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Local and geographical factors jointly drive elevational patterns in three microbial groups across subarctic ponds

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Abstract

Aim: Elevational biodiversity patterns are understudied in high-latitude aquatic systems, even though these systems are important for detecting very early impacts of climatic changes on Earth. The aim of this study was to examine the elevational trends in species richness and local contribution to beta diversity (LCBD) of three biofilm microbial groups in freshwater ponds and to identify the key mechanisms underlying these patterns.

Location: One hundred and forty-six ponds in subarctic Finland and Norway distributed across the tree line along an elevational gradient of 10–1,038 m a.s.l., spanning from forested landscape to barren boulder fields.

Time period: July–August 2015.

Major taxa studied: Diatoms, cyanobacteria and non-cyanobacteria.

Methods: Generalized linear models were used to identify the most important pond variables explaining richness and LCBD. Structural equation models were used to explore the direct and indirect effects of multiscale drivers on richness and LCBD.

Results: Diatom and cyanobacteria richness showed unimodal elevational patterns, whereas non-cyanobacteria richness decreased with increasing elevation. The LCBD–elevation relationship was U-shaped for all three microbial groups. Diatom and cyanobacteria richness and LCBD were best explained by local pond variables, especially by pH. Non-cyanobacteria richness and LCBD were related to pond variables, elevation as a proxy for climatic conditions, and normalized difference vegetation index as a proxy for terrestrial productivity.

Main conclusions: Aquatic autotrophs were primarily controlled by environmental filtering, whereas heterotrophic bacteria were also affected by terrestrial productivity and elevation. All studied aspects of microbial diversity were directly or indirectly linked to elevation; therefore, climatic changes may greatly alter aquatic microbial assemblages.

KEYWORDS

altitude, bacteria, beta diversity, biofilm, biogeography, cyanobacteria, diatoms, northern Fennoscandia, species richness

1 | INTRODUCTION

Understanding spatial patterns of biodiversity has been one of the central objectives for biogeographers and ecologists for decades (Gaston, 2000). Mountainous regions, which cover steep environmental gradients within a relatively small geographical area, present ideal natural systems for examining patterns of biodiversity and underlying mechanisms (Körner, 2000; McCain & Grytnes, 2010). Elevational gradients are not only highly useful for determining the underlying drivers of biodiversity but also for predicting how global changes in climate may influence biological communities (Lomolino, 2001; Rahbek, 2005; Sundqvist, Sanders, & Wardle, 2013). Elevational diversity has

traditionally been examined for large organisms, such as plants and mammals, which typically exhibit either a monotonically decreasing or a unimodal pattern (Rahbek, 2005). During the last decade, there has been an increasing interest in elevational trends in biodiversity of microbial organisms (e.g., bacteria, diatoms), groups of which are essential for multiple ecosystem functions (Bryant et al., 2008; Fierer et al., 2011; Hayden & Beman, 2016; Shen et al., 2013; Singh, Takahashi, Kim, Chun, & Adams, 2012; Wang et al., 2011, 2017). Nonetheless, no consensus has emerged over the generality in the patterns, and contrasting elevational diversity trends have been documented for different microbial groups within and across geographical regions (Wang et al., 2017).

Ecologists typically examine spatial biodiversity patterns separately for aquatic and terrestrial ecosystems, although these systems are linked by the fluxes of organisms, materials and energy (Polis, Anderson, & Holt, 1997; Soininen, Bartels, Heino, Luoto, & Hillebrand, 2015). For example, the amount of allochthonous nutrients and organic matter that enters water bodies can be greatly influenced by the soil properties and vegetation type of catchments (Bartels et al., 2012; Rautio et al., 2011). The close connections between aquatic and terrestrial systems are often apparent in mountainous regions, and such connections typically weaken towards high elevations (Rose, Williamson, Kissman, & Saros, 2015). Along elevational gradients, climate-induced changes in the terrestrial vegetation of catchments affect the chemical and physical properties of the respective water bodies, with implications for aquatic organisms (Bastidas Navarro, Balseiro, & Modenutti, 2014; Karlsson, Jonsson, & Jansson, 2001; Weckström & Korhola, 2001). Water bodies below the tree line are often influenced more by terrestrial subsidies compared with more autochthonous aquatic systems at higher elevations (Karlsson et al., 2001; Rose et al., 2015). Given such strong aquatic–terrestrial linkages, variables representing catchment characteristics may be highly useful in reflecting long-term environmental conditions in aquatic ecosystems, and should therefore be considered as possible predictors of aquatic diversity (Soininen & Luoto, 2012; Soininen et al., 2015). For example, Soininen & Luoto (2012) recently reported that the richness of planktonic organisms in small boreal lakes was related to the normalized difference vegetation index (NDVI), a widely used satellite-derived metric, which correlates with above-ground net primary productivity and absorbed photosynthetically active radiation (Kerr & Ostrovsky, 2003; Pettorelli et al., 2005).

