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Article

Nutrient enrichment, water clarity, and ecological risk in lakes, ponds, and reservoirs of the Winooski River Basin

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Abstract
The Winooski River Basin has a long history of eutrophication and associated ecological risk. This study evaluated nutrient dynamics, trophic state, and ecological risk across lakes, ponds, and reservoirs using water quality indicators and statistical analyses. Nutrient-related Risk Quotients (RQs) were used to assess the extent to which observed conditions exceeded ecological thresholds, allowing comparison of phosphorus, nitrogen, chlorophyll-a, and worst-case risk across waterbodies.
Results indicated that phosphorus is the primary driver of algal biomass, supported by a strong relationship between total phosphorus (TP) and chlorophyll-a (Chl-a), as well as consistent patterns across Carlson Trophic State Index metrics. Trophic conditions ranged from oligotrophic to eutrophic, with most sites classified as oligotrophic to mesotrophic. Principal Component Analysis (PCA) identified a dominant eutrophication gradient defined by TP, Chl-a, and water clarity, while secondary variation was associated with temperature and dissolved oxygen.
Ecological risk assessment showed that sites with elevated nutrient concentrations exhibited higher RQ values, with the worst-case metric (RQmax) indicating moderate to high risk in several systems. Overall, nutrient enrichment—particularly phosphorus—and reduced water clarity were strongly linked to ecological condition, highlighting the need for targeted nutrient management at the watershed scale.

Keywords*:* *eutrophication, phosphorus, chlorophyll-a, trophic state, ecological risk, freshwater*

INTRODUCTION

Freshwater ecosystems are increasingly threatened by nutrient pollution, particularly excess phosphorus and nitrogen delivered from surrounding watersheds. These nutrients accumulate in lakes, ponds, and reservoirs because they are closed systems that integrate runoff from agriculture, development, and atmospheric deposition. Phosphorus is often the primary limiting nutrient in temperate freshwater systems, meaning that even small increases can trigger large increases in algal biomass [1]. Nitrogen, while more abundant, becomes limiting during summer stratification or in systems dominated by nitrogen fixing cyanobacteria, enabling these taxa to outcompete other phytoplankton under high phosphorus conditions [2]. Internal loading from sediments further amplifies nutrient availability, particularly in shallow or stratified systems where anoxic bottom waters release phosphorus bound to iron minerals [3]. Seasonal dynamics also shape nutrient responses: spring snowmelt delivers large nutrient pulses, summer stratification traps nutrients in bottom waters, and fall turnover redistributes accumulated nutrients throughout the water column. Together, these processes create conditions that favor rapid bloom formation and sustained eutrophication.

Elevated nutrient concentrations stimulate rapid algal growth, often dominated by cyanobacteria, which respond strongly to warm temperatures and stable water columns. As Paerl and Otten [4] describe, cyanobacterial harmful algal blooms (cHABs) arise when nutrient enrichment interacts with environmental conditions that favor buoyant, bloom forming taxa. Beyond reducing water clarity,

nutrient driven blooms disrupt food web structure by shifting energy pathways from grazer based to microbial dominated systems, reducing the efficiency of energy transfer to higher trophic levels [5]. As blooms senesce, microbial decomposition increases ecosystem respiration, often driving nighttime or bottom water hypoxia. These low oxygen conditions can cause fish kills, particularly for cold water or sensitive species, and disrupt benthic invertebrate communities that support fish production [6]. Chronic eutrophication can also shift ecosystem metabolism toward net heterotrophy, where respiration exceeds primary production [7]. Over longer timescales, repeated bloom–hypoxia cycles may push systems toward alternative stable states dominated by cyanobacteria, turbid water, and reduced biodiversity, making recovery increasingly difficult even if nutrient inputs decline [8]. Smaller waterbodies are especially vulnerable because limited volume and slower flushing rates amplify the effects of nutrient inputs and accelerate ecological responses.

Ecological Risk Assessment (ERA) provides a structured and widely used framework for evaluating how environmental stressors influence ecological systems. In its classical formulation, ERA follows a causal sequence in which a stressor leads to exposure, resulting in an effect, which collectively determines ecological risk [9,10]. Within this framework, assessment endpoints define the ecological attributes to be protected—such as maintaining suitable oxygen conditions, preventing harmful algal blooms, or preserving fish habitat [11]. Exposure pathways describe how nutrients move from watersheds into waterbodies and how organisms encounter elevated concentrations, whether through direct contact, ingestion, or habitat degradation. Thresholds play a central role in ERA because they translate ecological understanding into actionable benchmarks that distinguish acceptable from unacceptable conditions. Nutrient thresholds, for example, identify concentrations above which the probability of harmful algal blooms, hypoxia, or other adverse effects increases sharply [12]. By comparing measured conditions to these thresholds, ERA provides a transparent, reproducible method for quantifying risk and identifying systems where nutrient enrichment is likely to impair ecological function.

Despite the well documented ecological consequences of nutrient enrichment, relatively few studies apply formal ERA frameworks to quantify nutrient related risk in freshwater systems. Most regional assessments rely on concentration based criteria, trophic state classifications, or statistical trend analyses, which describe conditions but do not explicitly quantify ecological risk or exceedance relative to established thresholds [13,14]. Threshold based approaches are widely used in toxicology and contaminant risk assessment, yet they remain underutilized for nutrient stressors, even though nutrients exhibit well defined nonlinear responses and ecological tipping points [2,12]. A second gap is the limited comparison of nutrient related vulnerability across different waterbody types. Lakes, ponds, and reservoirs are often grouped together in monitoring programs or analyzed independently, leaving uncertainty about whether these systems experience distinct levels of nutrient related risk [15]. Small waterbodies, in particular, remain understudied despite their heightened sensitivity to nutrient loading, rapid response times, and disproportionate influence on regional biogeochemical cycles [16]. Finally, no studies to our knowledge have applied a worst case nutrient risk quotient—a maximum RQ (RQ_{max}) that integrates multiple nutrient stressors into a single, conservative indicator of ecological vulnerability. Worst case or "maximum exposure" metrics are common in chemical risk assessment [9], but have rarely been adapted for nutrient driven eutrophication, where multiple stressors often interact to produce compounded ecological effects [5]. Incorporating (RQ_{max}) provides a novel way to identify systems where any single nutrient exceeds ecological thresholds, offering a transparent and precautionary tool for screening waterbodies at elevated risk.

