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Watershed, lake, and food web factors influence diazotrophic cyanobacteria in mountain lakes

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Abstract

Cyanobacterial blooms can occur in freshwater ecosystems largely isolated from development and not experiencing extensive cultural eutrophication. For example, remote mountain lakes can experience intense blooms of diazotrophic (nitrogen-fixing) cyanobacteria caused by factors acting at different spatial and temporal scales. In this study, we examined how cross-scale interactions among watershed, lake, and food web characteristics influence diazotrophic cyanobacteria biovolume in mountain lakes. We quantified diazotrophic cyanobacteria biovolume, zooplankton abundance, and physico-chemical variables for 29 lakes in the Cascade Mountains of Oregon, USA, in summer 2019. Watershed characteristics were compiled from historical datasets available for the region. Diazotrophic cyanobacteria biovolume ranged across the lakes from 0 to 1,930,000 μm^3 mL^{-1}; *Dolichospermum* was the most common genus. Random forest models showed that 11 watershed, lake, and food web characteristics explained 76% of the variance in diazotrophic cyanobacteria biovolume among the sampled lakes. Structural equation models suggested that the drainage ratio (i.e., the relative area of the lake to the watershed) was positively related to phosphorus concentrations and, in turn, to diazotroph biovolume. Among lakes, hypolimnetic dissolved oxygen was negatively correlated with diazotroph biovolume, possibly due to the release of nutrients, like phosphate and iron, bound to sediments. In addition, zooplankton grazers were negatively related to diazotrophic cyanobacteria biovolume, potentially reflecting the influence of stocked fish. Thus, lake management must account for bottom-up factors, such as nutrient loading, which is influenced by lake morphometry and watershed size, as well as top-down factors, such as fish stocking, to effectively mitigate diazotrophic cyanobacterial blooms.

A combination of abiotic and biotic factors can interact to cause harmful blooms of cyanobacteria in freshwater ecosystems (Paerl and Otten 2013; Ger et al. 2016). Increasing loading of limiting nutrients, notably nitrogen and phosphorus, is a major factor driving increases in cyanobacterial biomass and harmful blooms in Northern Hemisphere lakes (Reynolds 2006; Rigosi et al. 2014; Taranu et al. 2015; MacKeigan et al. 2023). While cyanobacteria often bloom in eutrophic conditions, there is increasing evidence of blooms in nutrient-poor waterbodies (Sterner et al. 2020; Reinl et al. 2021). Some species that bloom in mesotrophic and oligotrophic waters are diazotrophic, which refers to the ability to fix atmospheric nitrogen, allowing these taxa to overcome nitrogen (N) limitation (Carey et al. 2008; Sterner et al. 2020; Reinl et al. 2021). In addition, climate warming may favor cyanobacteria, which often have optimal growth temperatures higher than other phytoplankton (Reynolds 2006). Food web shifts, including non-native fish introductions, may also contribute to harmful blooms, but the connection to cyanobacterial dominance and blooms is less certain (Paerl and Otten 2013; Ger et al. 2016; MacKeigan et al. 2023). The specific nature of how abiotic and biotic factors interact to control cyanobacterial blooms requires further study, especially in regions with minimal anthropogenic development where blooms occur and external nutrient loading is low (Paerl and Otten 2013; Taranu et al. 2015; Sterner et al. 2020; Reinl et al. 2021).

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Additional Supporting Information may be found in the online version of this article.

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Mountain lakes represent model systems for examining potential drivers of cyanobacteria, with natural gradients of nutrients and temperature (Catalan et al. 2006). The topographic complexity and elevational gradient of mountains create distinct microclimates and vegetation zones, which, in turn, impact lake thermal regimes, productivity, and food webs (Catalan et al. 2006; Sadro et al. 2012; Symons and Shurin 2016). In addition, frequent and repeated stocking of non-native fishes in naturally fishless mountain lakes has been widespread throughout the world. This practice has altered lake food webs with declines in large invertebrates and amphibians, as well as shifts in nutrient cycling (Knapp et al. 2001; Schindler et al. 2001). The variation, remoteness, and sensitivity of mountain lakes have shed new light on climatic influences of ecosystem processes, including how warming enhances trophic cascades and increases in primary production from summer storm events (Symons and Shurin 2016; Oleksy et al. 2020). However, mountain lakes differ from most lowland lakes in several important ways: mountain lakes tend to have higher UV radiation, lower water temperatures, and lower dissolved organic matter, resulting from less vegetation cover at high elevations, especially wetlands, deciduous forests, and agricultural land (Sommaruga 2001; Juetten et al. 2022). Therefore, finding commonalities among lakes across a gradient of elevation requires careful consideration of environmental differences. Key determinants of bloom-forming cyanobacteria, such as nutrients, climate, and grazers, have not been well studied in mountain lakes, which could provide novel insights into the complex issue of harmful algal blooms across different lake types (MacKeigan et al. 2023).

External nutrient inputs, non-native fish stocking, and climate change-induced warming also interact with landscape and lake characteristics, which, in turn, alters the effects of these factors on cyanobacteria abundance and composition. Therefore, examination of cross-scale interactions could offer new information on drivers of cyanobacteria. Cross-scale interactions occur when influential factors operate at different spatial or temporal scales and produce a unique response that differs from the sum of individual effects (Soranno et al. 2014). For example, increasing amounts of agriculture within a region can shift wetlands from acting as a phosphorus source to a sink for adjacent waterbodies (Soranno et al. 2014). These interactions may elucidate why specific drivers have different effects in different locations. In low-productivity mountain lakes with minimal nutrient loading from the watershed, a combination of several factors that influence dissolved oxygen (DO), nutrients, and temperature at different scales from the landscape to the lake affect cyanobacteria responses (Reinl et al. 2021; Molot et al. 2021*b*).

Watershed characteristics along elevational gradients in mountain ranges can influence nutrient loads and thermal regimes of lakes, interacting with regional and global atmospheric processes that may affect cyanobacteria (Catalan et al. 2006; Sadro et al. 2012). Atmospheric deposition of nitrogen (N) has increased since the Industrial Revolution and remains a major source of N for western US mountain ranges (Baron et al. 2011; Ackerman et al. 2019). However, the extent of lake enrichment from atmospheric deposition of N depends not only on proximity to sources, but also upon the amount of vegetation within the watershed, as N often limits terrestrial plant productivity (Kopáček et al. 2000; Baron et al. 2011). Increasing vegetation density and soil depth increases terrestrial uptake and retention of N in watersheds with minimal development, potentially reducing N loading downstream (Baron et al. 2011). Yet the extent of terrestrial N limitation depends on the abundance of terrestrial N-fixing plant species. Therefore, lakes in the subalpine and montane regions surrounded by predominantly forested watersheds may respond less readily to elevated atmospheric N deposition than alpine lakes with barren watersheds (Baron et al. 2011). Thus, N-limited subalpine and montane lakes may be particularly vulnerable to diazotrophic cyanobacteria blooms (Hayes et al. 2015).

Remote mountain lakes may also be experiencing increased phosphorus (P) loading due to increasing atmospheric P deposition from dust, as well as the burning of biomass and fossil fuels (Brahney et al. 2015; Stoddard et al. 2016; Scholz and Brahney 2022). In addition, P originating from the weathering of underlying bedrock varies with geology, from relatively low in silica-rich rock to relatively high in young volcanic rock (Porder and Ramachandran 2013; Hartmann et al. 2014; Scholz and Brahney 2022). Ongoing climate warming increases weathering rates, which may increase P loading depending on watershed geology (Goll et al. 2014). In addition, watershed size and topography influence nutrient loads to mountain lakes (Kopáček et al. 2000; Sadro et al. 2012). For example, watersheds vary from U-shaped and bowl-shaped basins due to glacial scour to shallow depressions with volcanic activity, resulting in different slopes and morphometry (Wetzel 2001). Groundwater sources, which are influenced by watershed geology, can sustain levels of limiting nutrients like P as surface flows decline in the dry season (Holman et al. 2008; Nolin 2012). In addition, topography, hydrology, vegetation, and ice cover can modify lake responses to large-scale climate shifts, such as lake temperatures, which are somewhat buffered when groundwater is the major inflow to the lake (Schindler 2006; Nolin 2012). Therefore, the responses of cyanobacteria need to be placed in the context of the watershed.

Lake characteristics also regulate nutrient and thermal dynamics, mediating the effects of larger-scale factors that ultimately affect cyanobacteria (Reynolds 2006; Adrian et al. 2009; Kraemer et al. 2015). As lake depth increases, residence time as well as dilution capacity also generally increase (Wetzel 2001). Therefore, shallow lakes may be more susceptible than deep lakes to elevated nutrient loading as nutrients quickly cycle through the ecosystem, favoring eutrophic phytoplankton (Reynolds 2006). In particular, diazotrophic cyanobacteria are more sensitive to P loading than non-diazotrophic cyanobacteria, potentially because N fixation is energetically expensive and requires additional P (Rigosi et al. 2014; Wan et al. 2019). One example of a cross-scale interaction of global and local factors is climate change-induced warming mediated by lake depth (Kraemer et al. 2015). Warming will likely increase overall temperatures in most lakes, especially shallow lakes, as well as alter the thermal stability of deep lakes, potentially prolonging stratification (Wagner and Adrian 2009; Kraemer et al. 2015; Preston et al. 2016). Prolonged stratification favors cyanobacteria species that can regulate their buoyancy and compete in nutrient-depleted conditions via diazotrophy, luxury P uptake, and other adaptations (Carey et al. 2008, 2012; Litchman and Klausmeier 2008). However, cyanobacteria blooms can also occur in polymictic shallow lakes during calm conditions (Wagner and Adrian 2009). While temperatures are rising at faster rates in the mountains than at lower elevations, the consequences of warming will vary with mountain lake morphology (Adrian et al. 2009; Pepin et al. 2015).