In this paper, we explore the mechanisms and pathways by which elevation and other environmental factors at multiple scales affect benthic microbial communities in the subarctic ponds of Finland and Norway. Benthic microbial communities are essential for the ecological functions of high-latitude aquatic systems because they often play a key role in driving biogeochemical cycles (Rautio et al., 2011; Vincent, Hobbie, & Laybourn-Parry, 2008). Nonetheless, we know relatively little about how and why their diversity varies with elevation. This is unwarranted given that freshwaters at high altitudes and latitudes are sensitive to climatic warming and associated environmental changes (Wang, Pan, Soininen, Heino, & Shen, 2016), which may greatly alter their physical, chemical and biological characteristics (Rühland, Paterson, Keller, Michelutti, & Smol, 2013; Schindler & Smol, 2006; Weckström & Korhola, 2001). Regarding the simplicity of their food webs facing harsh environmental conditions, such systems are important for detecting very early impacts of climatic changes on Earth, especially because organisms there live near their distributional borders.

A specific objective of this study is to examine how microbial species richness and beta diversity vary with elevation and to determine the key drivers underlying these patterns. We hypothesize that the richness and beta diversity patterns along elevational gradients are shaped by a set of hierarchically structured factors, which are connected to each other by causal relationships. We envision that microbial communities are primarily structured by local pond variables, some of which in turn reflect catchment properties and vary along geographical (i.e., spatial and climatic) gradients. To test this hypothesis, we construct structural equation models (SEMs; Appendix S1 in Supporting Information). The SEM is a modelling framework for representing and evaluating hypotheses about causal relationships in systems (Grace, 2006). It has rarely been used in studies analysing aquatic microbial diversity (but see Stomp, Huisman, Mittelbach, Litchman, & Klausmeier, 2011), even though it may provide new insights into the mechanisms that generate and maintain biogeographical patterns. We also aim to unravel whether the forces that regulate microbial diversity are the same for aquatic autotrophs (diatoms and cyanobacteria) and aquatic heterotrophs (bacteria other than cyanobacteria). We hypothesize that the autotrophs are more controlled by within-pond factors (e.g., pH and nutrients), whereas heterotrophs also respond to catchment properties (e.g., terrestrial productivity) because they are fuelled by allochthonous carbon.

2 | MATERIALS AND METHODS

2.1 | Study area and ponds

The study area (68°55′–69°58′ N, 20°02′–26°25′ E) is located in northern Fennoscandia, comprising parts of Finland and Norway (Supporting Information Appendix S2). The climate of the region is characterized by long, cold winters and short, relatively warm summers. The ice- and snow-free period typically lasts from May–June to October–November. The short growing season is compensated by abundant light, as the sun stays above the horizon for more than 2 months in the summer. The annual mean temperature varies from −1.9 °C in Kilpisjärvi (Finland) to −0.5 °C in Skibotn (Norway).

The study region is situated in a diverse topographical setting as it constitutes a part of the Scandinavian mountains. Our sampling covered long climatic and environmental gradients; the sampled ponds were distributed across the tree line along an elevational gradient of 10–1038 m a.s.l. Near sea level, the catchments were characterized by mixed forests or peatlands, with a transition to a zone dominated by mountain birch, and to treeless tundra with increasing altitude (Supporting Information Appendix S3). Finally, at the highest altitudes (above 1000 m a.s.l.), ponds were embedded in barren, rocky catchments with scarce terrestrial vegetation. The sampled water bodies

were typically small, shallow, clear watered and hydrologically independent of one another (i.e., not connected by streams). We define them as ponds rather than lakes because they were generally smaller than 1 ha in area (Biggs, Williams, Whitfield, Nicolet, & Weatherby, 2005). Most of the ponds were pristine or close to pristine, with negligible anthropogenic activity in their catchments.

2.2 | Field sampling and laboratory methods

We sampled 102 ponds in the Kilpisjärvi–Skibotn region in August 2015 and 44 ponds in Rásttigáisá in July 2015. At each pond, 10 cobble-sized stones were randomly selected along the shoreline from depths of 15–30 cm, and biofilm was collected by scraping the surfaces of these stones with a sterilized sponge (25 cm^2 per stone). The accumulated suspension was pooled into a composite sample, which was subsequently divided into two sample containers: one for diatoms and one for bacteria (including cyanobacteria) at each site. After sampling, the diatom samples were stored in cold (+4 °C) and dark, and the bacteria samples were stored frozen (−20 °C) until laboratory analyses.

We collected epilithic samples because communities growing on stony substrata should presumably be more stable than, for example, surface-sediment communities (Soininen & Eloranta, 2004). Surface-sediment assemblages are often derived from multiple source communities and they are subject to taphonomic modification, which may result in a discrepancy between species composition and prevailing environmental conditions (Cameron et al., 1999; Soininen & Eloranta, 2004). Moreover, it has been shown that among algae, epilithon may be more responsive than either epipelon or phytoplankton to climate-induced variations in the abiotic environments of shallow mountain lakes and ponds (Vinebrooke & Leavitt, 1999).