To address these gaps, this study applies a set of nutrient related Risk Quotients (RQs) to evaluate phosphorus, nitrogen, chlorophyll a, and worst case conditions across lakes, ponds, and reservoirs. Specifically, we ask:

How do nutrient concentrations and water clarity relate to ecological risk across study sites?

Which nutrient stressors contribute most strongly to overall ecological risk?

Does a worst-case RQ metric (RQ_{max}) reveal patterns not captured by individual nutrient indicators?

Based on these questions, we tested the following hypotheses:

H1. Nutrient concentrations and water clarity are significantly associated with ecological risk, with higher nutrient levels and reduced transparency corresponding to increased risk.
H2. Nutrient stressors do not contribute equally to ecological risk, with phosphorus, nitrogen, and chlorophyll-a exerting a stronger influence.
H3. The worst-case risk metric (RQ_{max}) reveals patterns of ecological vulnerability that are not evident from individual nutrient-based RQs alone.
The goal of this study was to apply a nutrient focused ERA framework to evaluate the vulnerability of lakes, ponds, and reservoirs to phosphorus, nitrogen, and chlorophyll a enrichment. Using threshold based RQs, we quantified the degree to which measured conditions exceeded established ecological benchmarks and compared patterns of nutrient related risk across waterbody types. Together, these objectives provide a transparent, threshold based assessment of nutrient risk in small and mid sized freshwater systems and offer a comparative framework for identifying waterbodies that may warrant enhanced monitoring or management attention.

MATERIALS AND METHODS

Study site

The Winooski River Basin in northwestern Vermont drains approximately 2,300 km² into Lake Champlain, making it one of the lake's largest tributary watersheds [17,18]. The basin spans a pronounced east–west physiographic gradient, beginning in the high-elevation Green Mountains and descending through the foothills into the lower Champlain Valley. This gradient reflects the region's glacial history, which produced steep uplands, narrow valleys, and lowland depositional plains [19,20]. Elevations range from roughly 30 m to over 1,100 m, creating strong spatial variation in slope, soil drainage, and hydrologic response.
Land use is similarly heterogeneous. Forests dominate the upper watershed, while agriculture, rural development, and expanding suburban and urban areas are concentrated in the valley bottoms and lower basin [18,21]. These patterns create spatially variable nutrient pressures, with agricultural areas contributing nonpoint phosphorus and nitrogen loads, and developed areas contributing stormwater-driven sediment and nutrient inputs [22,23].
The basin experiences a humid continental climate characterized by cold winters, warm summers, and evenly distributed precipitation [24]. Seasonal snowmelt, summer convective storms, and autumn rainfall events all influence hydrologic connectivity and nutrient transport. The Winooski River and its tributaries form a dense drainage network that integrates these climatic and land-use influences, delivering water and associated nutrient loads to numerous lakes, ponds, and reservoirs throughout the watershed [17].
This combination of steep physiographic gradients, mixed land use, and hydrologically connected surface waters makes the Winooski River Basin an ideal setting for evaluating nutrient-related ecological risk. The basin contains waterbodies that vary widely in size, depth, watershed area, and exposure to nutrient sources, providing a natural gradient for assessing how nutrient concentrations, chlorophyll-a, and oxygen conditions respond to differing watershed pressures. The spatial extent of the basin, along with sampling locations and major hydrologic features, is shown in (Figure 1).

Data sources

Water quality data were obtained from the EPA Water Quality Portal (WQP), a national repository that aggregates monitoring records from federal, state, tribal, and local agencies [25]. The WQP compiles data from programs including the U.S. Geological Survey [21], U.S. Environmental Protection Agency (EPA), state environmental departments, and regional watershed organizations. These agencies contribute standardized measurements for nutrients, chlorophyll-a, dissolved oxygen, temperature, and related water quality parameters.
Spatial datasets used for mapping and watershed characterization were obtained from the Vermont Center for Geographic Information (VCGI). These included the 1-meter digital elevation model (DEM) and the Vermont Hydrography Dataset, which provides waterbody boundaries, tributaries,

and river centerlines [26,27]. Basin boundaries were derived from state-level watershed delineations and clipped to the Winooski River Basin for spatial analysis.
All datasets were downloaded in 2024 and processed to ensure consistent units, coordinate systems, and attribute structures prior to analysis.

