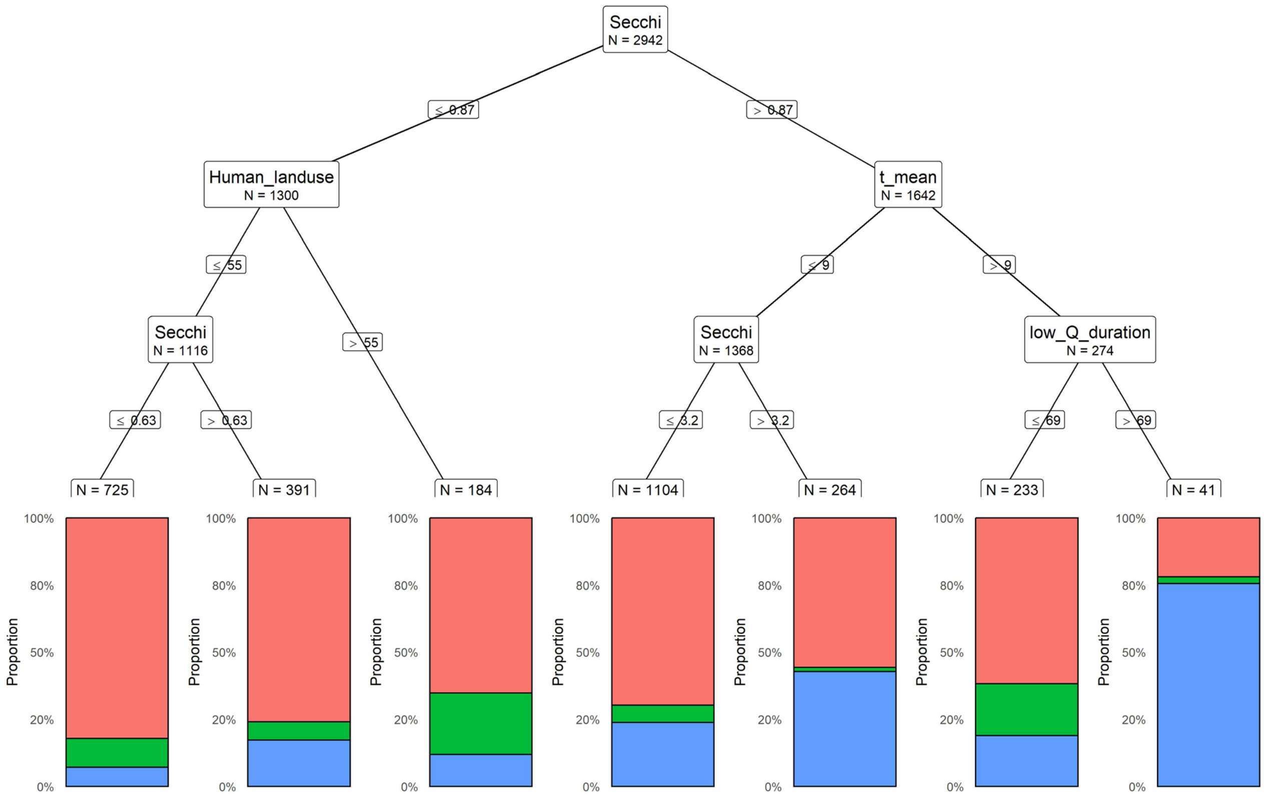


Figure 3.8. Conditional inference tree predicting limitation classes from water chemistry, hydroclimatic indicator and catchment characteristics. The final node shows the proportion of each limitation class. Blue: P-limitation, Green: N limitation, Pink: Co-limitation. For variable names see Table 2.2.

Ecological status (based on Chl-*a*) classification

P-limited lakes

Conditional inference tree results for P-limited lakes identified summer TN as the primary factor differentiating ecological status. Lakes with lower TN (< 1.2 mg L^{-1}) generally exhibited better ecological quality. Summer TP further influenced ecological classification for lakes with lower nitrogen concentrations with TP < 43 µg L^{-1} associated with a higher likelihood of good ecological status. For lakes with higher summer nitrogen levels, summer TP, the percentage of organic matter in catchment soils (pct_organic, a CAMELS-DK attribute related to soil infiltration capacity) and variation in flow, Q_var, were also important factors. Lakes with higher TN (> 3.9 mg L^{-1}) were mostly associated with degraded ecological status (Fig. 3.9). Overall, the accuracy of the tree was 0.48 and $\kappa = 0.25$, indicating fair agreement of the tree model.

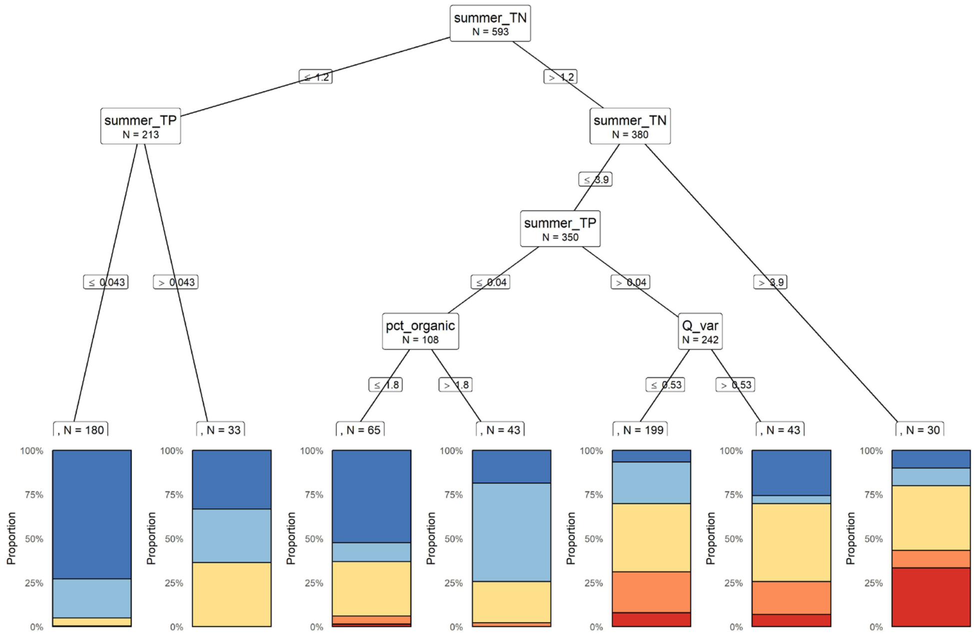


Figure 3.9. Conditional inference tree predicting ecological status filtered for P limited lakes. The final node shows the proportion of each ecological class. Colours indicate ecological status (blue = high, light blue = good, yellow = moderate, orange = poor, red= bad). For variable names see Table 2.2.

Co-limited lakes

For co-limited lakes, summer TN was also the most important factor determining ecological status. Lakes with low summer TN (< 1.2 mg L^{-1}) generally exhibited better ecological quality, whereas those with higher summer TN were more likely to have poorer conditions. Among lakes with lower TN, summer TP, QP_elasticity (indicating streamflow sensitivity to changes in precipitation), precipitation seasonality, colour and water temperature further distinguished the classes. Lakes with low TN (< 0.6 mg L^{-1}) and low TP (< 20 µg L^{-1}) were associated with better ecological quality, while higher colour was linked to poorer ecological quality. For lakes with higher summer TN, maximum depth and precipitation seasonality further differentiated the classes (Fig. 3.10). Overall, the tree achieved an accuracy of 0.42 and $\kappa = 0.25$, indicating fair agreement of the tree model.