Water temperature, conductivity and pH were measured in situ. Water samples were collected and analysed later in the laboratory for total nitrogen (TN) according to standard SFS-EN ISO 11905–1, and for Si, Ca, Mg and K according to standard SFS-EN ISO 11885.

2.3 | Buffer zone variables

NDVI was used as a catchment scale variable (i.e., buffer zone variable) to indicate terrestrial productivity. The used NDVI product was derived from Landsat 8 Surface Reflectance data provided by the U.S. Geological Survey (USGS; Masek et al., 2006). The data were captured by the Operational Land Imager sensor on 21 August 2015, shortly after the fieldwork ended. Landsat 8 Surface Reflectance data were generated from the L8SR algorithm, and the surface reflectance product was topographically, geometrically and radiometrically corrected by the USGS. The spatial resolution for the data is 30 m. The NDVI was calculated as a ratio between the red (R) and near infrared (NIR) values using the following equation: $NDVI = (NIR - R)/(NIR + R)$.

To extract the NDVI values for the ponds, 30 and 100 m non-overlapping buffer zones were first created around each study site. Then, maximal NDVI values were extracted for each buffer zone using ArcGIS 10.2.1 software (ESRI, 2015). We calculated maximal NDVI values because it has been shown that catchment productivity measured as the maximal NDVI may be related to aquatic diversity in boreal regions (Soininen & Luoto, 2012).

2.4 | Climate variables

Climate variables (average July mean temperature and average July precipitation) for each study pond were extracted from WorldClim global climate data (*c.* 1 km^2 spatial resolution, representative of 1950–2000; Hijmans, Cameron, Parra, Jones, & Jarvis, 2005) using ArcGIS 10.2.1 software (ESRI, 2015).

2.5 | Diatom analysis

Organic material was removed from diatom samples by boiling with hydrogen peroxide (30% H_2O_2) in the laboratory. The cleaned diatoms were mounted on slides using Naphrax. Then, using a phase contrast light microscope (Olympus BX40, Melville, NY, USA; magnification 1000×), 500 frustules per sample were counted and identified to species level (when feasible) following Krammer and Lange-Bertalot (1986-1991) and Lange-Bertalot and Metzeltin (1996). Diatom species richness was calculated as the sum of all species encountered within the 500 frustules.

2.6 | Analysis of bacteria and cyanobacteria

Bacterial analyses were performed according to previous references (Wang et al., 2017). Genomic DNA was extracted from biofilm using a phenol–chloroform method (Zhou, Bruns, & Tiedje, 1996). Bacterial 16S rRNA genes were amplified in triplicate using bacterial universal primers [515F, 5′-GTGCCAGCMGCCGCGGTAA-3′ and 806R, 5′-GGACTACHVGGGTWTCTAAT-3′] targeting the V4 region. Spacers of different length (0–7 bases) were added between the sequencing primer and the target gene primer in each of the eight forward and reverse primer sets. To ensure that the total length of the amplified sequences did not vary with the primer set used, the forward and reverse primers were used in a complementary fashion so that all of the extended primer sets had exactly seven extra bases as the spacer for sequencing phase shift. Barcodes were added to the reverse primer between the sequencing primer and the adaptor.

Positive polymerase chain reaction (PCR) products were confirmed by agarose gel electrophoresis. The PCR products from triplicate reactions were combined and quantified with PicoGreen (Molecular Probes, Eugene, OR). The PCR products from samples to be sequenced in the same Illumina MiSeq run were pooled at equal molality to maximize the even-sequencing effects for all samples. The pooled mixture was purified with a QIAquick Gel Extraction Kit (QIAGEN Sciences, Germantown, MD) and re-quantified with PicoGreen. Sample libraries for sequencing were prepared according to the MiSeq Reagent Kit Preparation Guide (Illumina, San Diego, CA).

Overlapped paired-end sequences from Miseq were assembled using FLASH (Magoč & Salzberg, 2011). Poorly overlapped and poor-quality sequences (such as sequence length < 150 and moving-window (5 bp) quality score < 29) were filtered out before de-multiplexing based on barcodes. Furthermore, the sequences were clustered into

operational taxonomic unit (OTUs) at 97% pairwise identity with the seed-based uclust algorithm (Edgar, 2010). After chimeras were removed via Uchime against ChimeraSlayer reference database in the Broad Microbiome Utilities, representative sequences from each OTU were aligned to the Greengenes imputed core reference alignment V.201308 (DeSantis et al., 2006) using PyNAST (Caporaso et al., 2010). The taxonomic identity of each representative sequence was determined using the RDP Classifier (Wang, Garrity, Tiedje, & Cole, 2007), and chloroplast and archaeal sequences were removed. The bacteria were rarefied at 8,000 sequences for each sample for following analyses. Given that cyanobacteria, as primary producers in aquatic ecosystems as diatoms, showed a high abundance (32.1 ± 18.7% of reads per sample) in bacterial communities, we separated cyanobacteria from the other components of bacteria (namely, non-cyanobacteria bacteria).