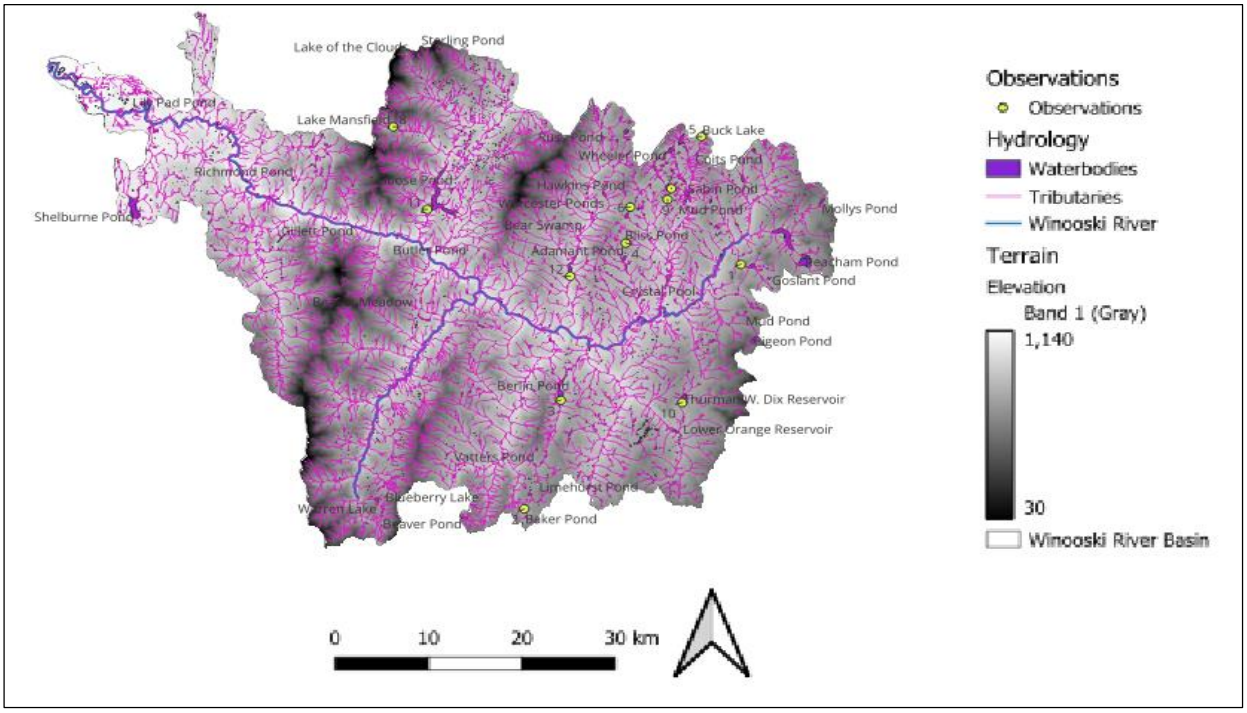

Fig. 1. Map of the Winooski River Basin

Note: *Sampling locations across the Winooski River Basin with site names labeled. Lakes, ponds, and reservoirs are differentiated using categorized symbology. Stream networks, basin boundaries, and terrain shading are shown for spatial context.*

Data Collection and Variables

All water quality data were obtained from the EPA Water Quality Portal (see Data Sources for details).
The dataset included key indicators of nutrient enrichment and ecological condition: depth (m), chlorophyll-a (µg/L), dissolved oxygen (mg/L), total nitrogen (µg/L), and total phosphorus (µg/L). These variables represent both nutrient inputs (nitrogen and phosphorus) and biological or ecological responses (chlorophyll-a, dissolved oxygen, and depth-related stratification effects), offering a comprehensive basis for evaluating nutrient-related ecological risk in freshwater systems [28,29].
The relevance of these indicators is heightened within the Winooski River Basin, where steep physiographic gradients, mixed land use, and hydrologically connected surface waters create strong spatial variation in nutrient pressures and ecological responses. Agricultural areas contribute nonpoint nitrogen and phosphorus loads, while developed areas influence stormwater-driven nutrient and sediment inputs [22,23]. As a result, chlorophyll-a and dissolved oxygen conditions can vary substantially among waterbodies depending on watershed position, elevation, and land-use context. These variables therefore serve as effective metrics for assessing nutrient stress across the basin's diverse lakes, ponds, and reservoirs.
All water quality records were screened for completeness, units were standardized, and only sampling events with full parameter coverage were retained. For sites with multiple measurements across the 15-year period, values were averaged to produce a single representative record per site, ensuring consistency across analyses and reducing temporal sampling bias.

Field and Lab Methods

Field and laboratory methods follow standardized protocols used by agencies contributing data to the EPA Water Quality Portal (see Data Sources).

Field measurements included depth (m) and temperature (°C), typically collected using calibrated multiparameter sondes or depth probes following EPA field protocols for in-situ water quality monitoring [30]. Dissolved oxygen (DO) concentrations (mg/L) were measured using optical or membrane-based DO sensors, with calibration and quality assurance procedures consistent with EPA Method 360.1 and Standard Methods 4500-O [34,35].

Laboratory analyses for nutrient parameters followed EPA-approved colorimetric and spectrophotometric methods. Total phosphorus (TP) was quantified using persulfate digestion followed by colorimetric detection [31]. Total nitrogen (TN) was measured using persulfate digestion with ultraviolet or colorimetric detection [32]. Chlorophyll-a concentrations (µg/L) were determined using acetone extraction and fluorometric or spectrophotometric analysis following EPA Method 445.0 [33].

All contributing agencies follow routine quality assurance and quality control (QA/QC) procedures, including field blanks, calibration checks, laboratory controls, and method detection limit verification. These standardized procedures ensure that measurements of TP, TN, chlorophyll-a, DO, depth, and temperature are comparable across sites and years, supporting robust long-term ecological assessment.

Spatial Analysis

Spatial analyses were conducted in QGIS 3.44 [36]. A 1-meter resolution digital elevation model (DEM) from the Vermont Center for Geographic Information (VCGI) was used to characterize topographic variation and watershed context across the Winooski River Basin [26]. The DEM was clipped to the basin boundary and rendered using a grayscale elevation ramp to provide terrain context without obscuring hydrologic features.

Hydrologic layers, including waterbodies, tributaries, and the mainstem Winooski River, were obtained from the Vermont Hydrography Dataset [27]. All spatial layers were projected into the NAD83 / Vermont State Plane Meters coordinate system to ensure consistent spatial alignment. Sampling locations were imported as point features and overlaid on the DEM and hydrology layers to visualize the spatial distribution of monitoring sites relative to watershed position, elevation gradients, and surface-water connectivity.

All cartographic products were produced in the QGIS Print Layout environment. The final map includes the basin boundary, hydrologic network, waterbody polygons, sampling locations, and a grayscale DEM background, providing a spatial framework for interpreting nutrient concentrations, chlorophyll-a, and dissolved oxygen patterns across the watershed.

Temporal Framework

Water quality records were compiled across a 15-year period (2010÷2024) to capture long-term variability in nutrient conditions and ecological responses within the Winooski River Basin. This temporal window encompasses a wide range of hydrologic conditions, including wet and dry years, seasonal extremes, and interannual climate variability, providing a robust basis for evaluating chronic nutrient pressures rather than short-term fluctuations. Because data were contributed by multiple agencies reporting to the EPA Water Quality Portal, sampling frequency varied among sites and years. To ensure comparability across locations, all measurements were screened for completeness, and only years with full parameter coverage were retained. For sites with multiple observations within the 15-year period, values were averaged to produce a single representative record per site. This approach reduces the influence of anomalous sampling events and aligns with long-term ecological assessment practices commonly used in nutrient-risk evaluation. The resulting dataset reflects multi-year mean conditions for total phosphorus, total nitrogen, chlorophyll-a, dissolved oxygen, depth, and temperature, providing a stable foundation for subsequent spatial, statistical, and risk-based analyses.