Although physical dynamics within lakes affect cyanobacteria, biotic processes can also have a substantial influence. Food webs can influence cyanobacteria and other phytoplankton directly through top-down predation and indirectly through nutrient cycling (Knapp et al. 2001; Schindler et al. 2001; Ger et al. 2016). These biotic interactions primarily occur in the euphotic pelagic zone of the lake, where phytoplankton mainly persist (Reynolds 2006). However, pelagic food web effects on cyanobacteria are also mediated by cross-scale interactions with large-scale drivers like climate change (Cavalli et al. 2001; Symons and Shurin 2016). Stocked fish in naturally fishless lakes can reduce the biomass of zooplankton by selectively feeding on larger taxa, releasing phytoplankton from grazing pressure in a trophic cascade (Knapp et al. 2001). Moreover, the effects of fish on lower trophic levels increase with temperature, suggesting that climate change will amplify trophic cascades (Symons and Shurin 2016). Stocking can result in higher P recycling even relative to lakes with naturally reproducing trout populations due to higher growth rates from ongoing stocking of planktivorous fingerlings (Schindler et al. 2001). Additional P may shift mountain lakes to be more N-limited, favoring diazotrophic cyanobacteria (Schindler et al. 2001). However, if lakes also receive elevated atmospheric deposition of both N and P, the combined inputs may favor other eutrophic phytoplankton (e.g., chlorophytes) (Nydick et al. 2011; Brahney et al. 2015). Therefore, biotic stressors, such as introduced predators, should be examined in the context of abiotic factors to determine the influence on phytoplankton biomass and composition.

Despite minimally developed watersheds, a subset of lakes in the Cascade Mountains (USA) have experienced toxic cyanobacteria blooms (> 300,000 μm^3 mL^{-1} in biovolume and toxin concentrations above public health thresholds) in recent years (Eilers et al. 2006*a*,*b*; Oregon Department of Environmental Quality 2011; Chorus and Welker 2021). Paleolimnological studies of two lakes in the region confirmed an increase in diazotrophic cyanobacteria from the 1970s to the 2000s (Eilers et al. 2006*a*,*b*). The affected lakes are located in the montane to subalpine zone at elevations between 1000 and 3000 m in the Southern Cascades (Oregon Department of Environmental Quality 2011). In contrast to alpine lakes, which lack extensive watershed vegetation and are relatively cold, lakes in the subalpine and montane have forested watersheds and warmer temperatures (Rose et al. 2009). Therefore, subalpine and montane lakes can be more readily compared to temperate lowland lakes than alpine lakes. Factors across multiple spatial scales (watershed, lake, food web of the pelagic zone) can drive cyanobacterial blooms in mountain lakes, with small-scale factors interacting with the effects of larger-scale factors (Adrian et al. 2009; Kraemer et al. 2015; Borrelli and Relyea 2022), that is, a cross-scale interaction. Lakes in the Cascades Mountains of Oregon serve as a model system to study the cross-scale interactions of factors influencing diazotrophic cyanobacteria.

Our study examined diazotrophic cyanobacteria in a subset of Oregon Cascade lakes and addressed the following question: How do cross-scale interactions between variables from the watershed, lake, and food web influence diazotrophic cyanobacteria biovolume in mountain lakes? We tested three hypothesized pathways (Fig. 1): (1) Food web: The top-down control of zooplankton by fish stocking affects diazotrophic cyanobacteria biovolume by reducing grazing pressure from large zooplankton in the pelagic zone (Knapp et al. 2001); (2) Nutrients: Fish stocking biomass, as well as watershed characteristics, drive changes in diazotrophic cyanobacteria biovolume by affecting nutrient concentrations. These effects vary across lakes and that variation is explained in part by lake thermal regime, as it may regulate internal nutrient loading (Kopáček et al. 2000; Kamenik et al. 2001; Schindler et al. 2001); and (3) Thermal regime: Relative groundwater contribution of the watershed as well as watershed topography and land cover drive changes in diazotrophic cyanobacteria biovolume by affecting lake temperatures. We predict that watershed effects vary across lakes and that variation is explained in part by the lake thermal regime as it regulates the warming of the water column (Wetzel 2001; Nolin 2012; Kraemer et al. 2015). Results from our study may inform the management of waterbodies mostly isolated from development yet still susceptible to diazotrophic cyanobacterial blooms.

Methods

Site description

The underlying geology of lakes in the Oregon portion of the Cascade Range is predominantly highly permeable basalt or andesite volcanic rocks, resulting in primarily groundwater-fed lakes (Johnson 1985; Nolin 2012). In addition, basalts tend to contain and weather significantly higher amounts of P compared to other types of rocks; therefore, the Cascade lakes tend to be less P-limited compared to mountain lakes underlain by other geologies (Porder and Ramachandran 2013). Oregon Cascade lakes range from ultra-oligotrophic to eutrophic, which is likely due to variations in specific geology and hydrology

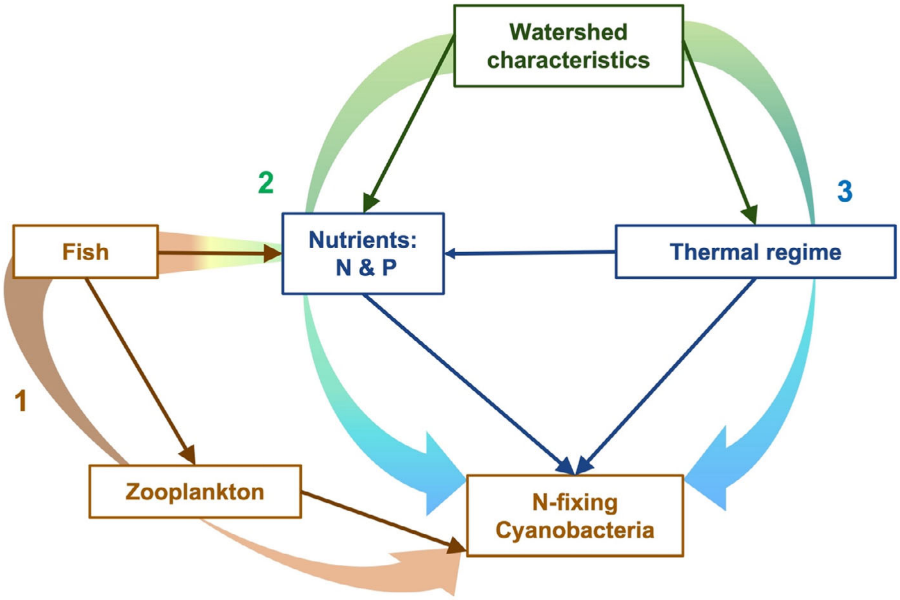

Fig. 1. Conceptual diagram of hypothesized pathways with cross-scale interactions (wide arrows with a color gradient representing the interaction between variables at different scales) and related direct effects (solid thin arrows) of the food web (orange), lake (blue) and watershed (green) on nitrogen-fixing cyanobacteria. We hypothesize that there are three key pathways by which N-fixing cyanobacteria are influenced (numbered and colored based on scale): (1) food web (brown), (2) nutrients (green/blue), (3) thermal (green/blue) (*see* text for further discussion).

(Johnson 1985). These lakes were primarily formed by glacial scour, such as morainal deposits damming river valleys and volcanic activity, creating depressions either from eruptions or collapses in lava flows (Johnson 1985).

Many of the Oregon Cascade lakes were originally fishless. Stocking began in the early 1900s, and today, several hundred lakes are regularly stocked with brook (*Salvelinus fontinalis*), rainbow (*Oncorhynchus mykiss*), and cutthroat (*Oncorhynchus clarkii*) trout (Oregon Department of Fish and Wildlife 2011). There are also multiple native amphibian species in Oregon Cascade lakes, including Western toads (*Anaxyrus boreas*), Oregon spotted frogs (*Rana pretiosa*), and Cascade frogs (*Rana cascadae*) (Adams et al. 2019). Macrophytes have been found in some of the more productive lakes in the Oregon Cascades (Johnson 1985; Portland State University 2020). Although amphibians and macrophytes have the potential to influence cyanobacteria (Bakker et al. 2010), surveys for these additional communities were beyond the scope of our project. We contend that their influence would likely be indirect and mediated by other variables that we directly measured in our study, such as nutrients and zooplankton (Takamura et al. 2003; Arribas et al. 2014).

The subalpine and montane of the Oregon Cascades are dominated by Douglas fir (*Pseudotsuga menziesii*) and Western hemlock (*Tsuga heterophylla*) in the west and Ponderosa pine (*Pinus ponderosa*) in the east. Red alder (*Alnus rubra*), which through a bacterial symbiosis can fix atmospheric nitrogen, is observed in low abundance up to 1300 m in the Oregon Cascades mountains, making up 0.4% of vegetation based on recent surveys (USDA Forest Service 2021). Given that our study lakes are largely above 1000 m in elevation (Supporting Information Table S1) and the small overall percentage of red alder in watershed vegetation, the contribution of nitrogen from red alder in the mountain lake watersheds is likely fairly limited. Most watersheds in the Oregon Cascades are undeveloped, and those with development either have public campgrounds, private resorts, cabins, or a mix of all three along the lakeshore (Portland State University 2020). All resorts, cabins, and campground facilities have either septic or contained sewage systems (Portland State University 2020).