2.7 | Data analyses

Before statistical analyses, pond variables other than pH (i.e., conductivity, TN, Si, Ca, Mg and K) were log10-transformed to reduce their skewed distributions. Statistical dependence between the explanatory variables was assessed using Spearman's rank correlation coefficients (r_s). Mg was highly correlated with conductivity ($r_s = .76$) and Ca ($r_s = .72$); thus, it was excluded from further analyses. Maximal NDVI values for 100 and 30 m buffer zones were highly correlated ($r_s > .9$); therefore, we chose to use the maximal NDVI values calculated using 30 m buffer radius (hereafter NDVI) in further analyses. July temperature was negatively correlated ($r_s = -.94$) with elevation, and July precipitation was positively correlated ($r_s = .93$) with elevation. We therefore decided to use elevation as a proxy for climatic variables in the statistical analyses, reflecting the joint influence of decreasing temperature and increasing precipitation with elevation. All other pairwise correlations were $|r_s| \leq .7$. Water temperature was not included in the analyses because the timing of the sampling and the prevailing weather conditions affect the snapshot measurements of water temperature.

As a measure of beta diversity, we used local contributions to beta diversity (LCBD), which represent the relative contribution of individual sampling units to beta diversity (Legendre & De Cáceres, 2013). LCBD enables the identification of sites that contribute more or less than average to overall beta diversity. A high LCBD value at a site indicates that the site harbours a unique community composition in the data set, thus comprising many rare species. To compute the LCBD values, we used Hellinger-transformed abundance data and the function *beta.div* in the R code provided by Legendre and De Cáceres (2013).

We first examined the relationships between richness or LCBD and elevation with simple linear regressions. We also constructed models that included a quadratic term of elevation to detect a possible non-linear elevational pattern.

Before using generalized linear models (GLMs) and multivariate analyses, we log10-transformed LCBD values to approximate a normal distribution better. The transformed values were subsequently standardized to a scale 0–1 to avoid negative values. Additionally, non-cyanobacteria richness was square-root-transformed because of its skewed distribution.

We used GLMs with Gaussian error distribution (McGullagh & Nelder, 1989) to identify the key local environmental variables explaining species richness and LCBD of diatoms, cyanobacteria and non-cyanobacteria in the ponds. The selection of the best approximating model was based on Akaike's information criterion (AIC; Akaike, 1974) and the function *stepAIC* with backward stepwise model selection in the R package MASS (Venables & Ripley, 2002). Only variables that were significantly related to the response variables were included in the final models.

To examine the causal relationships between richness or LCBD and pond variables, NDVI and elevation, we constructed structural equation models (SEMs). We first constructed an initial model for each taxonomic group that included all possible pathways between the response variable, the key pond variables (identified by GLM), NDVI and elevation. In addition to direct pathways, we considered indirect ones to see if variables that were not directly related to the response variable exerted some effects via other mediating variables. All variables were standardized before they were entered in the SEMs. From the initial model, non-significant paths were eliminated stepwise until all remaining paths were significant (if possible) and directly or indirectly related to the response variable. The goodness of fit of the final model was evaluated with a chi-square test; a non-significant *p*-value ($> .05$) indicates that there are no significant deviations between the model and data (i.e., the model is consistent with the data; Grace & Bollen, 2005). Both unstandardized and standardized path coefficients were calculated for each pathway in the final model. Unstandardized coefficients are in raw units, which makes comparing the effects of different factors on responses challenging because of these disparate scales. For standardized coefficients, the units are the same across pathways, which makes such coefficients more comparable in the SEM (Grace, 2006). The SEMs were constructed using the R package lavaan (Rosseel, 2012).

Finally, to ensure that the overall results were independent of the chosen method, we carried out variation partitioning (Borcard, Legendre, & Drapeau, 1992) to partition the variation in the response variables with respect to the pond variables, NDVI and elevation and their joint effects. Variation partitioning was conducted using the *varpart* function in the R package vegan (Oksanen et al., 2015). It was based on partial regression because we analysed single response variables.

All statistical analyses were conducted with R version 3.2.2 (R Development Core Team, 2015).

3 | RESULTS

Measured environmental variables varied considerably among the ponds (Supporting Information Appendix S4). Water pH ranged from 4.4 to 8.5 (*SD* 0.75), conductivity from 2.6 to 77.4 $\mu S\ cm^{-1}$ (*SD* 14.4), TN from 0.05 to 1.0 mg L^{-1} (*SD* 0.17), Si from 0.08 to 7.53 mg L^{-1} (*SD* 1.56), and K from 0.02 to 2.2 mg L^{-1} (*SD* 0.44). NDVI varied between 0.18 and 0.83 (*SD* 0.14). Several variables exhibited distinct elevational gradients. For example, conductivity and NDVI decreased with increasing elevation, whereas pH showed a unimodal trend (Supporting