Data Processing and Software

All data processing and preparation were conducted using a combination of R (Version 4.x; R Core Team, 2024) and QGIS 3.44 [36]. Raw water quality records downloaded from the EPA Water Quality Portal were imported into R for cleaning, filtering, and standardization. Duplicate entries, incomplete records, and measurements lacking essential parameters (total phosphorus, total nitrogen, chlorophyll-a, dissolved oxygen, depth, or temperature) were removed. Units were standardized across agencies to ensure comparability, and parameter names were harmonized to a consistent naming convention. Temporal filtering was applied to retain only observations collected between 2010 and 2024. For sites with multiple measurements within this 15-year period, values were averaged to produce a single representative record per site. This approach reduces temporal sampling bias and aligns with long-term ecological assessment practices. Summary statistics, data validation, and exploratory visualizations were generated in R. Spatial data processing, including clipping, projection, and integration of sampling locations with hydrologic and elevation layers, was performed in QGIS. Attribute tables were joined to spatial layers to link water quality measurements with their corresponding geographic features. Final datasets were exported as CSV and GeoPackage files for use in spatial analysis, risk calculations, and statistical modeling. This combined workflow ensured that all water quality, spatial, and temporal data were consistently formatted, quality-checked, and ready for subsequent ecological risk assessment.

Formulas

Together, these processing steps produced a fully standardized dataset suitable for quantitative evaluation of nutrient conditions across the Winooski River Basin. With all spatial, temporal, and water-quality variables harmonized, the next stage of the analysis involved applying a set of established ecological risk and water-quality formulas. These formulas quantify nutrient enrichment, biological response, and overall ecological risk, providing the mathematical foundation for the indicators and comparisons presented in the Results. The following section outlines the equations used in this study and the rationale behind each metric.

Carlson's Trophic State Index (TSI)

The trophic status of each waterbody was evaluated using Carlson's Trophic State Index (TSI), a widely applied metric that relates nutrient concentrations and algal biomass to overall lake productivity [37]. TSI values were calculated using chlorophyll-a (Chl-a), total phosphorus (TP), and Secchi Depth (SD):

$$TSI(Chl-a) = 9.81 * ln(Chl-a) + 30.6 \quad (1)$$

$$TSI(TP) = 14.42 * ln(TP) + 4.15 \quad (2)$$

$$TSI(SD) = 60 - 14.41 ln(SD) \quad (3)$$

where Chl-a and TP are expressed in µg/L, and SD is expressed in meters and ln denotes the natural logarithm.

TSI values were interpreted using standard trophic classifications:

- TSI < 40 → oligotrophic (low productivity)
- TSI 40–50 → mesotrophic (moderate productivity)
- TSI > 50 → eutrophic to hypereutrophic (high productivity)

Differences between TSI(Chl-a), TSI(TP), and TSI(SD) were examined to assess potential nutrient limitation or mismatches between nutrient availability and algal response.

Nutrient limitation (TN:TP Ratio)

Nutrient limitation was evaluated using the molar ratio of total nitrogen to total phosphorus (TN:TP), following the Redfield Ratio framework and freshwater adaptations [29]:

$$TN:TP = (TN/14.01)/(TP/30.97) \quad (4)$$

where TN and TP are expressed in µg/L, and 14.01 and 30.97 are the molar masses of nitrogen and phosphorus (g/mol), respectively.

Interpretation followed established freshwater thresholds:

- TN:TP < 10 → nitrogen limitation
- TN:TP > 20 → phosphorus limitation
- 10÷20 → potential co-limitation

This ratio was used to interpret whether nutrient imbalance influenced observed chlorophyll-a concentrations and trophic state.

Risk Quotient (RQ)

Ecological risk associated with nutrient concentrations was assessed using the Risk Quotient (RQ) framework, which compares measured environmental concentrations to established threshold values [38]:

$$RQ = MEC/PNEC \quad (5)$$

where: MEC = measured environmental concentration (e.g., TP, TN, Chl-a); PNEC = predicted no-effect concentration derived from regulatory criteria or literature.

RQ values were interpreted using standard ecological risk thresholds:

- RQ < 0.1 → Negligible Risk
- RQ > 1.0 → Potential ecological risk (threshold exceedance)

Separate RQs were calculated for TP, TN and Chl-a.

RQ_{max}

$$RQ_{max} = \max (RQ(TP), RQ(TN), RQ(Chl)) \quad (6)$$

The highest nutrient-related risk at each site defines the worst-case ecological vulnerability.

Statistical analysis

All statistical analyses were conducted in RStudio (Version 4.5.2). Summary statistics, including mean, standard deviation, and range, were calculated for all variables. Water quality parameters were grouped into nutrient variables (TP and TN), biological response variables (Chl-a), physicochemical variables (DO, temperature, and depth), and derived indices including the Carlson Trophic State Index (TSI) and nutrient-specific Risk Quotients (RQ). Relationships among variables were evaluated using Pearson correlation analysis to quantify linear associations between nutrient concentrations, algal biomass, trophic status, and ecological risk. Linear regression models were used to assess directional relationships between nutrient drivers (TP, TN) and response variables (Chl-a and TSI). Principal Component Analysis (PCA) was conducted on standardized (z-score) variables to identify dominant gradients in water quality and to reduce dimensionality among correlated parameters. PCA loadings and biplots were used to interpret multivariate structure and to evaluate whether nutrient enrichment, trophic state, and physicochemical conditions aligned along shared axes of variation. Statistical significance was assessed at $\alpha = 0.05$ for all analyses.

RESULTS AND DISCUSSION

Summary statistics

Nutrient and physicochemical conditions varied across sampling sites, indicating spatial heterogeneity within the system (Table 1).