Data compilation

We compiled a data set of 132 Oregon Cascade lakes ranging in elevation from 918 to 2127 m containing data on fish stocking, water chemistry, and watershed characteristics from various sources to identify a potential set of study lakes (Johnson 1985; USDA Forest Service 1996; US Environmental Protection Agency 2009). We only considered lakes > 10 ha and with a maximum depth of ≥ 3 m to exclude extremely small lakes or ponds that can be seasonally variable in depth and contain emergent vegetation (US Environmental Protection Agency 2009). We created a representative sample of lakes from this dataset using binary regression trees from the R package *rpart* v.4 (Therneau et al. 2019). A binary regression tree repeatedly divides the response data into nodes to reduce variation within nodes based on predictor variables. Total P concentration was the response variable as P is often a crucial lake characteristic for diazotrophic cyanobacteria and varies significantly in the Cascades (Johnson 1985; Williams et al. 2016). In addition, total P was the most commonly available chemical analyte for all lakes from the compiled datasets. We chose elevation, 10-yr annual average fish stocking biomass, and surface area as the predictor variables. From the resulting binary regression tree, we selected a similar number of lakes from each node to make up the 29 lakes of the study, which were sampled in summer 2019 (Supporting Information Figs. S1, S2).

We compiled lake morphometry data from the Atlas of Oregon Lakes database, including bathymetry, to calculate the Schmidt stability index (a measure of stratification strength) using the R package *rLakeAnalyzer* v. 11 (Johnson 1985; Winslow et al. 2019). We obtained annual fish stocking records from 1978 to 2019 for the study lakes, including biomass, count, and species (Oregon Department of Fish and Wildlife in press). Fish were mostly stocked as fingerlings < 250 mm long (fork length) and thus were assumed to be planktivores (Elser et al. 1995).

For many watershed variables, we extracted data from the LakeCat dataset, which provides watershed characteristics of lakes within the conterminous US derived from existing spatial data such as land cover, geology, and long-term climate (Supporting Information Table S1; Hill et al. 2018). As the watersheds have limited development, we combined the National Land Cover Dataset classes of developed open space, developed low intensity, developed medium intensity, and developed high intensity into one class: developed area. The average slope and aspect of watersheds were derived from Digital Elevation Models with 10-m resolution (US Geological Survey 2019). Maximum snow water equivalent (SWE) for each watershed in the previous winter (2018–2019) was derived from the Snow Data Assimilation System daily estimated SWE, selecting the days based on the maximum SWE at the nearest SNOTEL site for each watershed (National Operational Hydrologic Remote Sensing Center 2004; USDA Natural Resources Conservation Service 2020). Percent change in forest cover in each watershed for the past 20 yr was derived from the spatial Global Forest Change dataset for 2000–2019 (Hansen et al. 2013). We calculated the drainage ratio for each lake by dividing the watershed area by the lake's surface area.

Lake and food web: Field sampling

Each lake ($n = 29$) was sampled twice in summer 2019 to capture within-season variation, with the first sampling occurring between 24 June and 13 July and the second between 08 August and 31 August. We measured physical and chemical variables at the deepest spot in the lake, including a complete depth profile of temperature and DO with a YSI ProODO meter, as well as specific conductance and pH with a YSI ProQuatro multiparameter meter (Yellow Springs, Ohio, USA). We calibrated the YSI meters every 3–5 d based on standard protocols. For the stratified lakes, the profile data were divided into the thermal layers (epilimnion, metalimnion, hypolimnion) to calculate average epilimnion temperature, mixed layer depth (i.e., the bottom of the epilimnion), and average hypolimnetic DO. For the two unstratified lakes, average whole water column temperature was used in lieu of epilimnetic temperature and the bottom DO value was used in lieu of average hypolimnetic DO. The only temperature variable used for statistical analyses was average epilimnetic temperature.

We sampled for phytoplankton with a Van Dorn sampler (Wildco) at 1 m below the surface at the deepest point in the lake, identified using bathymetric maps and a depth sounder. Samples were collected in 250-mL brown Nalgene bottles and preserved for identification using Lugol's solution. An additional sample was taken at the deep spot and a known volume was filtered out of direct sunlight onto glass fiber filters (Whatman GF/C, 1.2-μm pore size) for chlorophyll *a* (Chl *a*) analysis. For Chl *a*, we collected samples in triplicate at each lake to account for in-lake variation. We collected samples for nutrient analyses (total N and P) from the top 5 m of the water column, using a 5-m long, 2.54-cm diameter Tygon tube and transferred them into 125-mL HDPE bottles, which were kept cool and preserved with H_2SO_4 until frozen in the laboratory at -18°C. For nutrients, we collected samples in duplicate at each lake. We conducted an integrated tow of the water column for crustacean zooplankton using a plankton net with 80-μm mesh and 25-cm diameter, starting two meters from the bottom and preserving the sample in 70% ethanol for identification.

Lake and food web: Lab analyses

We concentrated preserved phytoplankton samples before counting by gently mixing the sample for 5 min and then taking a 100-mL subsample for settling in a graduated cylinder. After 100 h of settling, the top 98 mL were removed via a vacuum pump and reserved to dilute, while the remaining 2 mL were used for counting. We counted and identified 300 natural units per concentrated sample in Palmer counting cells to the genus level or to the lowest taxonomic level possible, using taxonomic guides (Smith 1950; Wehr 2002; Matthews 2016) with a Leica DM1000 microscope at ×400 and ICC50 HD camera (Leica Microsystems Inc.). Diazotrophic genera were determined based on current literature (Reynolds 2006). We measured the dimensions of 20 individuals of each taxon in each sample to calculate biovolume using standardized equations based on the shapes of taxa (Supporting Information Table S2; Hillebrand et al. 1999). For filamentous and colonial taxa, we measured the dimensions for 10 cells of each individual. For zooplankton, we counted and identified 250 individuals from each preserved sample to the order level for Copepoda and to the family level for Cladocera using taxonomic guides (Balcer et al. 1984; Thorp and Covich 2014) with a Leica M165C microscope at ×100 and IC80HD camera. For further analyses, cladocerans were aggregated into two groups based on different feeding impacts, as *Daphnia*, large and efficient, and small cladoceran grazers, smaller and less efficient (e.g., *Bosmina*, *Holopedium*, *Ceriodaphnia*) (DeMott 1982; Reynolds 2006).

We extracted Chl *a* from filters using acetone for 20 h in a dark refrigerator and measured concentrations using a fluorometer following Arar and Collins (1997). We used a persulfate solution to digest total P samples heated to 100°C and then analyzed with a Shimadzu UV-1800 spectrophotometer (Kyoto, Japan) using the molybdenum blue colorimetric method (detection limit: $0.002\ \text{mg L}^{-1}$; precision limit: $\pm 0.004\ \text{mg L}^{-1}$) (APHA 2018*b*). We also used a persulfate solution to digest total N samples heated to 100°C and then analyzed with a SmartChem 200 discrete analyzer (Guidonia, Italy) for colorimetric determination of nitrate and nitrite (detection limit: $0.01\ \text{mg L}^{-1}$; precision limit: $\pm 0.01\ \text{mg L}^{-1}$) (APHA 2018*a*). In addition to field replicates, we analyzed a field blank of ultrapure deionized water for every five lakes sampled for Chl *a* and nutrients to account for any potential contamination.

Statistical analyses

The relative importance of lake, food web, and watershed characteristics to diazotrophic cyanobacteria biovolume was determined using a two-step process. First, machine learning methods were used to identify the most important variables for diazotrophic cyanobacteria. Second, these variables were used in a structural equation model (SEM) to test the hypothesized simple and interactive effects of characteristics from the three different pathways (Fig. 1).

First, we examined and ranked the potential influence of all variables using two complementary machine learning methods: binary regression tree and random forest models. This approach was chosen because a single binary regression tree provides an interpretable graphic on the entire regression function but is often unstable. In comparison, a random forest model, which generates hundreds of trees based on bootstrap samples, provides robust estimates of variable importance, but limited information on the overall regression function (Breiman 1996; De'ath and Fabricius 2000). Predictors were removed if they had zero or near zero variance based on the percent of unique values ($< 10\%$) and frequency ratio (i.e., frequency of the most common value divided by the frequency of the second most common value) (Kuhn 2008; Kuhn and Johnson 2013). Correlations between predictors were examined using Spearman's rank coefficient due to non-normal distributions, and one predictor of a pair was removed if $\rho > 0.80$ (Kuhn and Johnson 2013). For example, maximum depth was positively correlated with the Schmidt stability index ($\rho = 0.85$, $p < 0.001$), so the latter was dropped (Jansen et al. 2023). Lake depth influences the thermal stratification of lakes, so depth can serve as a factor for the thermal regime (Kraemer et al. 2015). Epilimnetic DO and pH were dropped as predictors as variation may be mainly a consequence of algal production, especially in poorly buffered lakes such as in the Cascades (Wetzel 2001; Reynolds 2006). Hypolimnetic DO was kept as a predictor as it is a key factor for internal P loading through anoxic release of sediment-bound P, though it can also be indirectly influenced by algal production (Nürnberg 1984). Yet summer hypoxia in the hypolimnion occurred with a range of phytoplankton biomass, suggestive of other factors such as lake thermal regime (Supporting Information Fig. S3).