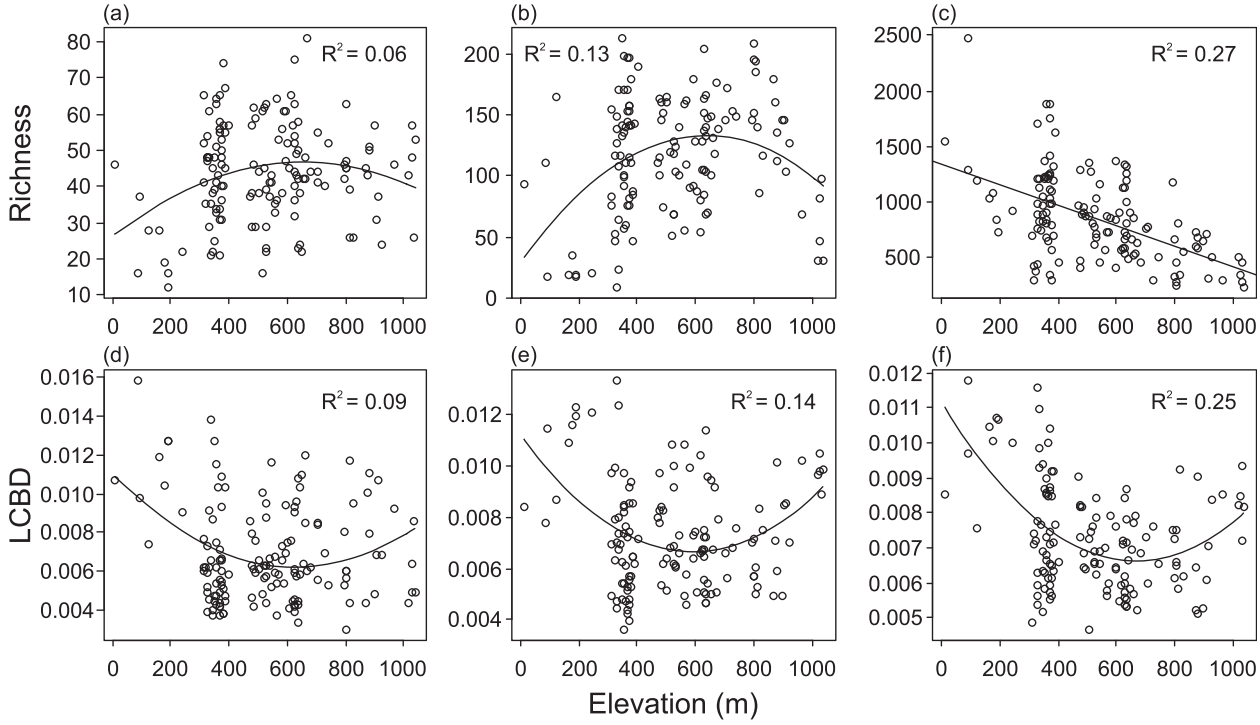

FIGURE 1 The relationships between species richness and elevation for (a) diatoms, (b) cyanobacteria and (c) non-cyanobacteria, and between local contribution to beta diversity (LCBD) and elevation for (d) diatoms, (e) cyanobacteria and (f) non-cyanobacteria in the subarctic ponds. Significant ($p < .05$) linear or quadratic models are shown with lines

Information Appendix S5). The mean number of diatom species in a pond was 44 (range: 12–81), and the mean number of cyanobacteria taxa was 118 (range: 8–213). The number of non-cyanobacteria taxa ranged from 228 to 2,477; the mean was 857.

Each response variable exhibited significant elevational patterns (Figure 1a–f). Diatom and cyanobacteria richness showed unimodal trends; richness peaked at mid-elevations and was lower at both ends of the elevational gradient (Figure 1a,b). Non-cyanobacteria richness decreased with increasing elevation (Figure 1c). The LCBD–elevation relationship was U-shaped for all three microbial groups (Figure 1d–f).

There was a negative relationship between richness and LCBD in diatoms ($R^2 = .35$, $p < .001$) and cyanobacteria ($R^2 = .54$, $p < .001$), whereas the relationship between non-cyanobacteria richness and LCBD was positive ($R^2 = .07$, $P = .002$).

Based on GLMs, pH was one of the major determinants of richness and LCBD across taxa (Supporting Information Appendix S6). In addition to pH, TN and Si were important drivers for diatom richness; TN for cyanobacteria richness; and conductivity, TN and K for non-cyanobacteria richness. Diatom and cyanobacteria LCBD were mostly affected by pH and K, and non-cyanobacteria LCBD by pH and conductivity.

The SEMs indicated that microbial communities in ponds were shaped by hierarchically structured factors, connected to each other by causal relationships (Figure 2a–f). Elevation governed NDVI and affected pond variables both directly and indirectly through NDVI.

For species richness, the final SEMs indicated that both diatom and cyanobacteria were controlled by pond variables, especially pH, whereas elevation exerted an indirect effect (Figure 2a,b). The final model explained 35% of the variation in diatom richness and 28% of the variation in cyanobacteria richness. Non-cyanobacteria richness was most strongly affected by elevation, with additional effects by pond variables (e.g., pH; Figure 2c). In addition to direct effects, elevation had indirect effects via NDVI and pond variables. The final model explained 41% of the variation in non-cyanobacteria richness.