Table 1. Summary Statistics of Selected Variables

Variable	Min	Max	Mean	SD
Chl-a	1.23	40.7	7.73	11.1
DO	6.71	11.0	8.81	1.46
Secchi Depth	1.42	9.91	4.21	2.70
Temperature	6.40	15.5	11.1	2.84
TN	0.18	0.40	0.288	0.070
TP	8.91	48.4	16.4	11.1

Chl-a concentrations ranged from 1.23 to 40.7 µg/L, with a mean of 7.73 µg/L, reflecting a broad gradient in algal biomass and primary productivity. TP concentrations exhibited substantial

variability (8.91÷48.4 mg/L; mean = 16.4 mg/L), suggesting that some sites experience elevated phosphorus enrichment. In contrast, TN concentrations were more constrained (0.18÷0.40 mg/L; mean = 0.288 mg/L), indicating comparatively lower variability across the basin.

Dissolved oxygen (DO) concentrations ranged from 6.71 to 11.0 mg/L (mean = 8.81 mg/L), indicating generally well-oxygenated conditions. Water temperature varied from 6.4 to 15.5 °C (mean = 11.1 °C), while Secchi depth ranged from 1.42 to 9.91 m (mean = 4.21 m), reflecting variability in water clarity and light penetration. Differences in temperature and water clarity suggest variation in physical conditions that may influence mixing dynamics, nutrient availability, and biological response.

Overall, the greater variability observed in TP and *Chl-a* relative to TN indicates that phosphorus availability and algal biomass are key factors structuring water quality conditions within the basin. Additionally, the wide range in Secchi depth suggests that water clarity varies substantially among sites, potentially reflecting both algal productivity and non-algal turbidity influences. These patterns provide the basis for evaluating trophic state dynamics and nutrient limitation across the basin.

TSI values

Trophic state conditions across the study sites were evaluated using the Carlson Trophic State Index. TSI values derived from chlorophyll-a ranged from 31.3 to 59.6, while TSI based on total phosphorus ranged from 31.7 to 54.4, indicating conditions spanning from oligotrophic to mesotrophic, with one site approaching eutrophic status. In contrast, TSI values calculated from Secchi depth were consistently higher, ranging from 40.0 to 57.0, suggesting reduced water clarity across multiple sites. The majority of sites fell within oligotrophic to mesotrophic classifications based on nutrient concentrations and algal biomass. However, one site exhibited elevated TSI values for both chlorophyll-a and total phosphorus, indicating localized eutrophic conditions likely associated with increased nutrient enrichment.

Notably, TSI(SD) values were frequently higher than corresponding TSI(Chl-a) and TSI(TP) values, indicating that water transparency was lower than expected based solely on algal biomass. This pattern suggests that factors other than phytoplankton, such as suspended sediments or non-algal turbidity, may contribute to reduced clarity in certain waterbodies. These findings highlight the influence of watershed processes on light availability and reinforce the importance of considering multiple trophic indicators when assessing ecological conditions. Site-level TSI values are provided in Table 2.

Table 2. Site-level Carlson Trophic State Index (TSI) values

Site	TSI (Chl)	TSI (TP)	TSI(SD)	Trophic Status	Site	TSI (Chl)	TSI (TP)	TSI(SD)	Trophic Status
1	36.7	38.1	53.3	Oligotrophic	7	35	41	40	Oligotrophic
2	31.3	41	55	Oligotrophic	8	45.3	37.4	48.6	Mesotrophic
3	33.7	35.7	44	Oligotrophic	9	36.4	45.4	42.3	Oligotrophic
4	47.8	50	50	Mesotrophic	10	38.8	36.8	49.6	Oligotrophic
5	33.5	40	46.7	Oligotrophic	11	59.6	60.1	49.3	Eutrophic
6	41.6	46.5	46.8	Mesotrophic	12	37.2	38.5	57	Oligotrophic

Note: TSI = Carlson Trophic State Index; Chl-a = chlorophyll-a; TP = total phosphorus; SD = Secchi depth. Trophic classifications follow standard thresholds (oligotrophic < 40, mesotrophic 40–50, eutrophic > 50).

TN:TP

TN:TP molar ratios ranged from 6.81 to 37.07 across the 12 sampled waterbodies. One site fell below the nitrogen-limitation threshold (TN:TP < 10), four sites were within the co-limitation range (10÷20), and the remaining seven sites exceeded 20, indicating phosphorus limitation. Overall, most waterbodies exhibited TN:TP ratios consistent with phosphorus-limited conditions, suggesting that phosphorus availability is the primary constraint on algal biomass across the study area. Site-specific TN:TP ratios and nutrient limitation categories are provided in Table 3.

Table 3. Molar nitrogen-to-phosphorus (TN:TP) ratios and nutrient limitation classification for sampled waterbodies

Site	N:P Ratio	Limitation Category	Site	N:P Ratio	Limitation Category
1	26.67	Phosphorus limitation	7	13.99	Co-limitation
2	17.20	Co-limitation	8	37.00	Phosphorus limitation
3	22.45	Phosphorus limitation	9	20.50	Phosphorus limitation
4	10.00	Co-limitation	10	32.19	Phosphorus limitation
5	22.58	Phosphorus limitation	11	6.81	Nitrogen limitation
6	15.87	Co-limitation	12	37.07	Phosphorus limitation

Note: TN:TP represents molar ratios of total nitrogen to total phosphorus. Nutrient limitation categories follow established thresholds: TN:TP < 10 (nitrogen limitation), 10÷20 (co-limitation), and > 20 (phosphorus limitation).

RQ/RQ_{max}

To assess how nutrient enrichment translates into ecological risk across the basin, we calculated risk quotients for phosphorus, nitrogen, and chlorophyll-a, along with the maximum risk quotient (RQmax) for each site. Risk levels for all 12 sites are summarized in (Table 4).