After the pre-processing steps, there were 38 predictor variables remaining from the lake, food web, and watershed categories (Table 1; Supporting Information Tables S1, S3, S4). The correlation between these final 38 predictors was reexamined using the Spearman rank coefficient (Jansen et al. 2023). Binary regression tree and random forest models are sensitive to non-constant variation in the response variable (De'ath and Fabricius 2000); therefore, diazotrophic cyanobacteria biovolume was natural log (ln)-transformed. In addition, one lake lacked bathymetry data (thus, mean depth was not calculated), and another lake had missing total N concentrations due to an analytical error. The missing data were imputed as the median value for the variable for the random forest models. The values of the variables were averaged between the two sampling bouts to avoid temporal pseudoreplication. Variation in diazotrophic cyanobacteria dominance was somewhat similar across bouts (Supporting Information Fig. S4).

We built binary regression tree and random forest models using variance explained, model error, and cross-validation to check and improve model fit. We adjusted the binary regression tree from the default settings to allow a minimum of eight observations per split due to the small sample size as well as 10-fold cross-validation and a complexity parameter of 0.01 to prevent overfitting (Breiman 1996; De'ath and Fabricius 2000; Therneau et al. 2019). The final model was selected based on the minimum cross-validated relative error and root mean squared error (RMSE). We created random forest models with the R packages *caret* v. 6.0, *randomForest* v. 4.6, and *pdp* v. 0.7 (Breiman 1996; Kuhn 2008; Greenwell 2017; Breiman and Cutler 2018). For the random forest models, we used 10-fold cross-validation repeated three times on different random subsets to reduce the error in the estimated mean model performance. We used the default of 500 trees. The number of predictors randomly selected at each split was set at the square root of the number of predictors. Variable importance was measured using the increase in mean squared error if the variable was randomly imputed. A reduced random forest model was developed to improve interpretation by dropping predictors iteratively based on their variable importance in the full model, starting with the least important, checking RMSE and pseudo-R^2. Once the model fit was no longer improved by the removal of a predictor (pseudo-R^2 decreased and RMSE increased), the process stopped.

Second, we used SEMs to test the hypothesized simple effects and cross-scale interactions of the food web, lake, and

Table 1. Summary statistics for diazotrophic cyanobacteria biovolume and the predictor variables that were candidates for the best binary regression tree and random forest models. Median, minimum, and maximum were calculated for variables by each sampling bout (early and late summer) and summer average (averaging across dates). Northness is a measure of direction with values ranging from 1 (due north) to −1 (due south).

	Early summer	Late summer	Summer average
Parameter	Median (min–max)	Median (min–max)	Median (min–max)
Diazotrophic cyanobacteria biovolume (μm^3 mL^{-1})	32 (0–217,000)	41 (0–1,930,000)	71 (0–967,000)
Food web			
Daphnia (# L^{-1})	771 (0–76,000)	1781 (0–13,100)	2140 (0–41,300)
Small cladoceran grazers (# L^{-1})	588 (0–18,300)	842 (0–24,400)	886 (0–18,500)
Lake environment			
Specific conductance (μS cm^{-1})	23.5 (2.0–492.0)	27.2 (2.3–590.0)	24.6 (2–541.0)
Total phosphorus (mg L^{-1})	0.010 (0.001–0.370)	0.003 (0.001–0.080)	0.010 (0.001–0.220)
Total nitrogen (mg L^{-1})	0.070 (0.001–0.161)	0.030 (0.001–0.136)	0.020 (0.000–0.360)
Mixed layer depth (m)	9.0 (1.0–19.2)	10.0 (1.0–19.2)	9.5 (1.0–19.2)
Maximum depth (m)	–	–	19.2 (4.9–86.0)
Hypolimnetic dissolved oxygen (mg L^{-1})	7.0 (0.3–11.4)	6.2 (0.3–20.4)	6.8 (0.3–14.7)
Watershed			
Latitude (°)	–	–	43.8 (42.4–45.5)
Drainage ratio	–	–	12.3 (2.0–206.0)
Developed area (%)	–	–	0.01 (0.00–4.17)
Base flow index (%)	–	–	79.7 (56.3–86.0)
Northness [cos(aspect)]			0.16 (−0.21–0.69)

watershed characteristics on diazotrophic cyanobacteria biovolume (Fig. 1). Cross-scale interactions are defined as influential factors that operate at different spatial or temporal scales producing a unique response that differs from the sum of individual effects (Soranno et al. 2014). SEMs were used as these models allow variables to be both independent and dependent, so simple and interactive effects can be separated (Grace 2006). Cross-scale interactions have been assessed previously using multilevel SEMs, as well as setting large-scale variables as exogenous variables acting indirectly through smaller-scale variables (Grace et al. 2016; Loewen et al. 2020). Thus, we interpreted the presence of a cross-scale interaction as a significant pathway involving multiple scales. The SEMs were restricted in complexity due to the small sample size; therefore, a single indicator variable for each main component in the conceptual model (watershed, nutrients, thermal regime, food web) was selected based on variable importance in the final random forest model and existing literature. We acknowledge that this approach may have missed some key variable interactions.

We scaled all variables by calculating the *Z*-scores to correct for different measurement scales and normalize variance because SEMs are limited to estimating linear relationships. In addition, we fitted the SEMs by fixing the independent variables' variances and covariances to one and zero, respectively, to limit the number of parameters estimated due to the small sample size. We started with a full model as described in Fig. 1. We examined nested models to test the relative importance of simple and interactive effects of predictors by constraining specific paths that are free in the full model (Grace 2006). We used the model Satorra–Bentler scaled χ^2, which is a robust test statistic for skewed data, as well as R^2 for endogenous variables to assess the goodness of fit for each model (Rosseel 2012). Model fit was also evaluated using root mean square error of approximation (RMSEA) as well as comparative fit index (CFI). CFI represents the amount of variance that has been accounted for in a covariance matrix (Rosseel 2012). For model comparison, we used the Satorra–Bentler scaled χ^2 difference test. All path coefficients in the SEMs were standardized. SEMs were developed using the R package *lavaan v.* 0.6 (Rosseel 2012). All statistical analyses were conducted in R 4.1.1 (R Development Core Team 2021).

Results

Data summary

Study lakes ($n = 29$) had similar watershed geology, mainly dominated by volcanic extrusive rock, and were mostly groundwater fed (Supporting Information Table S1). The elevation range of the final set of study lakes was 958 to 1983 m, located within the subalpine and montane zones (Supporting Information Table S1). The lakes varied notably in surface area and mean depth (Table 1). While the majority of lakes were oligotrophic, several were mesotrophic (Table 1). The dominant land cover of the watersheds was coniferous forest (Table 1). Most of the watersheds had minimal development, with a maximum of 4.2% developed area, which largely consisted of developed open space in the form of campgrounds and roads (Table 1; Supporting Information Table S1).

Biovolumes of diazotrophic cyanobacteria were similar between the early and late summer among all lakes (Wilcoxon signed-rank test: $W = 114$, $p = 0.20$), but the range in biovolume was greater in the late summer with a maximum of 1,930,000 $\mu m^3\ mL^{-1}$ (Table 1). The most common genera of diazotrophic cyanobacteria present were *Dolichospermum* and *Gloeotrichia*.

The most common zooplankton groups were cyclopoids and *Daphnia*, although a few lakes were dominated by small cladocerans and calanoids (Supporting Information Table S4). In terms of fish populations, 23 of the lakes have been actively stocked in the past 10 yr while the other 6 lakes have natural reproducing populations (Supporting Information Table S4;