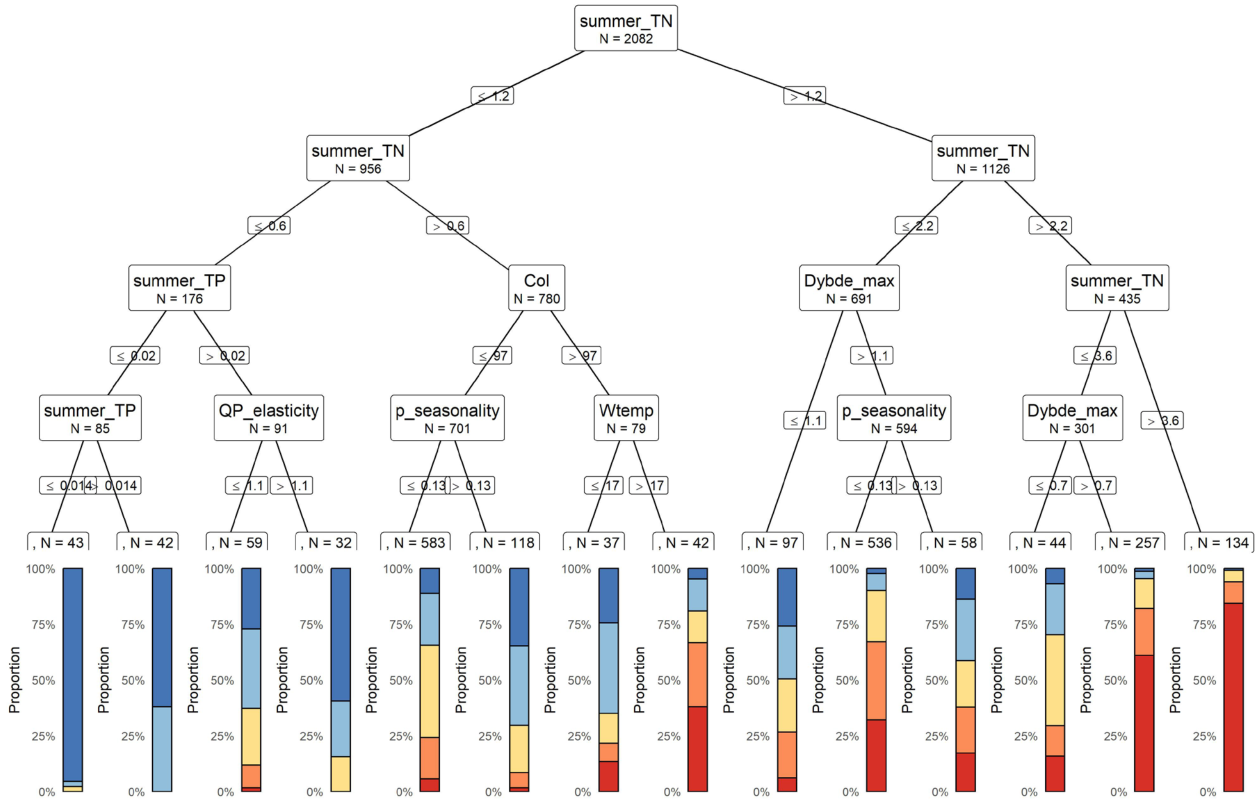


Figure 3.10. Conditional inference tree predicting ecological status filtered for co-limited lakes. The final node shows the proportion of each ecological class. Colours indicate ecological status (blue = high, light blue = good, yellow = moderate, orange = poor, red= bad). For variable names see Table 2.2.

N-limited lakes

For N-limited lakes, summer TN was also the most influential factor determining ecological status (Fig. 3.11). Although N-limited lakes generally showed lower ecological status compared to co-limited and P-limited lakes, those with summer TN < 1 mg L^{-1} typically exhibited better ecological quality. Among lakes with TN < 2.2 mg L^{-1}, root depth (a catchment characteristic related to water availability) also emerged as a significant factor. Lakes with catchments having shallower root depth tended to exhibit better ecological status. Higher summer TN concentrations were consistently associated with lower ecological status (Fig. 3.11). Overall, the tree achieved an accuracy of 0.39 and $\kappa = 0.19$, indicating only slight agreement beyond chance.

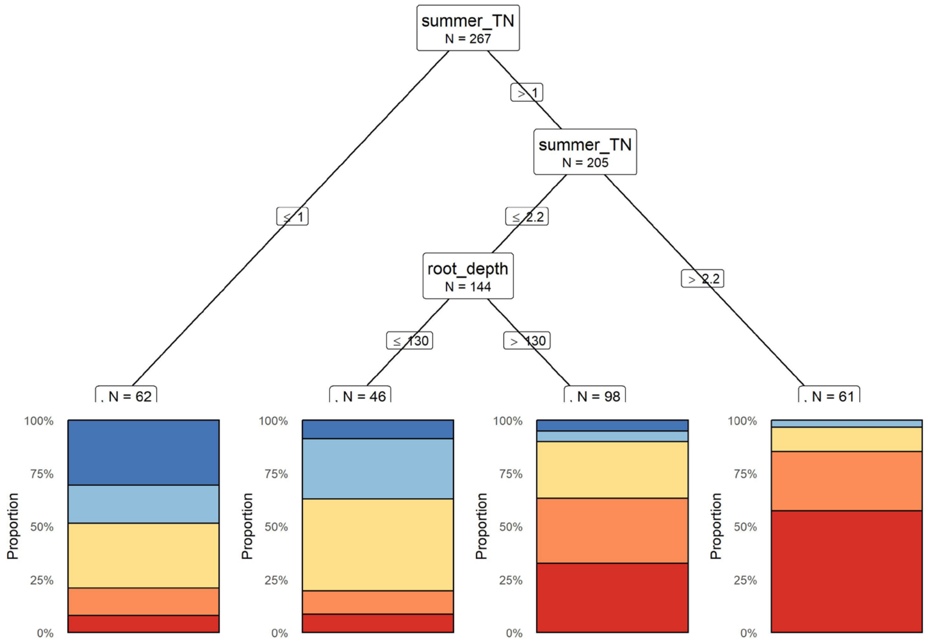


Figure 3.11. Conditional inference tree predicting ecological status filtered for N-limited lakes. The final node shows the proportion of each ecological class. Colours indicate ecological status (blue = high, light blue = good, yellow = moderate, orange = poor, red= bad). For variable names see Table 2.2.

P-limited shallow lakes

Conditional inference trees for P-limited shallow lakes identified summer TN as the primary factor differentiating ecological status. A TN concentration of 1.2 mg L^{-1} was identified as the main threshold, with lakes below this value showing good ecological status. For lakes with higher summer TN values, summer TP was also an important variable. Among these, lakes with TN < 4.3 mg L^{-1} but summer TP > 77 µg L^{-1} tended to have a lower ecological status. (Fig. 3.12). Overall, the tree achieved an accuracy of 0.49 and $\kappa = 0.28$, indicating fair model agreement.

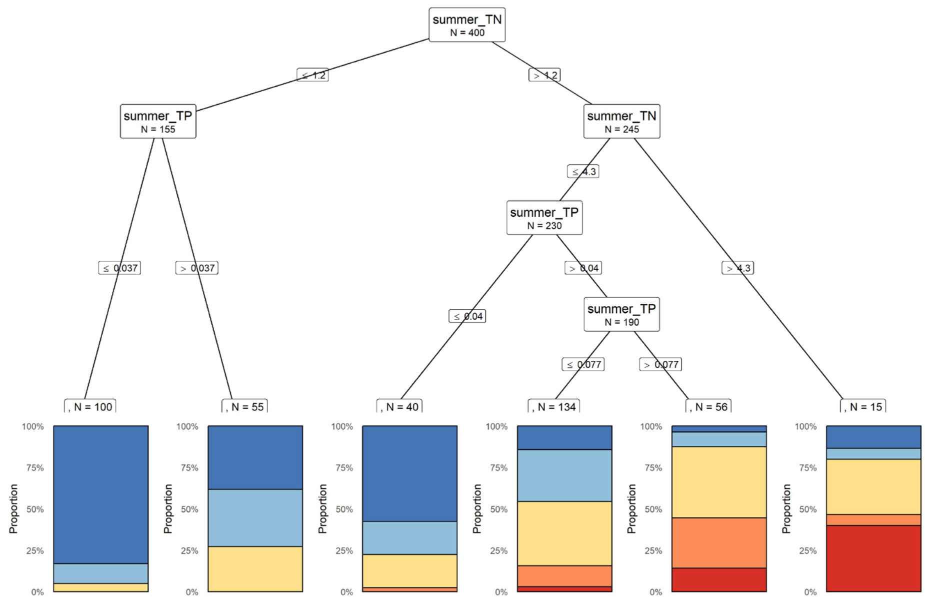


Figure 3.12. Conditional inference tree predicting ecological status filtered for P-limited shallow lakes. The final node shows the proportion of each ecological class. Colours indicate ecological status (blue = high, light blue = good, yellow = moderate, orange = poor, red= bad). For variable names see Table 2.2.