Table 4. Risk Level by Site

Site	RQ (TP)	RQ (TN)	RQ (Chl-a)	RQmax	Risk Level
1	0.58	0.85	0.44	0.85	Negligible
2	0.71	0.67	0.18	0.71	Negligible
3	0.50	0.61	0.26	0.61	Negligible
4	1.33	0.73	2.00	2.00	High Exceedance
5	0.66	0.82	0.26	0.82	Negligible
6	1.05	0.91	1.00	1.05	High Exceedance
7	0.72	0.55	0.31	0.72	Negligible
8	0.56	1.12	1.54	1.54	High Exceedance
9	0.98	1.09	0.42	1.09	High Exceedance
10	0.54	0.94	0.58	0.94	Negligible
11	2.69	1.00	5.81	5.81	High Exceedance
12	0.60	1.21	0.46	1.21	High Exceedance

Note: Risk Quotient TP (RQP): Calculated as (Measured TP / Threshold). The Level of Concern (LOC) threshold is set at 18 µg/L [17]. Risk Quotient TN (RQN): Calculated as (Measured TN / Threshold). The Level of Concern (LOC) threshold is set at 0.33 µg/L [39]. Risk Quotient Chl-a (RQChl): Calculated as (Measured Chl-a / Threshold). The Level of Concern (LOC) threshold is set at 7 µg/L [17].

Risk Quotient (RQ) values varied across nutrients and chlorophyll-a, with exceedances occurring in all three metrics. RQ (TP) ranged from 0.50 to 2.69, with three sites exceeding the threshold value of 1. RQ (TN) values were more consistently elevated, ranging from 0.55 to 1.21, and five sites exceeded the threshold. RQ(Chl) showed the greatest variability, spanning 0.18 to 5.81, with four sites above 1, including one site with a markedly high value (RQ = 5.81). These patterns indicate that nitrogen-related risk was the most widespread across the study area, while chlorophyll-a exhibited the strongest individual exceedances, suggesting localized biomass responses that may reflect internal loading, hydrologic retention, or other site-specific factors. RQ_{max} values, representing the worst-case nutrient or biomass risk at each site, ranged from 0.61 to 5.81. Six of the twelve sites exceeded the threshold value of 1, indicating high nutrient-related risk, while the remaining six sites fell below the threshold. The highest RQmax value (5.81) occurred at a site where chlorophyll-a strongly exceeded its threshold, reflecting an intense localized biomass response. Other exceedances were driven by elevated nitrogen or phosphorus RQs, depending on the site. Overall, RQmax revealed a clear split between low-risk and high-risk waterbodies, highlighting spatial heterogeneity in nutrient and biomass stress across the study area.

Correlation

Pearson correlation analysis revealed clear relationships among nutrient concentrations, algal biomass, and physicochemical conditions across the study sites (Table 5).

Table 5. Correlation matrix of selected variables

Variable	TP	TN	Chl-a	DO	Temp	Secchi Depth
TP	—					
TN	0.142	—				
Chl-a	0.938*	0.246	—			
DO	−0.144	−0.264	0.036	—		
Temp	0.341	0.099	0.329	−0.109	—	
Secchi Depth	−0.158	−0.377	−0.273	−0.507	−0.383	—

Note: *p < 0.05. Only the lower triangle is shown.

TP exhibited a strong positive correlation with chlorophyll-a (r = 0.938, p < 0.05), indicating that phosphorus is the primary driver of algal productivity within the system. In contrast, TN showed a much weaker relationship with chlorophyll-a (r = 0.246), suggesting a comparatively limited role in controlling algal biomass.

Secchi depth showed negative relationships with multiple variables, including chlorophyll-a (r = -0.273), temperature (r = -0.383), and dissolved oxygen (r = -0.507), indicating that reduced water clarity is associated with increased biological activity and changing physicochemical conditions. The moderate negative correlation between Secchi depth and temperature suggests that warmer conditions may contribute to decreased transparency, potentially through enhanced biological production or increased suspended material.

Dissolved oxygen exhibited weak correlations with most variables, including chlorophyll-a (r = 0.036), suggesting that oxygen dynamics are influenced more by physical processes such as mixing and temperature rather than direct biological production alone. Temperature showed moderate positive relationships with both TP and chlorophyll-a, indicating that warmer conditions may enhance nutrient availability and biological response.

Regression

A simple linear regression showed that total phosphorus significantly predicted chlorophyll-a concentrations, $F(1,10) = 73.44$, $p < 0.001$, with TP explaining 88% of the variance in Chl-a ($R^2 = 0.88$). The model indicated that chlorophyll-a increased by 0.94 µg/L for every 1 µg/L increase in TP ($\beta = 0.94$, $p < 0.001$) (Figure 2).

Chlorophyll-a was strongly and positively related to total phosphorus. The regression model was highly significant ($F(1,10) = 73.44$, $p < 0.001$) and explained 88% of the variation in chlorophyll-a. TP was a strong predictor ($\beta = 0.94$, $p < 0.001$), indicating that algal biomass increased nearly one-to-one with phosphorus concentrations. This relationship reflects a tight coupling between phosphorus availability and phytoplankton biomass across the sampled waterbodies.

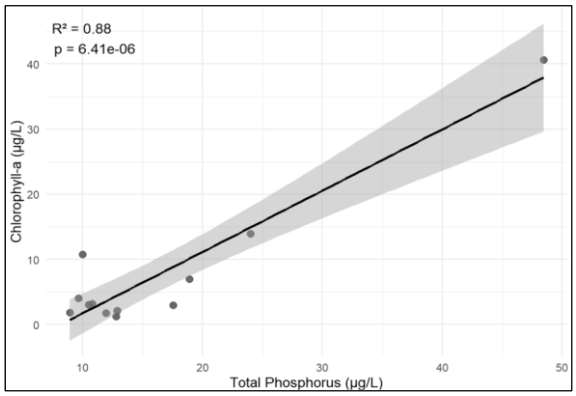

Fig. 2 Linear Relationship Between TP and Chl-a

PCA

Principal component analysis revealed clear multivariate structure among the environmental variables (Table 6).