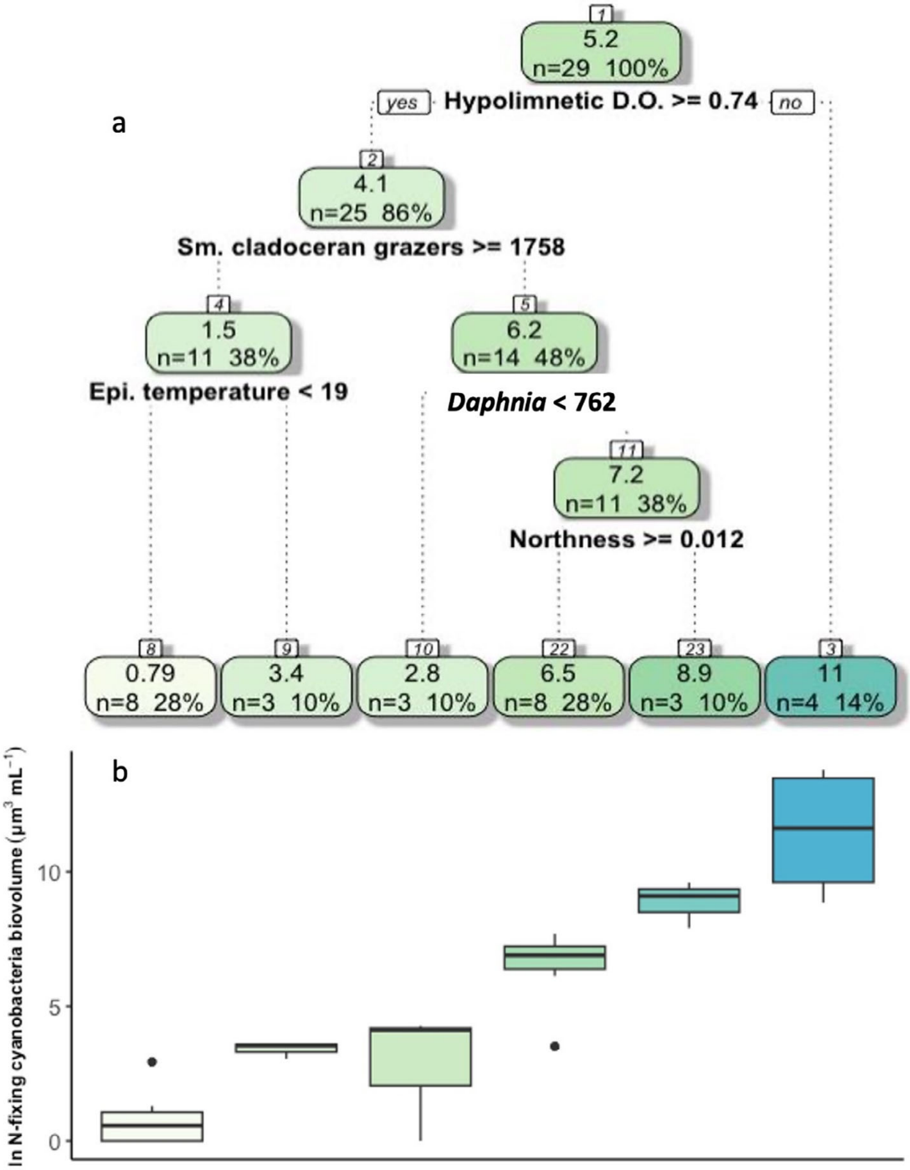

Fig. 2. (**a**) Binary regression tree for diazotrophic cyanobacteria with the five predictors driving the splits: hypolimnetic dissolved oxygen (D.O., mg L^{-1}), small (Sm.) cladoceran grazers (# L^{-1}), average epilimnetic (epi.) temperature (°C), *Daphnia* (# L^{-1}), and degree of northern aspect (cos [aspect]). Each node at the bottom of the tree represents the average diazotrophic cyanobacteria biovolume ($\mu m^3\ mL^{-1}$, ln-transformed) and (*n*) number of lakes for that cluster. (**b**) Boxplot of ln-transformed (unit) total biovolume of diazotrophic (N-fixing) cyanobacteria averaged across sampling bouts of lakes within each terminal node. The median is the bold line within the box and the bottom and top of the box defined by the first and third quartiles. The whisker extends no further than 1.5 times the interquartile range. The boxplots in (**b**) correspond to the nodes in (**a**).

Oregon Department of Fish and Wildlife 2011). Stocking rates varied from less than 0.01 to 135.1 kg ha^{-1} (Supporting Information Table S4).

Identification of top predictors

The optimal binary regression tree included the following lake, watershed, and food web predictors: hypolimnetic DO, small cladoceran grazers, epilimnetic temperature, *Daphnia*, and northness (Fig. 2). The model had a pseudo-R^2 of 0.89 and an RMSE of 1.40 with five splits selected. The highest diazotrophic cyanobacteria biovolumes (range: 7040–967,000 μm^3 mL^{-1}) occurred in lakes with low hypolimnetic DO as well as relatively high densities of *Daphnia* (Supporting Information Table S5). These lakes also had summer epilimnetic temperatures close to the overall median of 17.6°C (Supporting Information Table S5). In contrast, the lowest diazotrophic cyanobacteria biovolumes (range: 0–20 μm^3 mL^{-1}) occurred in lakes with some of the highest densities of small cladoceran grazers and the lowest summer epilimnetic temperatures.

We found the fit of the reduced and the full random forest model to be similar, as was the variance explained, so the reduced random forest model was used for further analyses. The final random forest model contained 11 predictors and explained 76.1% of the variance in diazotrophic cyanobacteria biovolume with an RMSE = 2.57 (Fig. 3). The predictors were a mix of food web, lake, and watershed characteristics with the six most important predictors being hypolimnetic DO, drainage ratio, small cladoceran grazers, specific conductance, total P, and the relative amount of total developed area (% of watershed). Epilimnetic temperature and degree of northern aspect (i.e., northness), which were predictors from the binary regression tree, were not among the predictors in the final random forest model, suggesting limited influence. In addition, the nodes of the single binary regression tree beyond the primary and secondary splits are especially unstable (Breiman 1996). Therefore, these variables may not be important once averaging across hundreds of trees based on bootstrap samples in the random forest model.

The marginal relationship, which is the relationship when all other predictors are kept fixed, that each predictor in the

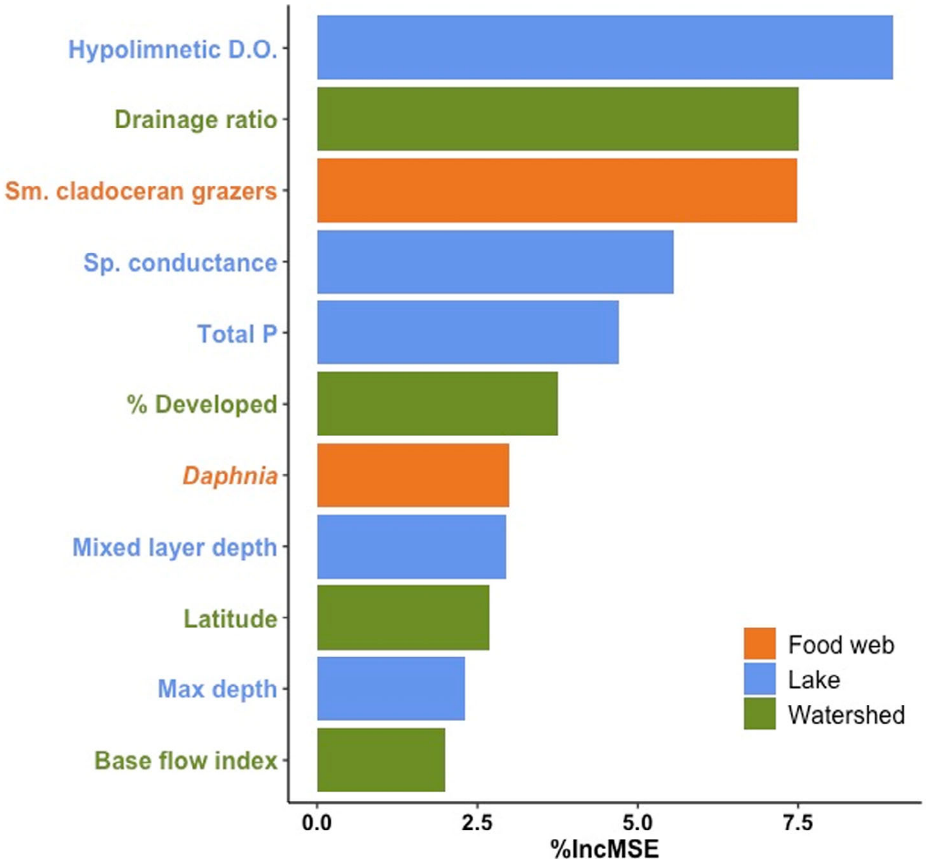

Fig. 3. Variable importance plot of 11 most important predictors for nitrogen-fixing cyanobacteria biovolume (ln-transformed) based on the final reduced random forest model, categorized by food web, lake, and watershed. %IncMSE = percent increase in mean squared error, a measure of the decrease in model accuracy when each variable is dropped. Abbreviations: hypolimnetic dissolved oxygen (D.O.), small (sm.) cladoceran grazers, and specific (sp.) conductance.

random forest model had with diazotrophic cyanobacteria biovolume varied in directionality and shape (Fig. 4). The marginal relationship of hypolimnetic DO with diazotrophs was negative, with biovolume declining most sharply as DO increased from 0.3 to 1.5 mg L^{-1} (Fig. 4a). Drainage ratio, specific conductance, and *Daphnia* abundance had similar positive relationships with diazotroph biovolume (Fig. 4b,d,g). The marginal effect of small cladoceran grazer abundances was negative, with biovolume declining as these grazers increased from 0 to 3000 L^{-1} (Fig. 4c). The relative amount of developed area had a more gradual positive relationship overall, but this relationship should be interpreted with caution as 83% of the lakes have $< 1\%$ developed area in their watershed (Fig. 4f). Total P had a positive marginal effect from 0.001 to 0.040 mg L^{-1}. Latitude, which annual precipitation was highly correlated with (Jansen et al. 2023), had a gradual negative relationship with biovolume overall (Fig. 4). Maximum depth and mixed layer depth had similar negative relationships with biovolume, although mixed layer depth had a more complex shape (Fig. 4h,j). The relative flow from groundwater (% base flow) had a positive marginal effect, although this relationship should be interpreted with caution as $> 90\%$ of the watersheds obtain 70% of their flows from groundwater (Fig. 4k; Supporting Information Table S1).

Potential cross-scale interactions

We found that the SEM, which was based on the original conceptual model in Fig. 1, explained a substantial part of the variation in diazotrophic cyanobacteria biovolume across the study lakes ($n = 29$). A single variable was selected to represent each component in the full and nested SEMs based on the final random forest model and existing literature (Fig. 5). The variables included drainage ratio (watershed characteristics), maximum depth (lake thermal regime), total P (nutrients), and small grazing cladocerans (zooplankton). The full model produced a good fit with the data (Satorra–Bentler $\chi^2 = 5.69$, df $= 5$, $p = 0.34$, RMSEA $= 0.08$, CFI $= 0.96$) and explained 37% of the variance in the response variable.