Co-limited shallow lakes

For co-limited shallow lakes, summer TN was again the most important factor determining ecological status (Fig. 3.13). Lakes with low summer TN (< 1.7 mg L^{-1}) and summer TP (< 43 µg L^{-1}) generally exhibited better ecological quality. Among lakes with lower summer TN, colour and maximum depth further distinguished the classes. Lakes with shallower depths (< 2.6 m) were associated with better ecological quality, whereas higher colour was linked to lower ecological quality. For lakes with higher summer TN, variation in flow further differentiated the classes (Fig. 3.13), with lower flow variability typically associated with poorer water quality. The overall accuracy of the tree was 0.43, and κ = 0.29, indicating fair model performance.

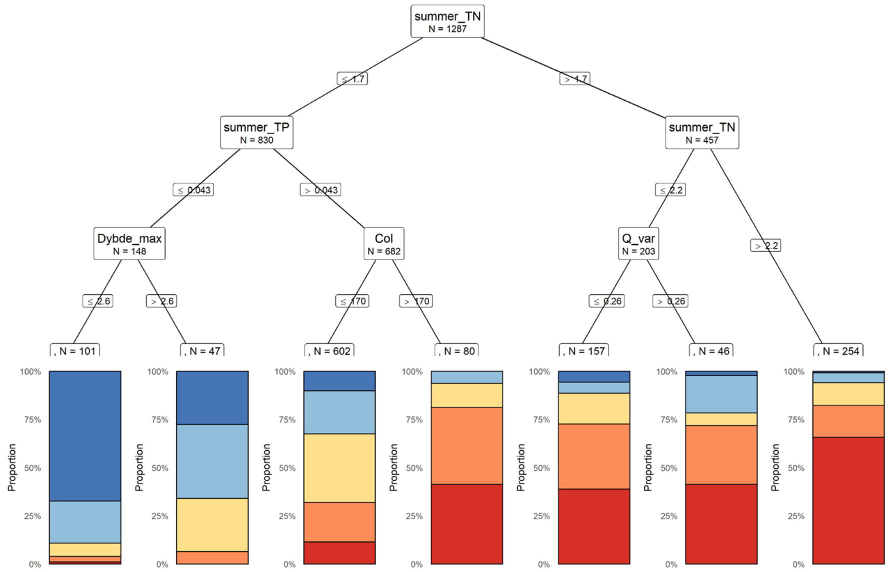


Figure 3.13. Conditional inference tree predicting ecological status filtered for co- limited shallow lakes. The final node shows the proportion of each class. Colours indicate ecological status (blue = high, light blue = good, yellow = moderate, orange = poor, red= bad). For variable names see Table 2.2.

N-limited shallow lakes

or N-limited shallow lakes, total nitrogen (TN) was the only significant fac determining ecological status (Fig. 3.14). Lakes with lower summer TN (< 0.93 mg L^{-1}) generally exhibited higher ecological status, whereas those with summer TN > 1.8 mg L^{-1} were mostly associated with poorer ecological status. (Fig. 3.14). Overall, the accuracy of the tree was 0.35 and κ = 0.10, indicating fair model performance.

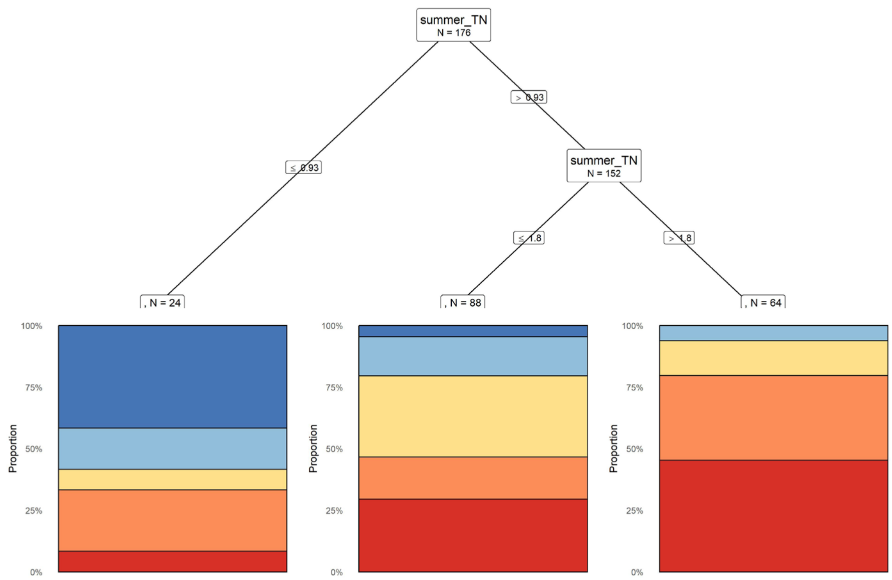


Figure 3.14. Conditional inference tree predicting ecological status filtered for N-limited shallow lakes. The final node shows the proportion of each class. Colours indicate ecological status (blue = high, light blue = good, yellow = moderate, orange = poor, red= bad). For variable names see Table 2.2.

P-limited deep lakes

Conditional inference tree results for P-limited deep lakes identified summer TP as the primary factor differentiating ecological status, which contrasts with the pattern observed in shallow lakes (Fig. 3.15). Lakes with lower summer TP (< 22 µg L^{-1}) generally exhibited better ecological quality, whereas those with summer TP > 44 µg L^{-1} showed poorer ecological quality. Colour and lake area further influenced the ecological classification, with lakes of lower colour and smaller areas being more likely to have better ecological status (Fig. 3.15). Overall, the tree achieved an accuracy of 0.53 and κ = 0.37, indicating fair model performance.

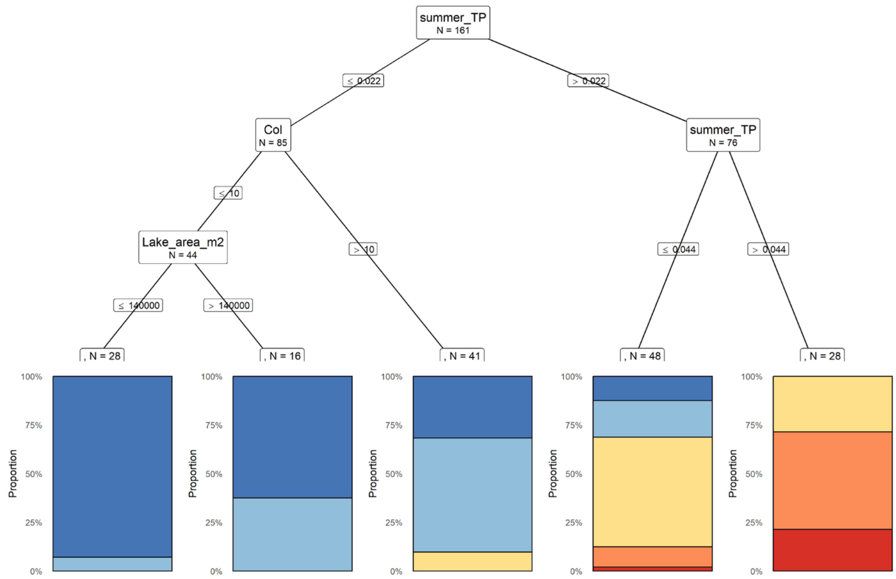


Figure 3.15. Conditional inference tree predicting ecological status filtered for P-limited deep lakes. The final node shows the proportion of each class. Colours indicate ecological status (blue = high, light blue = good, yellow = moderate, orange = poor, red= bad). For variable names see Table 2.2.