Table 6. PCA explained by Variables

Component	Eigenvalue	Proportion of Variance	Cumulative Variance
PC1	2.40	0.400	0.400
PC2	1.48	0.246	0.646
PC3	1.11	0.184	0.831
PC4	0.83	0.139	0.969
PC5	0.16	0.027	0.996
PC6	0.02	0.004	1.00

Note: Eigenvalues were calculated as the squared standard deviations of each principal component.

Components with eigenvalues greater than 1 (PC1÷PC3) were retained for interpretation.

The first three components explained 83.1% of the total variance. PC1 (40.0%) represented a strong eutrophication gradient, with high positive loadings for total phosphorus, chlorophyll-a, and temperature, and a negative loading for Secchi depth, indicating that nutrient-rich, warm, turbid sites clustered together. PC2 (24.6%) captured a clarity–oxygen gradient, with Secchi depth loading positively and dissolved oxygen loading negatively. PC3 (18.4%) was dominated by total nitrogen, reflecting a secondary nutrient axis independent of the TP–Chl-a relationship. Together, these components describe distinct ecological gradients in productivity, water clarity, and nitrogen availability across the sampled sites.

Multivariate relationships among environmental variables are illustrated in (Figure 3), which presents the principal component biplot for the first two axes.

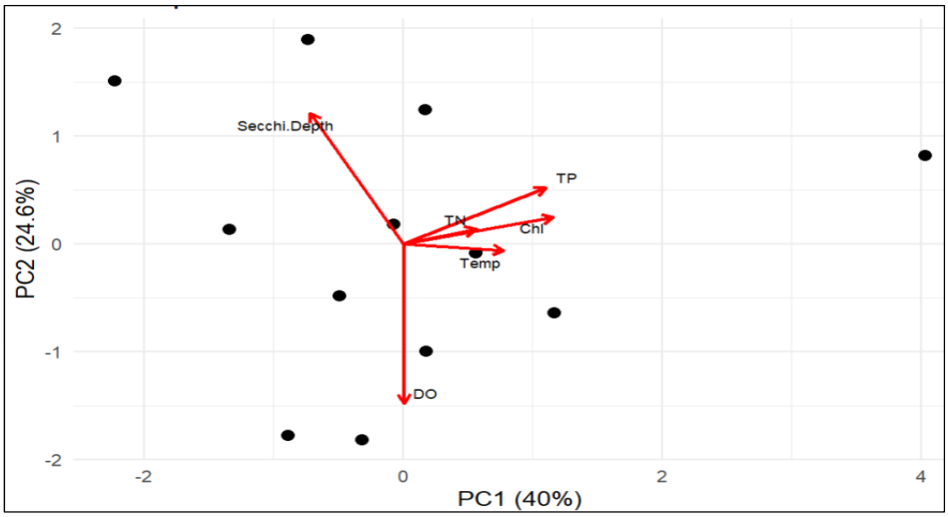

Fig. 3. Principal component biplot showing multivariate relationships among environmental variables

The biplot displays site scores (points) and variable loadings (arrows) for the first two principal components, which together explain 64.6% of the total variance. PC1 (40.0%) represents a strong eutrophication gradient, with positive loadings for total phosphorus, chlorophyll-a, and temperature, and a negative loading for Secchi depth. PC2 (24.6%) reflects a clarity–oxygen gradient, with Secchi depth loading positively and dissolved oxygen loading negatively. Arrow length indicates the strength of each variable's contribution to the ordination.

Spatial distribution of nutrient-related ecological risk

Spatial patterns of nutrient-related ecological risk varied across the Winooski River Basin, with several localized hotspots exhibiting elevated worst-case Risk Quotient values (RQ_max) (Figure 4). Sites in the lower basin and areas influenced by agricultural or mixed land use showed the highest nutrient-driven ecological concern, consistent with known nutrient loading pathways. In contrast, upland and forest-dominated watersheds generally exhibited lower RQ_max values, reflecting reduced nutrient pressures in less developed catchments.

The hotspot map highlights clear spatial clustering of elevated phosphorus- and chlorophyll-a-related risk, indicating that nutrient enrichment is not uniformly distributed across the basin. These patterns reinforce the need for targeted, watershed-scale nutrient management focused on high-risk sub-basins where nutrient exceedances are most pronounced.

How do nutrient concentrations and water clarity relate to ecological risk across study sites?

Across study sites, ecological risk was strongly structured by nutrient enrichment and declining water clarity. Higher total phosphorus and chlorophyll-a consistently aligned with reduced Secchi depth, indicating that increased nutrient availability and diminished light penetration correspond to elevated ecological risk. These patterns were reinforced across correlation analysis, regression, and PCA, where nutrient enrichment and transparency loss formed the dominant environmental gradient, supporting H1. Evidence from Lake Champlain mirrors these findings: long-term monitoring shows that elevated phosphorus reduces water clarity and increases bloom risk, demonstrating a direct nutrient–clarity–risk pathway [40].