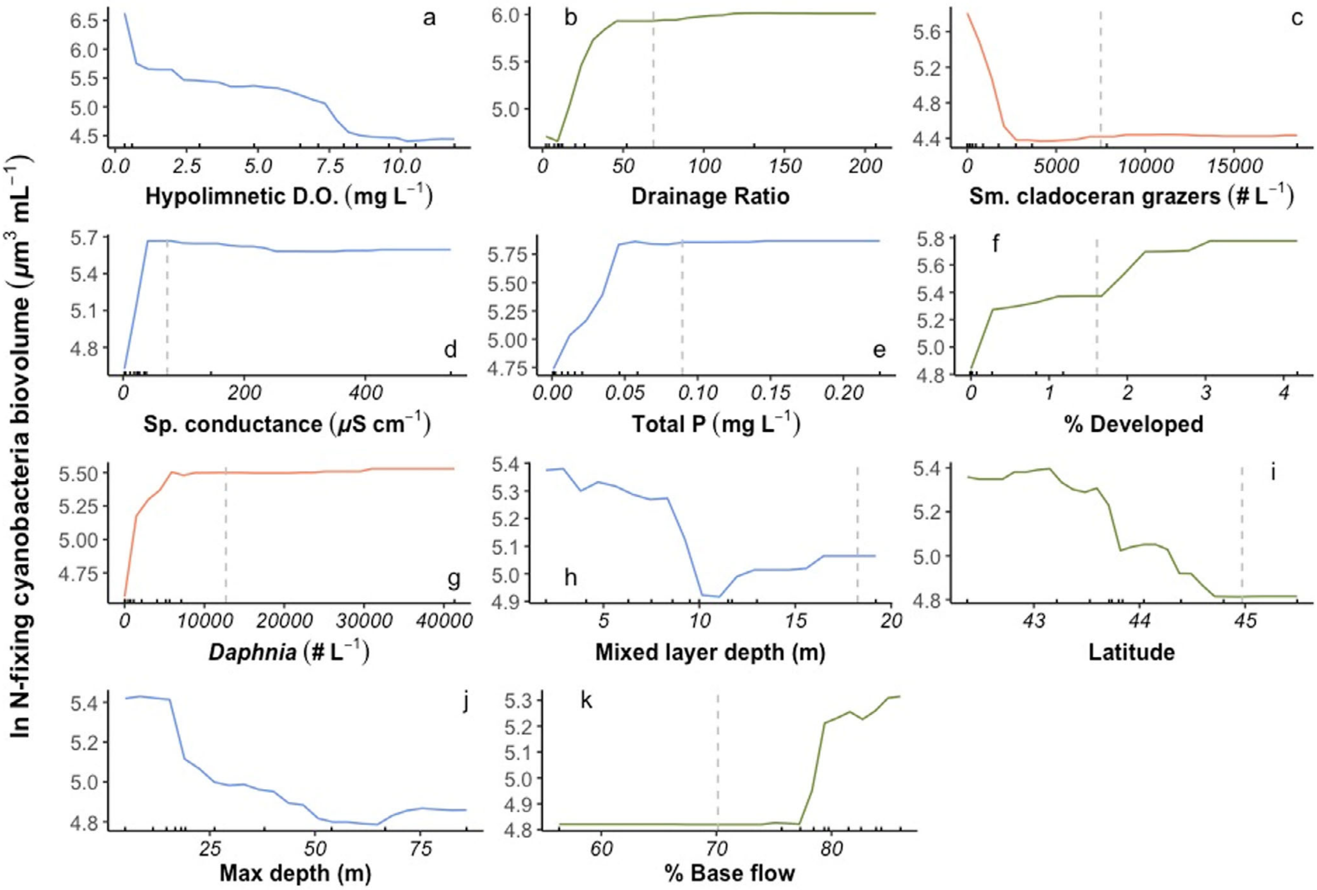

Fig. 4. Partial dependence plots of the 11 most important predictors (**a–k**) based on the final random forest model. The *y*-axis shows the amount of change in the response variable relative to the change in the predictor so the *y*-axes vary with each variable. The dashed line to the right demarks outliers based on 1.5 times the interquartile range above the third quartile or below the first quartile (Tukey 1977). The dashed line represents the upper outlier; for all predictors except Base flow and Max depth, the curve to the right of the line should be interpreted with caution. The curve to the left of the dashed outlier line (lower outlier) for Base flow should be interpreted with caution. Abbreviations: hypolimnetic dissolved oxygen (D.O.), small (sm.) cladoceran grazers, and specific (sp.) conductance.

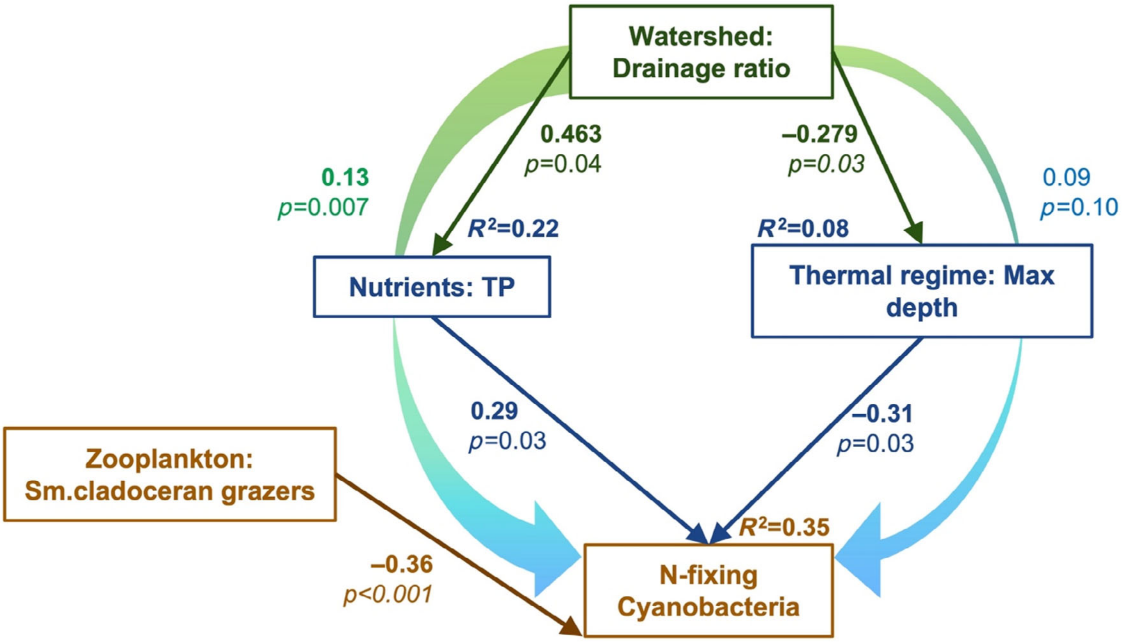

Fig. 5. Path diagram of the final SEM of the effects of drainage ratio, small cladoceran grazers, total phosphorus, and maximum depth on naturally log transformed (ln) diazotrophic cyanobacteria biovolume. The paths are colored by the scales: food web (orange), lake (blue) and watershed (green). The values near each path are the standardized coefficients with the associated p values below ($p < 0.05$ in bold and thick lines) and the variance explained (R^2) being reported for total phosphorus (TP), maximum depth, and diazotrophic cyanobacteria. The cross-scale interactions are represented as in Fig. 1 by the gradient arrows ($p < 0.05$ in thicker lines) along with the coefficients and p values.

Among the nested models, the reduced model that dropped the thermal regime–nutrient path and the zooplankton-nutrient path had the best fit ($\chi^2 = 7.93$, df $= 7$, $p = 0.34$, RMSEA $= 0.06$, CFI $= 0.95$) and explained the same amount of variance ($R^2 = 0.35$) (Fig. 5). The difference between the likelihood ratios for the reduced and full models was nonsignificant so the reduced model was the preferred final model (S-B scaled difference $\chi^2 = 2.28$, df $= 2$, $p = 0.32$).

The final SEM showed multiple significant associations with diazotrophic cyanobacteria biovolume through each pathway as well as a potential cross-scale interaction through the nutrient pathway (Fig. 5). Drainage ratio was negatively related to maximum depth and positively related to total P. Small cladoceran grazers and maximum depth were negatively associated with diazotrophic cyanobacteria biovolume. Total P was positively associated with biovolume. Drainage ratio explained 22% of the variation in the total P and 8% of the variation in maximum depth. The cross-scale interaction of drainage ratio on diazotrophs via maximum depth was not significant (path coefficient $= 0.087$, $p = 0.101$), while the cross-scale interaction of drainage ratio on diazotrophs via total P was significant (path coefficient $= 0.133$, $p = 0.007$) (Fig. 5).

As hypolimnetic DO was identified as the top predictor in the regression tree and final random forest model, the association of hypolimnetic DO with diazotrophs and the potential interaction with total P were examined in a separate SEM, as the sample size was insufficient to include hypolimnetic DO in the full model (Supporting Information Fig. S5). This model produced a good fit with the data (Satorra–Bentler $\chi^2 = 4.46$, df $= 3$, $p = 0.22$) and explained 33% of the variance in diazotroph biovolume. Only the negative association of hypolimnetic DO and diazotrophs was significant (Supporting Information Fig. S5; path coefficient $= -0.59$, $p < 0.001$).