Co-limited deep lakes

For co-limited deep lakes, summer TP also appeared as the most important factor determining ecological status, similar to P-limited deep lakes (Fig. 3.16). Lakes with lower summer TP (< 30 μg L^{-1}) generally exhibited better ecological quality, whereas those with higher summer TP were more likely to have poorer conditions. Among lakes with lower summer TP, colour further distinguished the classes. For lakes with higher summer TP, summer TN, maximum depth and colour were additionally important variables. Specifically, lakes with summer TP > 110 μg L^{-1} and maximum depth < 9 m tended to have lower ecological status. In contrast, among lakes with higher summer TP, those with lower TN (< 1 mg L^{-1}) and lower colour generally showed better ecological quality (Fig. 3.16). Overall, the tree achieved an accuracy of 0.45 and κ = 0.29, indicating fair model performance.

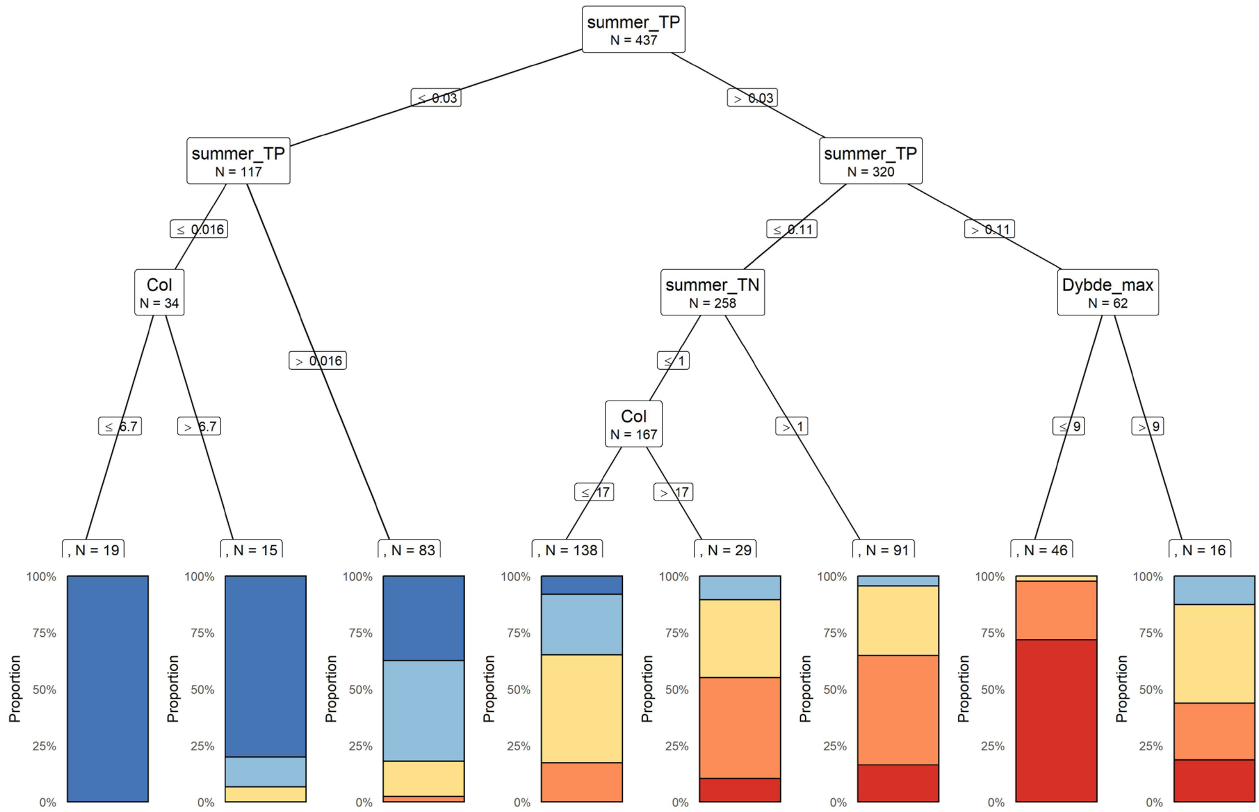


Figure 3.16. Conditional inference tree predicting ecological status filtered for co- limited deep lakes. The final node shows the proportion of each class. Colours indicate ecological status (blue = high, light blue = good, yellow = moderate, orange = poor, red= bad). For variable names see Table 2.2.

Ordinal regression

To identify how changes in nutrient concentrations could improve the ecological status of Danish lakes, we used a statistical approach called the ordinal (cumulative link) regression model. This model builds on the results from the conditional inference trees and estimates how changes in total phosphorus (TP) and total nitrogen (TN) concentrations affect the probability of a lake achieving good or high ecological status, while accounting for other important factors such as water temperature, lake depth, human land-use in the catchment, baseflow index (BFI) and root depth.

The cumulative link model achieved good convergence, although with some indications of multicollinearity, which is not surprising as average summer TN and TP concentrations are causally linked to each other (correlation around 0.51). TN and TP values were filtered to include all data up to the 95th percentile, respectively. The accuracy of the model in predicting ecological conditions (high/good or moderate/poor/bad) was approximately 76% when using TN, TP, water temperature, lake depth, human land-use, BFI and root depth. When other factors were kept constant, decreasing TP concentrations consistently increased the probability of achieving a higher ecological state, the uncertainty band narrowing at TP concentrations below 0.2 mg L^{-1} (Fig. 3.17). For example, reducing TP from 0.6 mg L^{-1} to 0.03 mg L^{-1} increased the probability of achieving good or high status from less than 5% to more than 40%. The effect of TP reduction was strongest when TN levels were low, with probabilities exceeding 60% for TN concentrations below the median. In contrast, at high TN levels, the benefit of lowering TP was much smaller (below 20%). Similarly, TN reductions increased the probability from less than

5% up to > 50% (Fig. 3.17), ranging from 4 to 0.18 mg L^{-1}. Unlike TP, the effect of reducing TN was less sensitive to TP levels.