For LCBD, the outcome was highly similar for diatoms and cyanobacteria (Figure 2d,e). Both groups were primarily associated with pond variables pH and K, with an indirect effect by elevation. The SEM explained 18% of the variation in diatom LCBD and 20% of the variation in cyanobacteria LCBD. Elevation and NDVI had direct negative effects on non-cyanobacteria LCBD (Figure 2f). Among the local factors, pH emerged as the key variable. The model explained 15% of the variation in non-cyanobacteria LCBD. In all the SEMs, the test statistics indicated good model–data fits.

In variation partitioning, the pure effects of pond variables accounted for most of the explained variation in diatoms (richness = 31.5%; LCBD = 13.4%; Figure 3a,d) and cyanobacteria (richness = 24.4%; LCBD = 18.9%; Figure 3b,e). Non-cyanobacteria richness was most strongly related to the effects of elevation (13.1%), the effects of pond variables (10.9%) and the joint effects of all the explanatory variables (9.9%; Figure 3c). The joint effects of elevation and pond variables (8.0%) captured the largest fraction of the explained variation of non-cyanobacteria LCBD (Figure 3f). The proportion of unexplained variation (adjusted R^2) was consistently lower for richness (diatoms 68.0%; cyanobacteria 74.5%; non-cyanobacteria 61.2%)