Discussion

Our results suggest that cross-scale interactions between watershed, lake, and food web characteristics influence diazotrophic cyanobacteria biovolume. There was mixed support for the hypothesized pathways. We found diazotroph biovolume was related to nutrients, which was, in turn, influenced by watershed size as well as hypolimnetic oxygen (Figs. 3–5). We hypothesized that the mechanisms behind these relationships are that larger watersheds likely have higher external loading, whereas depleted hypolimnetic oxygen may contribute nutrients via internal loading. In addition, grazing zooplankton may have also driven diazotroph biovolume, potentially reflecting differences in fish populations (Figs. 3–5). Temperature and related watershed characteristics appeared to have relatively less influence on diazotrophs, although the mixing regime of the lake likely drove some variation in diazotrophs. Thus, food web and nutrient factors may be more important than thermal factors for diazotrophic cyanobacteria in mountain lakes.

Nutrient pathway

P appeared to have a greater influence on diazotrophic cyanobacteria biomass than N in our study, concurring with other studies (Rigosi et al. 2014; Wan et al. 2019; MacKeigan et al. 2023). In addition, epilimnetic P had an asymptotic nonlinear relationship with diazotrophic cyanobacteria, which may be due in part to accessible sediment P. We recognize not all total P is bioavailable. Orthophosphate concentrations were, on average, 60% of total P concentrations in 12 of the same lakes resampled in 2020, suggesting most of the total P was bioavailable (Jansen, unpublished data). We also acknowledge that because phytoplankton contain nutrients, then total P and N may reflect algal biovolume, so future studies should examine dissolved inorganic components of N and P. Surprisingly, N was not a significant factor for diazotrophic cyanobacterial biomass in the study lakes. Diazotrophs tolerate low N and, therefore, we expected a negative relationship with N (Reynolds 2006; Rigosi et al. 2014). The limited influence of N may be due to most of the lakes being fairly low in N (< 0.04 mg L^{-1}) and having a ratio of N to P less than 30 (Supporting Information Table S3), which is a known threshold below where diazotrophic cyanobacteria can be prevalent (Smith 1983; MacKeigan et al. 2023). Therefore, P was likely more influential on diazotrophic cyanobacteria than N in these lakes that are likely N-limited, like other mountain and lowland lakes in forested watersheds (Hayes et al. 2015; Williams et al. 2016).

The expected effect of fish on diazotrophic cyanobacteria (via increasing P cycling) was not supported (Vanni and Layne 1997; Schindler et al. 2001; Sharitt et al. 2021). When planktivorous fish are relatively abundant, they are the dominant source of recycled P through excretion and decomposition (Vanni and Layne 1997). However, when planktivorous fish are less abundant or absent, zooplankton are significant contributors to P recycling (Vanni and Layne 1997). In addition, the amount of P excreted relative to N varies with zooplankton taxa: *Daphnia* excretes N : P at a higher ratio relative to copepods, and therefore, lakes dominated by *Daphnia* may be less favorable for diazotrophic cyanobacteria (Vanni and Layne 1997). By contrast, *Daphnia* and diazotrophic cyanobacteria had a positive relationship in our study (Fig. 4), suggesting other pathways, such as the limited consumption of diazotrophs by *Daphnia*, may be more influential. We also found no significant relationship between stocked fish biomass and TP (Jansen et al. 2023). The effect of fish and zooplankton on P cycling is highly variable across and within lakes (Vanni and Layne 1997; Schindler et al. 2001; Sharitt et al. 2021), providing support for our findings.

Sources of N may be relatively low compared to P in the lakes of the Cascades and similar environments. Atmospheric deposition is a major source of N in mountain lakes (Kopáček et al. 2000; Baron et al. 2011). Watersheds in our study spanned gradients of precipitation and atmospheric N deposition, increasing from south to north. Only the most northern watersheds have exceeded the critical N loads of atmospheric deposition estimated for the Cascades (Williams et al. 2016, 2017; NADP Program Office 2022), which may be due to their proximity to the Portland metropolitan area. High atmospheric N deposition in the most northern watersheds may partially explain the low biomass of diazotrophic cyanobacteria in these lakes (Fig. 4). In addition, terrestrial vegetation can be N limited, dependent on the abundance of N-fixing species, in minimally disturbed areas like the subalpine and montane, reducing the effect of atmospheric N deposition on lakes (Kopáček et al. 2000; Baron et al. 2011; Sadro et al. 2012; Williams et al. 2016). Rock weathering is another important source of N in relatively undeveloped mountains (Kopáček et al. 2000). However, the igneous bedrock of the Oregon Cascades and other mountain ranges (e.g., Sierra Nevadas, western Andes) are also low in N relative to sedimentary rocks (Holloway et al. 1998; Hartmann and Moosdorf 2012). Therefore, downstream runoff may also be low in N, which was reflected in most of our study lakes with low total N. In addition, nearly all study watersheds were dominated by relatively young volcanic bedrock, which is high in P (Catalan et al. 2006; Nolin 2012; Porder and Ramachandran 2013). Increasing watershed size may have amplified the effects of geological contributions of high P relative to N. Some of the largest watersheds also had the greatest amount of developed area, but there were only five watersheds with more than 1% developed area (Table 1). In addition, the total developed area was only weakly correlated with TP and not at all with TN (Jansen et al. 2023). Yet we acknowledge that even relatively little ($< 5\%$ of the watershed area) anthropogenic land use, concentrated around the lakeshore, may positively influence diazotrophic cyanobacteria (Doubek et al. 2015). Further study is needed to disentangle the role of limited development relative to the other natural watershed drivers of diazotrophic cyanobacteria (Doubek et al. 2015).

Hypolimnetic DO has a crucial but complex relationship with diazotrophic cyanobacteria via internal nutrient cycling and decomposition. Hypoxia and anoxia in the hypolimnion were more common in our lakes, which often have limited inputs of organic matter and nutrients, than expected based on prior studies of mountain lakes (Catalan et al. 2006; Juetten et al. 2022). Anoxic conditions release sediment-bound P that can be mixed physically as well as taken up by migrating cyanobacteria, such as certain diazotrophs; however, migration by cyanobacteria may be limited beyond 15 m, so the hypolimnion was likely inaccessible in the nine deeper study lakes with a mean depth > 16 m (Nürnberg 1984; Karlsson-Elfgren et al. 2004; Carey et al. 2012). In addition, anoxia releases sediment-bound ferrous iron, which is essential for phytoplankton growth, especially diazotrophs, which have higher iron requirements than other phytoplankton due to the iron-rich protein synthesis of the N-fixation enzyme nitrogenase (Molot et al. 2014, 2021*b*). Relatively shallow stratified lakes with an anoxic hypolimnion had the highest biovolumes of diazotrophic cyanobacteria. However, the depletion of hypolimnetic DO is partly influenced by the decomposition of sinking organic matter such as algae in the hypolimnion (Reavie et al. 2016; Paerl 2017). Blooms of algae, including cyanobacteria, can indirectly result in hypolimnetic DO depletion as the bloom senesces (Paerl 2017). Hypolimnetic anoxia and hypoxia occurred across a range of total phytoplankton and diazotroph biovolume in our lakes (Supporting Information Fig. S3). Therefore, diazotrophic cyanobacteria blooms may have contributed to hypolimnetic DO depletion but were likely not always the primary cause. Spring blooms of other algae, such as diatoms, can drive summer hypoxia in the hypolimnion (Reavie et al. 2016). We could not fully disentangle the response and role of diazotrophic cyanobacteria with hypolimnetic DO depletion as we were limited to only two sampling points. We recommend further study with higher frequency sampling of lakes to discern the relationship and mechanisms involved (Reavie et al. 2016).

Watershed size may mediate the effect of hypolimnetic DO depletion on nutrient loading at the lake level as a potential cross-scale interaction. Increasing watershed areas, especially in P-rich areas, can produce greater pools of legacy P that can be mobilized from the sediments under anoxic conditions (Porder and Ramachandran 2013; Sharpley et al. 2013). When watershed inputs attenuate either seasonally or due to drought, anoxia can sustain P availability through internal cycling (Sharpley et al. 2013). However, hypolimnetic DO was only significantly correlated with diazotrophs, not TP (Supporting Information Fig. S5), suggesting anoxia may be more important in releasing other limiting nutrients for diazotrophs such as iron (Molot et al. 2014). Regardless, lakes prone to hypolimnetic anoxia and with relatively large watersheds, may be among the most susceptible characteristics to diazotrophic cyanobacteria dominance and blooms.