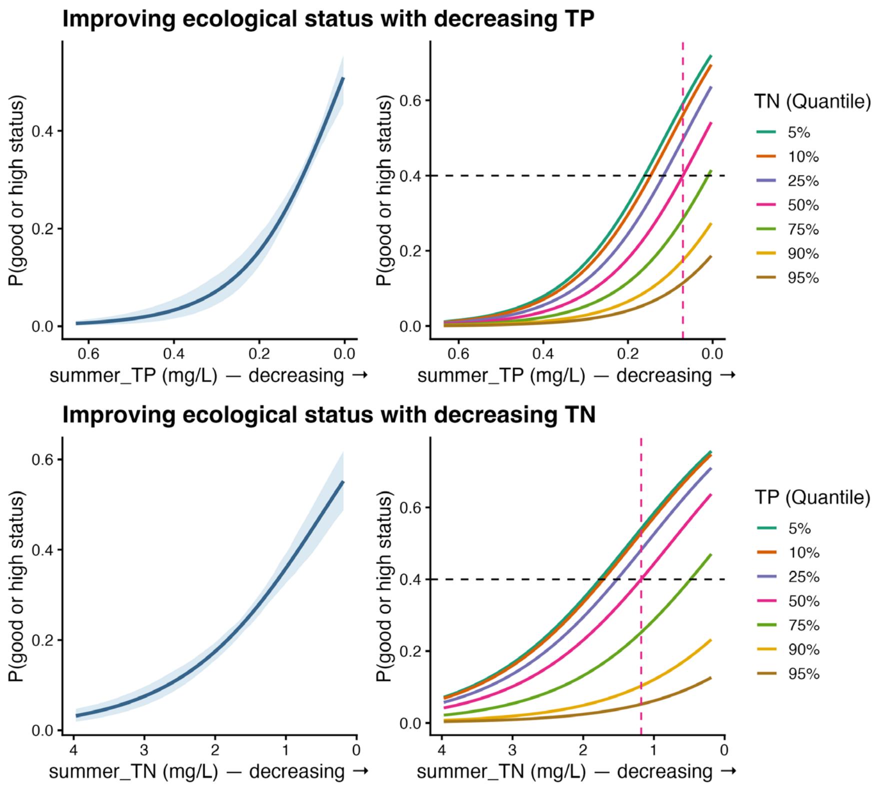
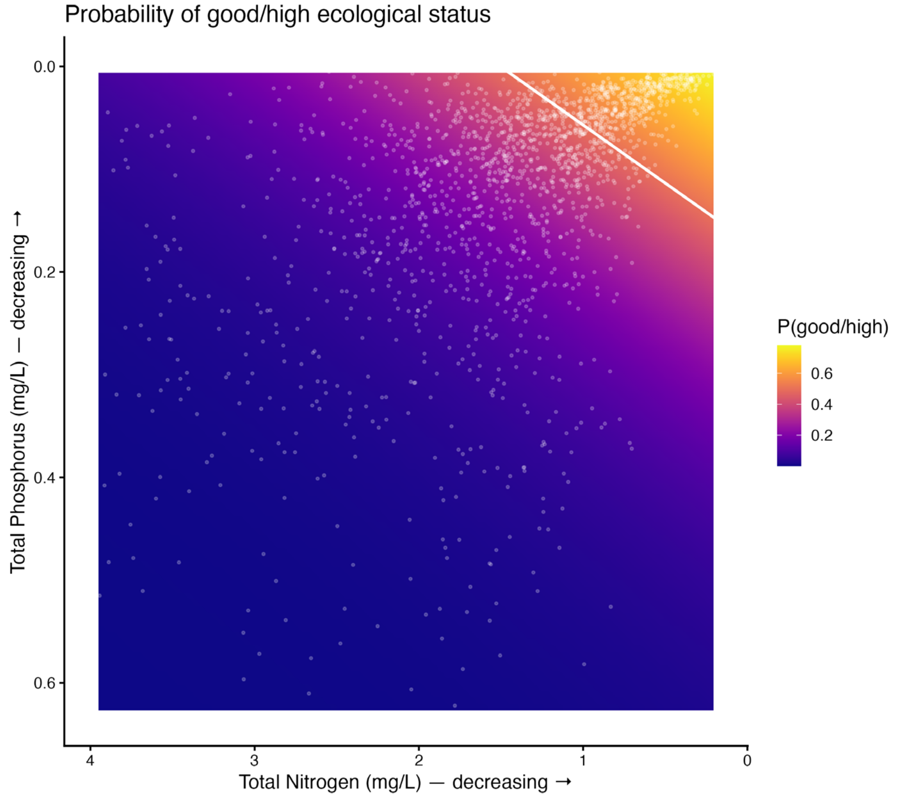


Figure 3.17. Probabilities of achieving good and/or high ecological state based on Chl-a. The first row illustrates probabilities for decreasing TP and the second row for decreasing TN. The first column indicates probabilities of nutrient reductions based on average covariates, uncertainty (shaded area) quantified using bootstrapping. The second column displays probabilities of nutrient reductions based on average covariates but stratified by specific quantiles of the other nutrients. In the upper-right figure, lines show predictions from the cumulative link model across the observed TP gradient for different TN percentiles (from 5th to 95th). In the lower-right figure, lines represent predictions across the observed TN gradient for different TP percentiles (from 5th to 95th). The horizontal dashed line indicates a 40% probability threshold, and the vertical dashed line indicates the TP or TN concentration at which this threshold is approximately reached.

To further explore the combined influence of nitrogen and phosphorus on ecological status, we used ordinal logistic regression by simultaneously varying TN and TP concentrations across a broader range. The response surface (Fig. 3.18) revealed a strong interaction between the two nutrients. The probability of a good or high ecological status increased as both TN and TP concentrations decreased, with the highest probabilities in lakes when both nutrients were simultaneously low (Fig. 3.17, and Fig. 3.18). Most observed lakes (white dots) converged to a high probability (> 0.6) at TN below 1.5 to 1 mg L^{-1}, which is similar to the TN thresholds identified by the inference trees in "Conditional inference trees" (0.93-1.7 mg L^{-1}). Similarly, TP reductions below 0.1 mg L^{-1} at TN near 1 mg L^{-1} yielded a probability of 40% for achieving good/high ecological status.

Figure 3.18. Surface contour plot of decreasing TN to decreasing TP concentrations and their associated probabilities of achieving good and/or high ecological status based on Chl-a. The white contour line indicates a probability of 40%. White dots represent observed TN to TP concentrations.



Double machine learning

To assess whether nutrient limitation types (P-, N-, co-limited) influence the likelihood of improving ecological status, we applied a double machine learning approach. Again, it is important to note that this method is primarily used in economics and assumes that all causal covariates are included in the dataset, which cannot be fully guaranteed in environmental research. Therefore, the resulting probabilities serve as qualitative indicators to infer causal inference rather than definitive predictions.

Double machine learning revealed that nutrient limitation type influences ecological status. The average treatment effect – defined as "effect of limitation type" – is approximately 24% (± 1.5%), meaning that being P-limited, in contrast to N-limited, increases the probability of achieving a "good to high" ecological state by, on average, 24%, controlling for covariates. This effect is statistically significant with $p < 0.001$.

Similarly, P-limited lakes had a 4% (±1%) higher probability of being in good condition compared to co-limited lakes, whereas N-limited lakes had an 11% (±5%) lower probability compared to co-limited ones. Overall, lakes have a higher probability of being in a good state if they are P-limited, followed by co-limited and, finally, N-limited.

We further quantified the causal estimates of achieving an improved ecological (good or high) state through changes in either molar TN or TP concentrations through double machine learning. The results were expressed as changes in TN and TP concentrations equivalent to 10% of their average concentrations for N-, P- or co-limited lakes (Table 3.2). The results indicate that reductions in nutrient concentrations generally increased the likelihood of ecological improvement, but the magnitude of this effect varies by limitation type. Statistically significant improvements were found in co-limited lakes (p

< 0.05) for both TN and TP reductions and for phosphorus reduction in N-limited lakes ($p \approx 0.1$), where a 10% decrease in TP improved ecological status by approximately 1%.

Table 3.2. Average treatment effects of 10% TN or TP reductions on improving the ecological state of a lake towards good or high condition. Average values represent the mean causal effect across all model iterations and lakes. Bold numbers indicate statistically significant results with *** $p < 0.05$ and ** $p < 0.5$. The corresponding concentration numbers show the needed 10% change to achieve the probability. N.s. refers to not significant.

		N-limited	**Co-limited**	**P-limited**
TN reduction	Probability of better ecological state	n.s.	1.4% ***	n.s.
	Corresponding concentration change (if significant)		Δ0.16 mg N L^{-1}	
TP reduction	Probability of better ecological state	0.8% **	1.2% ***	n.s.
	Corresponding concentration change (if significant)	Δ75 µg P L^{-1}	Δ17 µg P L^{-1}	

4 Discussion

4.1 Nutrient limitation

This study evaluated whether lakes limited in their productivity regarding algal biomass by nitrogen or phosphorus or both are suitable targets for lake management in Denmark to achieve good ecological status. In contrast to previous reports that investigated seasonal patterns, this advisory report addressed the research question by using long-term, annual summer averages of water quality parameters, supplemented with data on land-use, catchment characteristics and hydrological connectivity. To guide future restoration, the limitation ratio, modified from the previous exchange ratio/"vekselkurs", did not seem to offer additional insight compared to the 'classical' molar ratio of TN to TP for this analysis. To classify nutrient limitation in lakes, five methodologies were compared to each other, each with its own assumptions and underlying theoretical framework. Ultimately, the **stoichiometric balance methodology for classifying nutrient limitation was chosen for this current analysis** as it (a) can be applied specifically to different lake types, (b) uses the full dataset as it assumes that both TP and TN are bioavailable, (c) has no fixed concentration thresholds for limitation and (d) provides the most conservative classifications with the majority of lakes defined as co-limited. The **choice of nutrient limitation classification is critical**, as highlighted by our PERMANOVA analysis, which showed that lake characteristics differ significantly when accounting for both classification and ecological state. This indicates that lakes in different ecological states and nutrient limitation categories have distinct water quality characteristics.