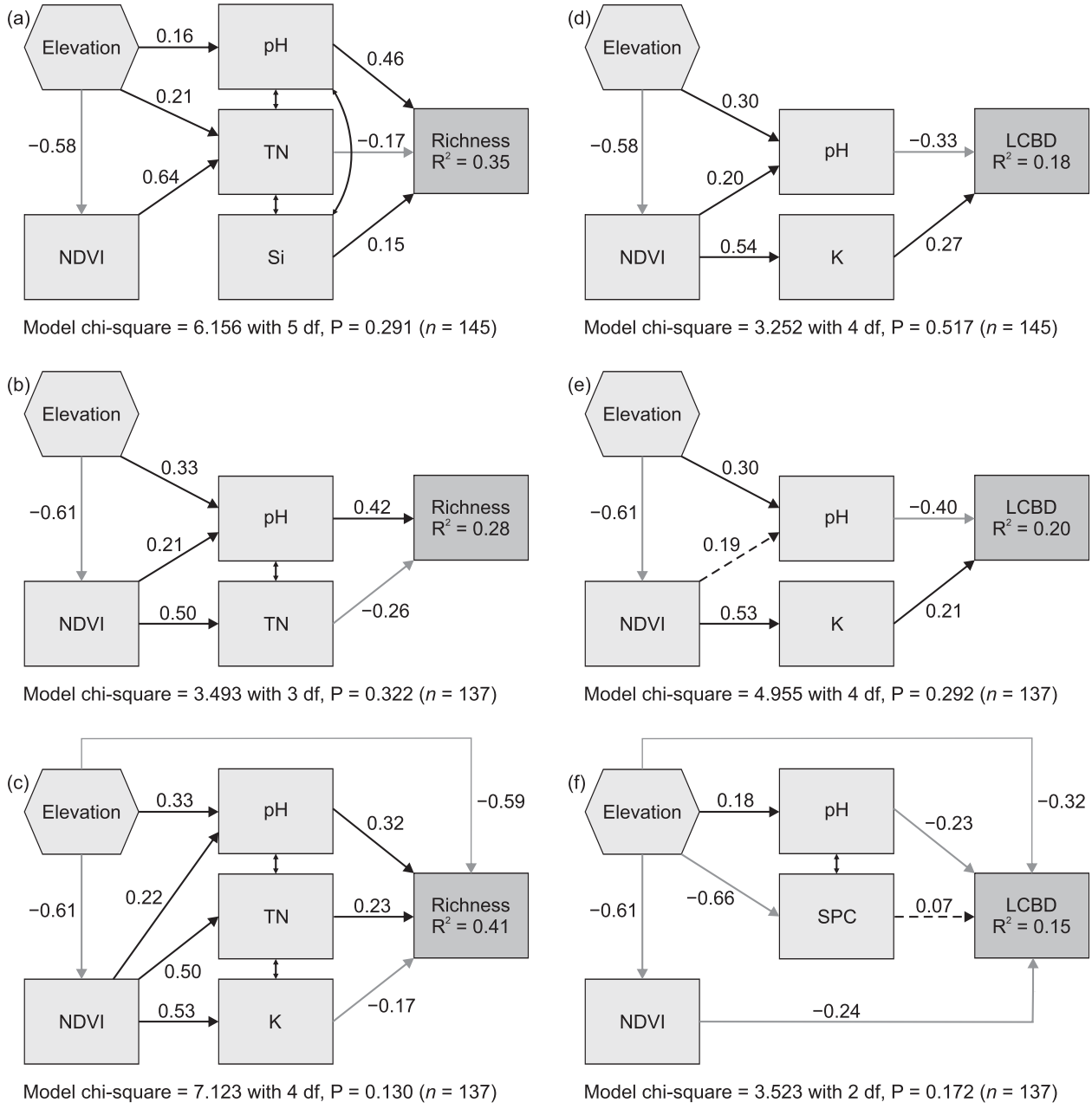

FIGURE 2 Structural equation models in explaining species richness and local contribution to beta diversity (LCBD). These models represent connections between explanatory variables and species richness of (a) diatoms, (b) cyanobacteria and (c) non-cyanobacteria, and between explanatory variables and LCBD of (d) diatoms, (e) cyanobacteria and (f) non-cyanobacteria. The values corresponding to the pathways are standardized path coefficients. Black arrows indicate positive effects, grey arrows denote negative effects, and dashed arrows denote non-significant effects. R^2 values indicate the amount of explained variations in the response variables. Double-headed arrows indicate covariance. NDVI = normalized difference vegetation index; SPC = conductivity; TN = total nitrogen

compared with LCBD (diatoms 86.0%; cyanobacteria 82.5%; non-cyanobacteria 86.4%).

4 | DISCUSSION

To our knowledge, the present study is the first one to examine pond microbial diversity along an extensive elevational gradient across three taxonomic groups representing broadly different functional roles. Our results show that the microscopic organisms in subarctic ponds exhibit clear elevational diversity patterns, the shapes and drivers of which vary across the focal taxonomic groups. The overall results were strikingly similar for diatoms and cyanobacteria, even though these data were obtained using differing methods (morphological versus molecular identification). This outcome suggests that the same mechanisms govern the diversity of these two functionally similar groups of aquatic autotrophs in the studied systems. The unimodal richness–elevation pattern that we found for diatoms and cyanobacteria is typical among

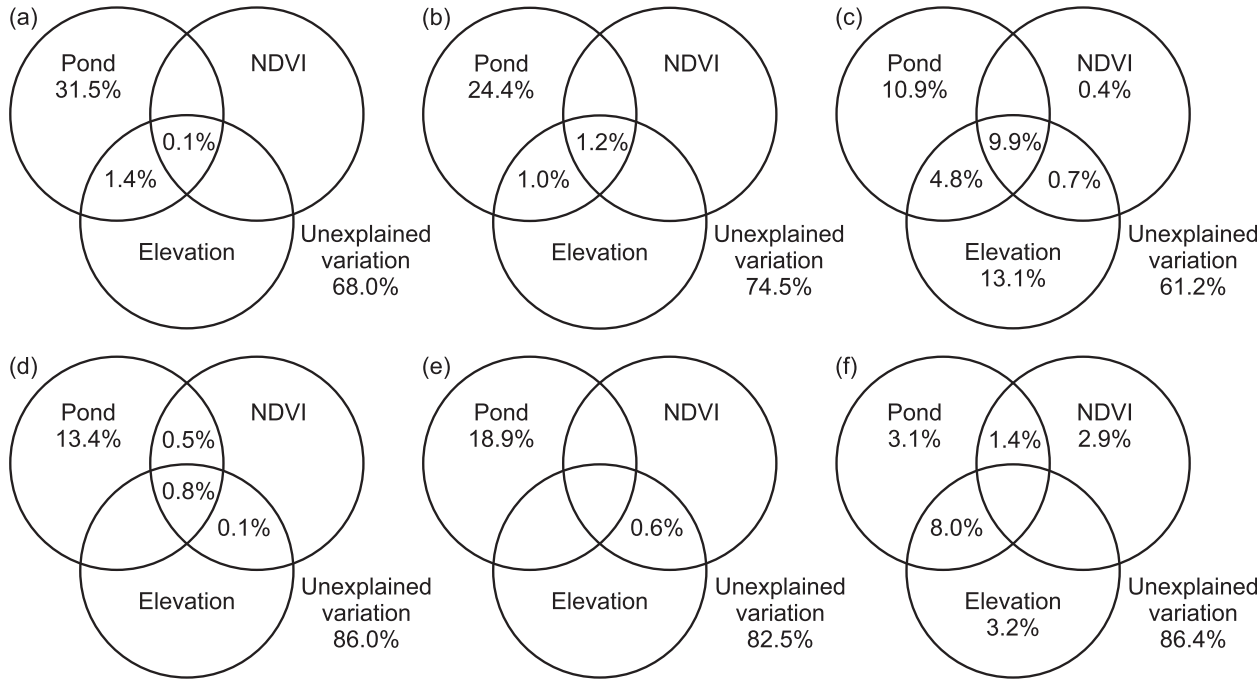

FIGURE 3 Results of variation partitioning showing the percentages of explained variation for species richness of (a) diatoms, (b) cyanobacteria and (c) non-cyanobacteria, and for local contribution to beta diversity of (d) diatoms, (e) cyanobacteria and (f) non-cyanobacteria. Variation was explained by pond variables, normalized difference vegetation index (NDVI) and elevation. Shown are adjusted R^2 values; negative values are not displayed

larger organisms (Rahbek, 2005). It has also been discovered for aquatic microorganisms, such as lake diatoms in northern Fennoscandia (Weckström & Korhola, 2001) and stream diatoms in China (Wang et al., 2017). However, in the study by Wang et al. (2017) only a minority (16%) of the studied elevational gradients revealed unimodal trends for stream diatoms. The results of variation partitioning indicated that the variability in both diatom and cyanobacteria richness was best explained by the pure effects of pond variables, which were also identified as the key factors driving richness in the SEMs. These findings are in line with previous studies, which have emphasized the role of environmental filtering for primary producers in aquatic systems (Stomp et al., 2011; Verleyen et al., 2009; Wang et al., 2017).