Food web pathway

Contrary to trophic cascade predictions, *Daphnia* had a positive relationship with diazotrophic cyanobacteria, while small cladocerans had a negative relationship, which may be due to different grazing modes (Figs. 4, 5). *Daphnia* in mesotrophic and eutrophic waters are more efficient grazers than other zooplankton due to filter feeding (DeMott 1982; Reynolds 2006). The non-selective feeding of *Daphnia* reduces phytoplankton biomass and diversity (Sarnelle 2007). This generalist feeding mode often leaves only a few indigestible species, such as filamentous cyanobacteria, to dominate, especially in the presence of fish (Vanni et al. 1990; Sarnelle 2007). *Daphnia* can locally adapt to fish predation such as by reducing body size over generations, therefore reducing their capacity to consume large colonies and filaments (De Meester et al. 1999). High dominance and biomass of colonial and filamentous cyanobacteria can also lead to declines in these large cladocerans, likely due to the low edibility of these cyanobacteria taxa, suggesting top-down control may only occur at lower densities (Gliwicz 1990; Ghadouani et al. 2003). Yet diazotrophic cyanobacteria, which were made of only filamentous genera in our lakes, were often dominant when *Daphnia* made up a major portion of the zooplankton community (Fig. 2), similar to some Canadian lakes (MacKeigan et al. 2023). Other experimental studies observed *Daphnia* populations expand with high levels of colonial cyanobacteria, suggesting at least some species or genotypes are well adapted to consume these cyanobacteria (Chislock et al. 2013; Ekvall et al. 2014). By contrast, small cladocerans are less efficient grazers, but more selective, so they support a greater diversity of phytoplankton, especially smaller species that are also well suited to resource-limited environments, competing with diazotrophic cyanobacteria (DeMott 1982; Bergquist et al. 1985; Reynolds 2006). When small cladocerans were abundant, diazotrophic cyanobacteria were less abundant, suggesting other phytoplankton taxa made up more of the phytoplankton community (Fig. 2). Therefore, top-down control may have a role in driving the dominance of diazotrophic cyanobacteria.

The relationships of cladocerans with diazotrophic cyanobacteria also may have been due to varying tolerance to resource limitation. Zooplankton can be reflective of trophic status, with *Daphnia* more dominant in eutrophic lakes and small cladocerans more dominant in oligotrophic lakes (DeMott 1982; Reynolds 2006). While *Daphnia* abundances were positively correlated with total P, small cladocerans were only weakly correlated with this trophic status indicator (Jansen et al. 2023). Therefore, zooplankton may only partially reflect trophic status. Yet, as this study was observational, further experimental studies are needed in mountain lakes or similar remote lakes to determine if grazing by different major zooplankton groups alters the biomass of filamentous diazotrophic cyanobacteria, including under varying nutrient conditions (Ghadouani et al. 2003).

While stocked fish were not among the top predictors of diazotrophs, we hypothesized that differences in zooplankton composition may reflect fish predation pressures in relation to lake habitat. However, *Daphnia* abundance was not correlated with fish stocking biomass, which is mostly made up of planktivorous trout fingerlings (Oregon Department of Fish and Wildlife in press; Jansen et al. 2023). A lack of observed relationship between stocked fish and zooplankton may be due in part to fish stocking records being weak proxies for actual population sizes, as fish have variable survival in mountain lakes such as in the Cascades (Liss et al. 1999). There also have been illegal introductions of bait fish in the region, but data on fish populations are lacking for most lakes (Eilers et al. 2011). Large zooplankton such as *Daphnia* are able to persist with fish when planktivory is fairly low, and there are predation refugia, such as vegetated littoral areas (Burks et al. 2001; Mushet et al. 2020). *Daphnia* abundances were highest in the shallowest lakes and negatively correlated with lake depth (Jansen et al. 2023). However, if refugia are minimal and planktivory is high, then smaller cladocerans, such as *Bosmina*, may become more abundant because they can largely avoid fish predation (Burks et al. 2001). Therefore, the role of lake morphometry (i.e., depth) in shaping zooplankton communities, which consequently affected cyanobacterial abundance, may reflect a cross-scale interaction between characteristics of the whole lake and pelagic zone food web.

Thermal pathway

The biovolume of diazotrophic cyanobacteria may be less constrained by temperature than by the mixing regime of lakes (Wagner and Adrian 2009; Reinl et al. 2023). While the binary regression tree indicated temperature can impact diazotrophs, this was not confirmed by the random forest model, suggesting temperature has a limited effect. Temperature may explain the temporal variation of diazotrophic cyanobacteria within lakes more so than variation across lakes (Taranu et al. 2015; Monchamp et al. 2019). Certain species of diazotrophic cyanobacteria are well adapted to cool temperatures, so temperatures may be less influential than nutrients (Carey et al. 2012; Reinl et al. 2023).

Lake depth may have more of an indirect effect on diazotrophic cyanobacteria by regulating lake mixing regimes. Maximum depth was strongly positively correlated with stratification stability (Jansen et al. 2023), but had a negative relationship with diazotrophic cyanobacteria (Fig. 5). Increasing lake depth constrains access to hypolimnetic nutrients for migrating cyanobacteria, as well as the recruitment of resting stages, like akinetes, from the sediments (Wetzel 2001; Karlsson-Elfgren et al. 2004; Carey et al. 2012). Stratification, which depends on lake depth, often favors buoyant cyanobacteria, including diazotrophs like *Dolichospermum* (Wagner and Adrian 2009; Carey et al. 2012). However, the Schmidt stability index does not account for the effective fetch of a lake; thus, physical mixing may not have been captured (Schmidt 1928). While turbulent conditions often favor other taxa, such as diatoms, over cyanobacteria, lake mixing with even a brief period of stratification (~ 2 weeks) can facilitate cyanobacteria recruitment and dominance (Wagner and Adrian 2009; Reinl et al. 2021). Further work is needed to examine the effect of other important aspects of lake thermal regimes, such as stratification duration, on diazotrophic cyanobacteria.

Development in the watershed, especially in nearshore areas, can increase lake temperatures (Doubek et al. 2015). Although the developed area was concentrated around the lakeshore in our study, there was no significant correlation with average lake temperatures, and development was fairly limited within the watersheds (Jansen et al. 2023). Watershed characteristics may be less of a factor for temperature than for nutrients in relation to diazotrophic cyanobacteria, but this may depend upon regional variation in key aspects such as groundwater contributions to lakes.

Conclusions and implications

This study offers insights relevant to the management of lakes removed from most direct anthropogenic impacts but where diazotrophic cyanobacteria and related toxic cyanobacterial blooms occur. Research on toxic cyanobacterial blooms in mountain lakes provides a way to understand the roles of climatic and natural environmental factors on bloom-forming taxa due to gradients of temperature, precipitation, and productivity within a single mountain range (Catalan et al. 2006). While many Cascades watersheds are naturally P-rich, P deposition from the atmosphere is increasing in many regions to the point that formerly oligotrophic waterbodies, including mountain lakes, are becoming mesotrophic (Stoddard et al. 2016; Scholz and Brahney 2022). Lakes in other mountain ranges are often low in P relative to the Cascades, due in part to the former typically having well-weathered granitic geology compared to the young volcanics in the Cascades. Therefore, lakes in other mountainous regions can be more sensitive to relatively small increases in atmospheric deposition in P (Catalan et al. 2006; Porder and Ramachandran 2013; Scholz and Brahney 2022). Diazotrophic cyanobacteria could become more prevalent in such mountain lakes where P is currently limiting (Scholz and Brahney 2022). However, atmospheric N deposition may also be elevated in mountain ranges and other isolated lakes (Baron et al. 2011; Ackerman et al. 2019); therefore, P deposition rates should be compared in relation to N deposition rates to assess vulnerability to diazotrophic cyanobacteria. While our study was conducted on montane and subalpine lakes, our findings may transcend mountain environments as similar conditions, such as N-limited watershed vegetation, can occur elsewhere, such as in the boreal forests of North America and Asia (Näsholm et al. 1998). However, lowland lakes, including boreal lakes, likely have greater inputs of dissolved organic matter as well as lower UV radiation than mountain lakes. These aspects should be considered when applying our findings beyond the mountain environment.

A combination of watershed, lake, and food web factors can drive diazotrophic cyanobacteria biomass and even blooms in lakes, especially in the absence of cultural eutrophication. Increasing watershed size in relatively undisturbed landscapes with forest cover may enhance the vulnerability of lakes to diazotrophic cyanobacteria due to greater P loading relative to smaller watersheds. In addition, montane and subalpine lakes can be impacted by logging and low-density development. Improved understanding of the influence of watershed size on diazotrophic cyanobacteria allows managers to identify and prioritize lakes susceptible to small changes in land cover and use. At the lake level, hypolimnetic anoxia may magnify the vulnerability of lakes to diazotrophic cyanobacteria by increasing internal P and Fe loading, especially in relatively shallow lakes (Carey et al. 2012; Molot et al. 2021*a*, *b*). In addition, large crustaceans like *Daphnia* may release filamentous diazotrophs from resource competition with other phytoplankton that are more readily grazed. This top-down regulation has implications for fisheries management as fish can alter the composition, biomass, and size of zooplankton communities (Vanni et al. 1990; Knapp et al. 2001; Symons and Shurin 2016). Diazotrophic cyanobacteria, which constitute most freshwater HAB species, may not be constrained by the conditions that are generally assumed to promote HABs, e.g., warm water and eutrophic conditions (Paerl and Otten 2013; Reinl et al. 2021, 2023). Therefore, scientists and managers must broaden their consideration of factors that can promote HABs when evaluating lake health and restoration. Lake management must account for abiotic factors, such as lake morphometry and watershed size, as well as biotic factors, such as fish stocking, to effectively mitigate diazotrophic cyanobacterial blooms.

Data availability statement

Data are available on a Dryad repository. The repository can be found here: https://datadryad.org/stash/share/04yFvIJ_Lu7yEw_SVz6r0gT-GPA8oTDTm4veFlhf5aQ.

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Conflict of Interest

None declared.

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