The nutrient limitation approach chosen, the stoichiometric balance methodology, assumes that all TN and TP are bioavailable for algae. However, as discussed in Moon et al. (2021), a large portion of TN and TP is not bioavailable and therefore does not, for example, accelerate the growth of algal biomass. TN often includes a significant recalcitrant fraction of dissolved organic nitrogen, which is not bioavailable (Lewis & Wurtsbaugh, 2008). Furthermore, unlike using, for instance, orthophosphate or nitrate, TN and TP inherently include N and P bound in biomass, which further complicates their correlation with Chl-*a*. As noted in Moon et al. (2021): "*TN/TP may also be misleading if both concentrations are very high, or if a nonnutrient factor (e.g., light, time, or another element) limits phytoplankton biomass. These uncertainties apply to any recommendation/guideline based on a TN/TP ratio, and [our] estimated tipping point N/P lines are no exception*." Following this reasoning, we argue that applying the stoichiometric approach to a continental dataset of lakes still provides a probabilistic analysis, as general nutrient dynamics can be assumed to relate to TN and TP.

The current study suggests that **N-limited lakes are generally in poorer ecological states than lakes classified as co- or P-limited**. This is assumed to be a byproduct of eutrophication, as N-limited lakes receive high external loads of nutrients. These loads shift their stoichiometry toward theoretical limitation of nitrogen, which, however, does not seem to limit algae growth because nitrogen remains sufficiently available for growth. Most N-limited lakes in types 9 and 10 are either in moderate, poor or bad ecological states. In contrast, P-limited lakes mostly range from moderate to high status. This pattern is caused by the strong correlation between N and P. Lakes with low P concentrations also often have low N concentrations, whereas the reverse is less pronounced.

4.2 Nutrient thresholds by inference trees

The inference trees furthermore highlighted that TN is the most important parameter determining ecological status across limitation types, with higher TN generally linked to lower ecological status. A concentration of about 1 to 1.2 mg TN L^{-1} acts as the main differentiating threshold. Secondarily, lakes in good to high ecological states are further characterised by low TP concentrations, typically below 50 µg TP L^{-1}. Additional factors such as colour, catchment and flow characteristics also affect the classification, highlighting the combined effects of water quality and catchment processes on ecological status. Lakes in poorer ecological states (poor to bad) are mainly characterised by high TN concentrations, greater maximum depth or specific flow characteristics of the catchment. The inference trees underscore hierarchical clustering: **TN first separates higher from lower ecological states, followed by TP concentrations as a threshold for lakes in good to high conditions.** However, for deep lakes, summer TP was found to be the most important parameter, indicating that phosphorus control becomes increasingly important with depth. We used decision trees for exploratory purposes; therefore, the results should be interpreted as identifying predictors that best discriminate among ecological states rather than as causal drivers.

The inference trees identified TN as the most significant split for ecological types in shallow lakes and TP in deep lakes. This is in agreement with general limnological theory given the data used – summer average concentrations of TN and TP sampled near the lake surface. Shallow lakes are mostly polymictic systems, characterised by frequent mixing and unstable summer stratification (Søndergaard, Nielsen, Johansson, & Davidson, 2023). Due to their lower volumes than deeper lakes, they are more strongly affected by external nutrient pulses. Their biological communities often alternate between clear and turbid states, which are dominated by either macrophytes or algae, respectively, making these systems sensitive to light limitation. Therefore, rapid mixing can accelerate resuspension of sediment-bound nutrients into the water column (Søndergaard, Jensen, & Jeppesen, 2003), which increases the biological availability of both TN and TP near the surface layers and promotes algae growth. As nitrogen cycles fast through the water column and is a "visible" proxy for external nutrient loading and algae biomass, TN separates shallow lakes in higher ecological states from lakes in poor states. In contrast, deeper lakes stratify more strongly, causing organic matter to settle into deeper water layers, where it is mineralised. Redox reactions can trigger an internal flux of these bound nutrients from sediments into the water column, but due to stratification, vertical mixing between deeper, nutrient-rich and shallower, nutrient-poor layers is limited. Therefore, TP becomes the key distinction between deep lakes in better ecological states from those in poor states, because the surface layers are TP poor. This pattern can be more profound in ecosystems already experiencing P limitation.

4.3 Co-reduction of nutrients

This study further explored how changes in TN and TP concentrations could improve ecological status using ordinal logistic regression models. These models highlighted that TP reduction consistently increased the probability of achieving good or high ecological quality. TN reduction had similar effects on increasing the probability of improved status but was more pronounced at lower TN concentrations. The models indicate that lakes with TN concentrations below 1 to 1.5 mg L^{-1} and TP below 0.1 mg L^{-1} have the highest likelihood – up to 60% – of reaching good or high ecological status. Note that TP should be lowered to at least 50 µg TP L^{-1} to shift to high ecological states, as underscored by the inference trees. The ordinal logistic models showed exponential patterns for TP, highlighting that further reductions at low TP concentrations can substantially increase the probability of improving ecological lake status. In contrast, TN exhibits linear behaviour at TN below 1 mg L^{-1} (Fig. 3.17), underscoring that further TP reductions are more critical than TN if both nutrients are low. A combined reduction of TN and TP strongly increased the probability of improving ecological status at TN below 1 mg L^{-1}, consistent with the results of the inference trees. Our observed data confirm that the lakes with TN below 1 mg L^{-1} and TP below 0.05-0.02 mg L^{-1} are most likely to have good/high ecological status.

To confirm our findings using causal inference, double machine learning was applied, which estimated higher average treatment effects for lakes being P-limited and in a better ecological state compared to N-limited conditions with about 24% (+- 1.5%). Further, the results underscore that TN and TP reductions are significantly effective for achieving a better ecological status primarily in co-limited lakes. For example, reducing a lake's TN by about 0.16 mg L^{-1} increases the chance of achieving a better state by about 1.4% [2]. We reiterate that the data were split into two groups, "high and good ecological state" (better ecological state) and "moderate to bad ecological state" (worse ecological state). Hence, this method cannot answer the question of whether lakes in specifically moderate, poor or bad conditions can be improved, but can generally give an indication if a specific nutrient change can increase the probability for a lake to change between worse to better ecological states, as defined above. Similarly, by reducing lake TP by 0.017 mg L^{-1}, the chance of reaching good or high ecological state improves by 1.2%. TP reductions for N-limited lakes were also slightly significant but less feasible, as only a reduction of lake TP of 0.075 mg L^{-1} would improve the chance of better status by 0.8%. The results highlight that, statistically, changes of TN and TP, especially in co-limited lakes, cause a statistically significant chance of improving ecological conditions (though the percentages are low, 1.4 and 1.2 % for TN and TP reduction, respectively). Nonetheless, they underscore that nutrient reduction in co-limited lakes is a valid management strategy to improve ecological states. Reduction needs seem specific for lakes depending on nutrient limitation type: For N-limited lakes, further TP reduction appears to be a sound strategy, whereas for co-limited lakes, reductions of both nutrients are necessary. TP reduction was identified as a valid management for even N-limited lakes, highlighting that even in lakes with very low N concentrations, a co-reduction strategy of both nutrients was deemed optimal to achieve a shift in the ecological state. For co-limited lakes, the results do not suggest that an only-P reduction strategy is not valid, but rather that reducing either nutrient or, ideally, both is an effective management approach. For P-limited lakes, management options are less obvious judging from the current analysis. Still, further nutrient reduction can be a valid management strategy as low TP conditions are generally associated with good or high ecological status.

[2] We emphasise that these values are only linear approximations and not causal estimates.