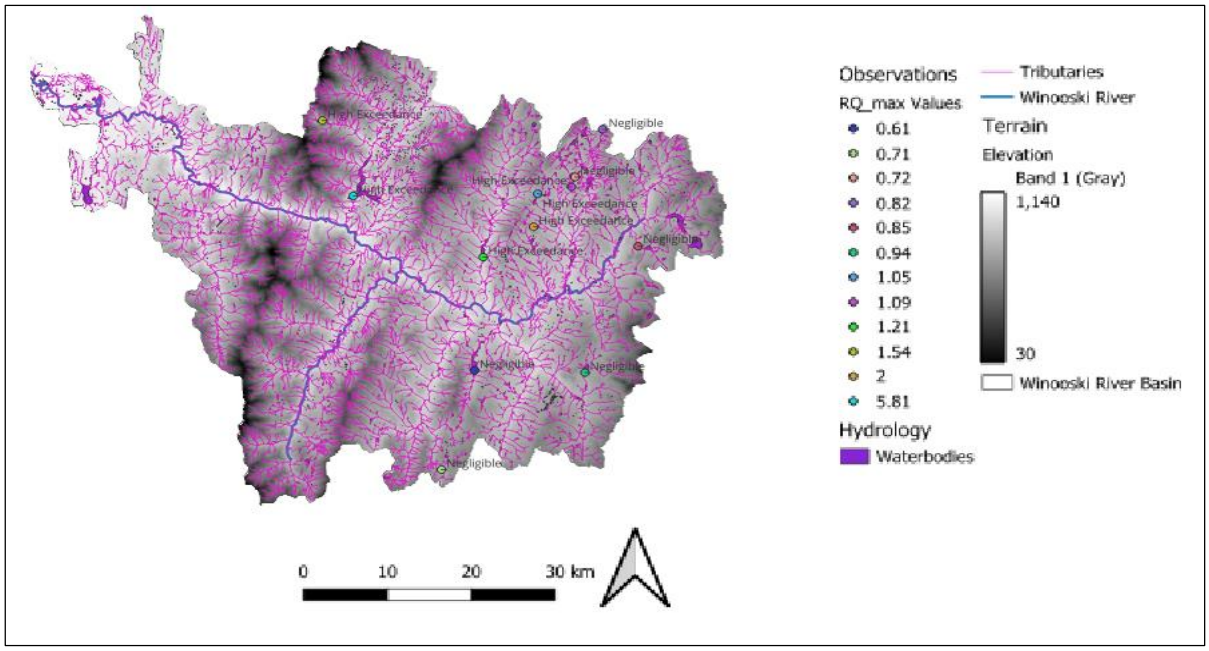

Fig. 4. Spatial hotspots of nutrient-related ecological concern based on worst-case Risk Quotients (RQ_max)

Note: Spatial distribution of nutrient-related ecological risk across the Winooski River Basin. Points represent sampling locations colored by worst-case Risk Quotient (RQmax), highlighting localized hotspots of nutrient-driven ecological concern.

Which nutrient stressors contribute most strongly to overall ecological risk?

Nutrient stressors did not contribute equally to ecological risk, supporting H2. Total phosphorus emerged as the primary driver of trophic state and algal biomass, showing the strongest positive association with chlorophyll-a and the greatest influence in the multivariate structure. Total nitrogen displayed weaker relationships with biological response variables, indicating a secondary role in productivity control, while chlorophyll-a exhibited the highest variability and most frequent risk exceedances. This pattern aligns with findings from the Yahara Lakes, where phosphorus consistently

explains the majority of variation in algal biomass, bloom frequency, and water-clarity decline, confirming its disproportionate influence on ecological condition [41].

Does a worst-case RQ metric (RQ_{max}) reveal patterns not captured by individual nutrient indicators?
The worst-case risk metric (RQ_{max}) revealed patterns of ecological vulnerability not captured by individual nutrient-based RQs, strongly supporting H3. While single-nutrient RQs often indicated low or moderate risk, RQ_{max} identified multiple sites with elevated vulnerability by capturing the highest exceedance among nutrient and biological indicators. This produced clearer separation between low-risk and high-risk waterbodies and highlighted locations where localized nutrient enrichment or biological response would otherwise be underestimated. Similar dynamics have been documented in lake systems where extreme precipitation and phosphorus-loading events were associated with later increases in cyanobacteria, demonstrating the value of worst-case approaches for detecting threshold-driven risk [42].

CONCLUSIONS

This study evaluated nutrient dynamics, trophic state, and ecological risk across waterbodies within the Winooski River Basin using a combination of water quality indicators, trophic indices, and statistical analyses. Results consistently demonstrated that phosphorus is the primary driver of algal biomass and trophic condition, as evidenced by strong relationships between total phosphorus and chlorophyll-a, as well as alignment across trophic state metrics derived from the Carlson Trophic State Index.

Trophic state conditions ranged from oligotrophic to mesotrophic, with localized instances approaching eutrophic conditions, indicating spatial variability in nutrient enrichment across the basin. Nutrient limitation analysis suggested that the system is generally co-limited to slightly phosphorus-limited, reinforcing the dominant role of phosphorus in controlling primary productivity. Ecological risk assessment further indicated that while overall risk levels were moderate, certain sites exhibited elevated Risk Quotient values, highlighting the presence of localized hotspots of nutrient-driven ecological concern.

Multivariate and statistical analyses supported these findings, identifying a primary gradient associated with nutrient enrichment and biological response, alongside secondary influences from physicochemical conditions such as temperature and dissolved oxygen. Together, these results demonstrate that water quality and ecological conditions within the basin are shaped by interactions among nutrient inputs, watershed processes, and environmental controls.

Management implications

The results highlight the need for targeted phosphorus-reduction strategies as the most effective pathway for mitigating eutrophication and lowering ecological risk across the basin. Because total phosphorus emerged as the dominant nutrient stressor and the strongest predictor of chlorophyll-a, water-clarity decline, and elevated risk quotients, management efforts should prioritize reducing nonpoint source inputs from agricultural land, developed areas, and shoreline disturbance. Sites identified as high-risk through RQ and RQ_{max} warrant continued monitoring to track nutrient trajectories and detect early signs of ecological deterioration. Incorporating temporal dynamics—particularly seasonal variability, storm-driven loading, and episodic nutrient pulses—will further refine risk assessments and improve the ability to anticipate harmful algal blooms and clarity declines. Collectively, these findings support a management framework centered on phosphorus control, targeted watershed interventions, and adaptive monitoring to maintain water quality and ecological integrity across freshwater systems.

Data Availability Statement

All water quality data used in this study were obtained from the EPA Water Quality Portal (https://www.waterqualitydata.us/), which aggregates monitoring records from federal, state, tribal, and local agencies. Spatial datasets, including the digital elevation model and hydrology layers, were

obtained from the Vermont Center for Geographic Information (VCGI). All processed datasets and analysis scripts used in this study are available from the corresponding author upon reasonable request. Dataset can be found at https://data.mendeley.com/datasets/6m85spf4fr/1.

AI Use Statement

I used an AI assistant to support sentence structure correction, improve grammar, and enhance clarity during manuscript preparation. All AI-generated suggestions were carefully reviewed, and all final editorial decisions were made by the authors. I confirm that the content, interpretations, and any remaining errors remain solely the responsibility of the authors.

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