5 Conclusions and recommendations

Co-reduction of nutrients, hence TN and TP, in Danish lakes is an important management consideration for achieving improved ecological states as (a) most lakes are classified as co-limited, and (b) all machine learning methodologies highlighted that both nutrients should be reduced to achieve better ecological states. A conceptual summary of these results is visualized in Fig. 5.1. Here, the stoichiometric method did classify most lakes in Denmark as co-limited, followed by P-limited (these lakes are mostly in good or high ecological states) and N-limited (these lakes are mostly in worse than moderate ecological states). Three machine learning methods were applied to these results. Inference trees underscored in general that lakes in moderate to high ecological states have lower TN concentrations. Once a low TN threshold is reached, inference trees highlighted that most lakes in high ecological states have low TP concentrations. Ordinal regression underscored that TN and TP should both be reduced to improve ecological states. Double machine learning quantified that TP reduction is important for N-limited lakes to achieve an improved ecological state, whereas for co-limited lakes, TN and TP reduction are important.

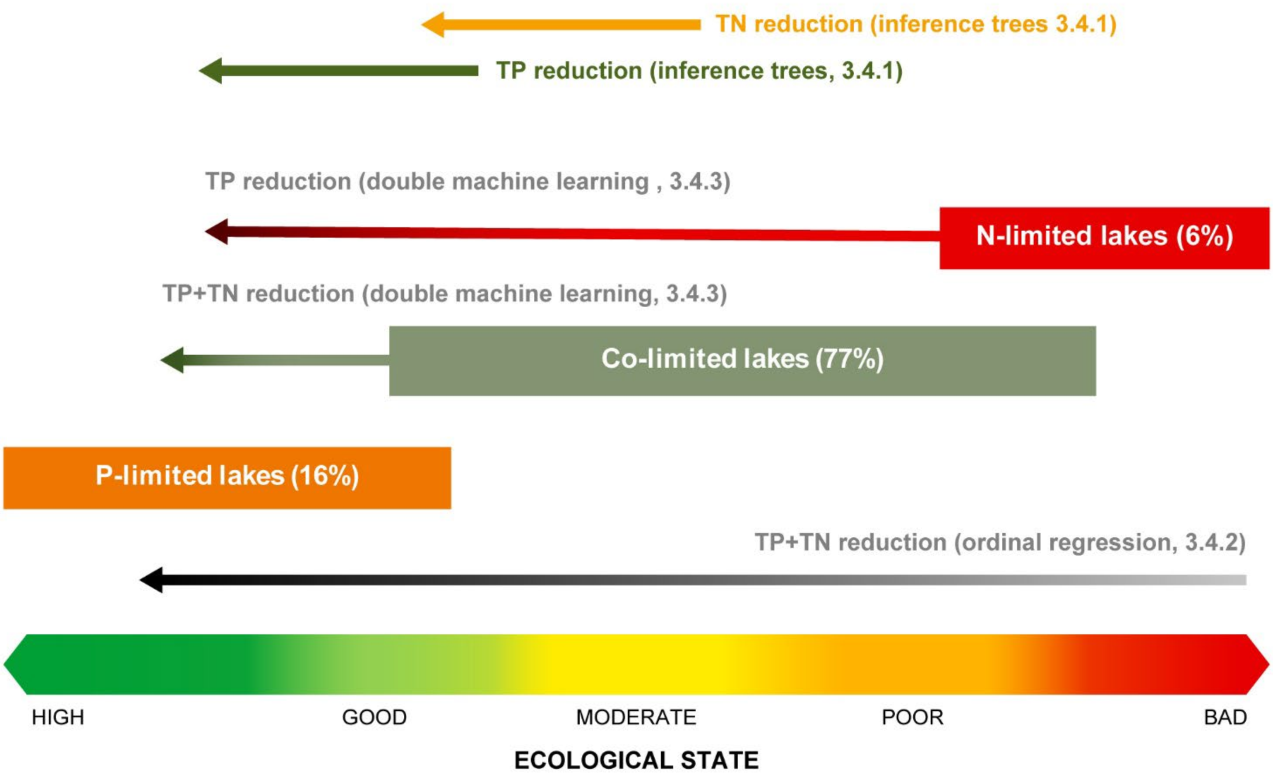


Figure 5.1. Conceptual summary of results based on the stoichiometric method. The boxes show the proportion of lakes investigated that are either N-limited, co limited or P-limited placed according to most dominant ecological states of those lakes (bad to high). Arrows and text above the arrows indicate whether reductions of TP, TN or both, TN and TP, are deemed efficient in terms of increasing the probability for a lake to shift to a higher ecological state, depending on the lakes ecological state and limitation type. In brackets a reference to the statistical method on which the conclusion is drawn is shown.

It is important to note that the statistical analyses in the project, which provided statistically significant insights, are based on **specific assumptions** (i.e., use of summer average concentrations mostly from the surface layer, assuming that TN and TP are bioavailable). Follow-up analyses could potentially provide diverging insights based on alternative assumptions. Further, dynamics in deep and shallow lake ecosystems can differ due to **internal processes**, such as enhanced or suppressed mixing (i.e., stratification), which can further affect apparent nutrient limitation.

5.1 Perspectives for management

In this study, 676 lakes (77%) were classified as co-limited, 58 as N-limited (6%) and 141 as P-limited (16%)[3]. Based on the analysis in this study, it is recommended to focus management efforts initially on co-limited lakes to achieve improvements in their ecological states. To give a rough estimate on which lakes could be used for management strategies targeting nutrient co-reduction, thresholds were calculated based on nutrient distributions across high, good, moderate, poor and bad ecological states. The threshold represents the midpoint between the medians of neighbouring distributions – for example, the high-good threshold is the midpoint between median nutrient concentrations in high and good ecological states. TN thresholds were 0.84, 1.04, 1.30 and 1.86 mg L^{-1} and TP thresholds were 0.048, 0.075, 0.101 and 0.160 mg L^{-1}, differentiating between high-good, good-moderate, moderate-poor and poor-bad ecological states, respectively. Tables 5.1 and 5.2 highlight nutrient thresholds for each limitation type. Note that the small number of N-limited lakes in the current analysis may bias the thresholds. See Supplementary Material Fig. 7.1 for a graphical overview of the TN and TP thresholds across all lake types.

Table 5.1. TN thresholds (mg L^{-1}) differentiating ecological states based on midpoints between the medians of neighbouring distributions.

	High-good	Good-moderate	Moderate-poor	Poor-bad
Co-limited	0.84	1.04	1.30	1.86
N-limited	1.15	1.34	1.45	1.77
P-limited	1.13	1.50	1.93	2.41

Table 5.2. TP thresholds (mg L-1) differentiating ecological states based on midpoints between the medians of neighbouring distributions.

	High-good	Good-moderate	Moderate-poor	Poor-bad
Co-limited	0.048	0.075	0.101	0.160
N-limited	0.335	0.382	0.430	0.507
P-limited	0.031	0.046	0.060	0.084

To illustrate how the results from this study can be applied cautiously in lake management, the distribution of TN and TP across ecological states for co-limited lakes, the majority nutrient limitation type in Denmark according to this study, is visualized in Figure 5.2. Accordingly, the locations of a subset of lakes in moderate ecological condition and their molar N:P ratios are also shown. These lakes were filtered to include those with differences of less than 1 mg TN L^{-1} and less than 0.050 mg TP L^{-1} from the good-moderate thresholds of 1.04 mg TN L^{-1} and 0.075 mg TP L^{-1}, respectively (see Tables 5.1 and 5.2). Hence, co-limited lakes with concentrations between 1.04 to 2.04 mg TN L^{-1}, and between 0.075 to 0.125 mg TP L^{-1} are shown. Notably, these threshold

[3] Note that the low sample size of N-limited lakes affects the statistical analyses in this study as fewer N-limited lakes are in high or good ecological condition compared to those in moderate or bad condition.

criteria for good ecological states are very similar to those identified by the inference trees and the ordinal regression modelling, confirming the robustness of these exploratory findings. These lakes seem to be favourable targets for management aimed at reducing TN and TP simultaneously. However, considering that the median TN concentration of co-limited lakes is approx. 1.33 mg L^{-1} and TP is 0.100 mg L^{-1} (Table 3.1), we acknowledge that reducing TN and TP to these low thresholds is ambitious, but not unfeasible.

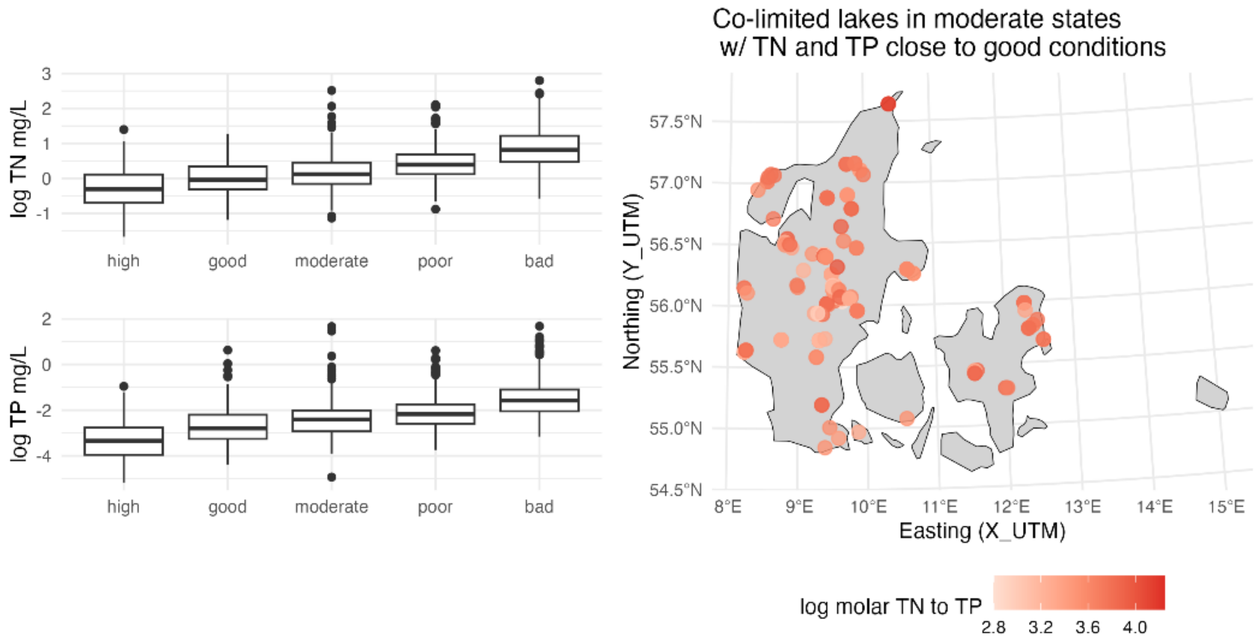


Figure 5.2. Boxplots of summer-averaged TN and TP concentrations for co-limited lakes ordered by ecological state, along with a map of Danish lakes in moderate condition that have TN and TP concentrations close to good ecological states. These were filtered to include those with differences of less than 1 mg TN L^{-1} and less than 0.050 mg TP L^{-1} from the good-moderate thresholds of 1.04 mg TN L^{-1} and 0.075 mg TP L^{-1}, respectively.

It is recommended to focus future lake management efforts on co-limited lakes, in line with global recommendations (McCullough, Sun, Hanly, & Soranno, 2024; Rock & Collins, 2024), to achieve ecological state improvements in the near future. The analyses of the current study suggest that a reduction of TN below 1 mg L^{-1} and a subsequent further reduction of TP could potentially yield the best results – especially for lakes classified as co-limited. Both nutrients are, of course, causally linked to each other regarding algal growth, and reducing one without the other is generally not feasible. For N- and P-limited lakes (both having similar numbers of lakes in moderate ecological states), management measures should also focus on both nitrogen and phosphorus, with lake-specific considerations depending on limitation, hydrological regime or current ecological state. To improve ecological status, external loads, hence nutrients from the catchment, must be reduced.

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7 Supplementary material

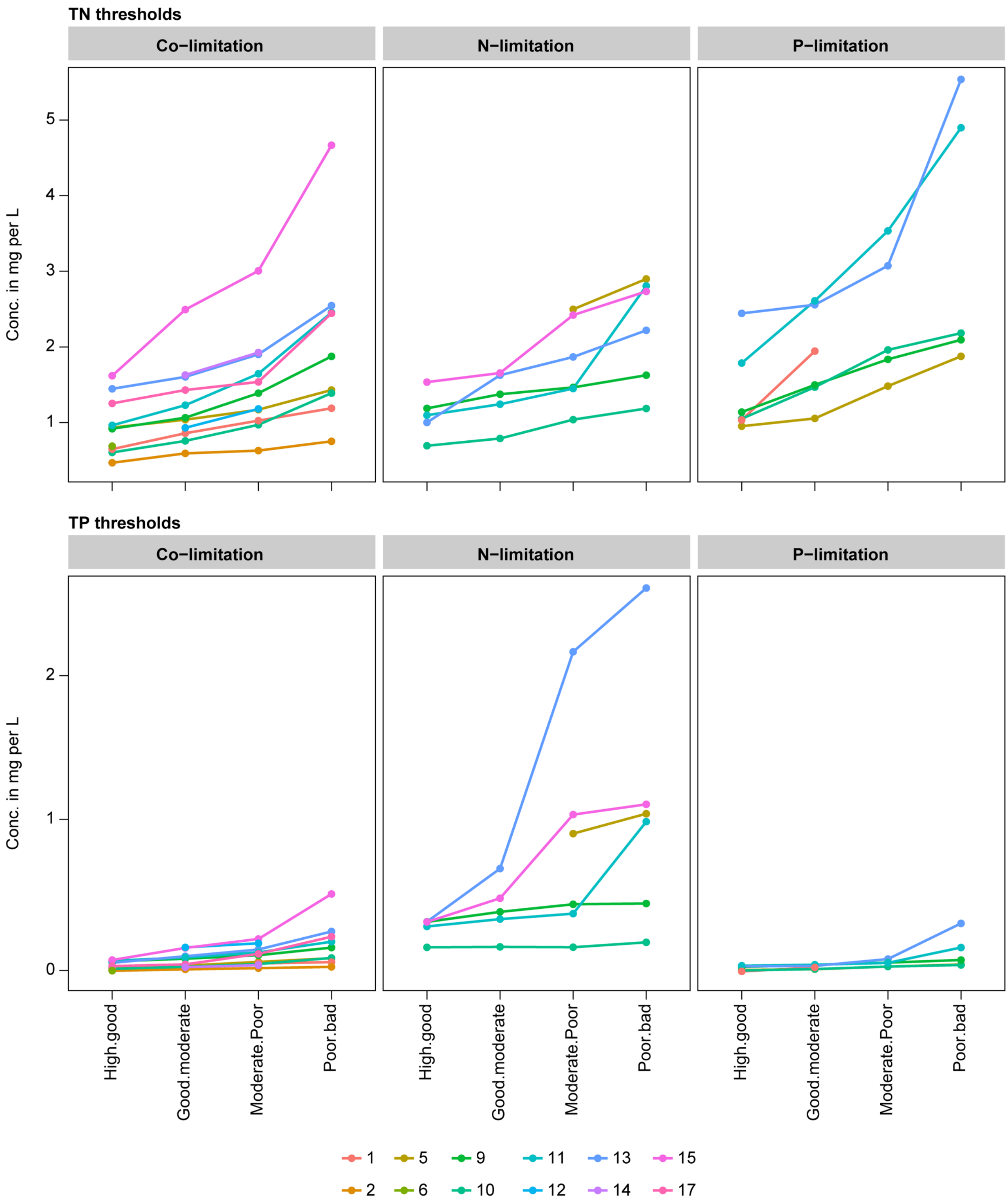

Figure 7.1. Nutrient thresholds in mg L^{-1} differentiating between ecological states and coloured by lake type based on mid-points between the medians of neighbouring distributions.

NITROGEN AS A LIMITING NUTRIENT IN DANISH LAKES

This report explores the role of nitrogen for nutrient limitation in Danish lakes by quantifying and discussing potential nutrient thresholds, which split lakes in different ecological states, and probabilities for shifting ecological state by either reducing nitrogen, phosphorus, or both. Co-reduction of both nutrients seems favourable to achieve ecological state improvements.

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