Among pond variables, pH had an overriding effect on diatom and cyanobacteria richness; the number of species declined with decreasing pH. Considering that the ponds with the lowest pH were located at both ends of the elevational gradient, the unimodal richness–elevation trend may reflect the positive richness–pH relationship. Low pH can act as an environmental filter excluding species that are sensitive to low pH, hence leading to impoverished communities largely dominated by species that are physiologically adapted to pond water acidity. Overall, the results imply that the number of diatoms and cyanobacteria in subarctic ponds is more constrained by pH-induced stress rather than limited by resources, and agree with earlier reports on the importance of pH for aquatic autotrophs (Soininen, Jamoneau, Rosebery, & Passy, 2016; Stokes, 1986; Wang et al., 2017; Weckström, Korhola, & Blom, 1997).

Although the primary producers showed unimodal patterns, non-cyanobacteria richness decreased with increasing elevation. This declining trend has previously been observed for soil bacteria in the Colorado Rocky Mountains, U.S.A. (Bryant et al., 2008) and stream bacteria in Meili Mountain, China (Wang et al., 2017), although most of the stream elevational gradients studied by Wang et al. (2017) showed other than decreasing trends for bacteria. Microbial diversity patterns contradictory to the present findings have also been reported in studies along hydrological continua (Besemer et al., 2013; Crump, Amaral-Zettler, & Kling, 2012). In variation partitioning, the explained variation in non-cyanobacteria richness was mostly accounted for by the pure effects of elevation, the pure effects of pond variables and the joint effects of all explanatory variables. The SEM suggested, however, that richness was primarily related to the direct effect of elevation. Unexpectedly, conductivity did not have a significant effect on richness in the SEM, although the GLM indicated that it was highly important for non-cyanobacteria richness. This may be because conductivity was correlated with elevation; the direct effect of elevation on richness might have masked the effect of conductivity. Therefore, we assume that the elevation effect in SEMs reflects not only climatic factors, such as decreasing temperature, but also the effects of conductivity and other variables, which we did not measure but which are known to covary with elevation and influence bacterial communities. One such factor is the concentration of dissolved organic carbon, which typically decreases with increasing elevation and has been identified as an essential variable for bacterial communities in subarctic lakes and ponds (Karlsson et al., 2001; Roiha, Tiirola, Cazzanelli, & Rautio, 2012). The low supply of organic carbon to high-elevation ponds attributable to low terrestrial productivity may limit the growth of aquatic bacteria. In contrast, lower-elevation ponds below the tree line are presumably more influenced by organic carbon subsidies from the surrounding

terrestrial environment (Bastidas Navarro et al., 2014; Karlsson et al., 2001; Rose et al., 2015), which may serve as a source of energy for heterotrophic bacteria and subsequently facilitate higher bacterial diversity. Alternatively, the effect of elevation could be interpreted as the outcome of biogeographical processes, such as dispersal limitation and colonization dynamics. For example, it has been shown that upslope soils may serve as sources of microbial diversity for downslope freshwaters (Crump et al., 2012). In our study, such dispersal across ecosystem boundaries may have increased richness in low-elevation ponds compared with high-elevation ponds, which were surrounded by barren boulder fields and shallow soils.

In addition to richness, we observed significant elevational trends in LCBD for all three microbial groups. The LCBD–elevation relationship was U-shaped across taxa. In general, ponds located in the middle of the elevational gradient had low LCBD values, whereas low-elevation and high-elevation ponds scored higher values, especially for cyanobacteria and non-cyanobacteria. Given that LCBD indicates uniqueness of the pond communities, with large values representing sites with highly different species compositions from other sites (Legendre & De Cáceres, 2013), this finding may indicate that ponds at mid-elevations often harbour communities with more generalist species, whereas specialists are relatively more prevalent in ponds located at both ends of the elevational gradient.

The results of variation partitioning and SEMs demonstrated that diatom and cyanobacteria LCBD values were primarily linked to pond variables. In SEMs, diatom and cyanobacteria LCBD were associated with pH, followed by K. Water pH had a negative coefficient whereas K had a positive coefficient in the models, indicating that ponds with high uniqueness of species composition were acidic or had high K concentration, or both. Moreover, LCBD and richness were negatively related, suggesting that ponds with high uniqueness of species composition were often species poor. It thus appears that among the autotrophs, taxonomically unique species-poor communities prevail in specific ecological conditions, such as those found in acidic ponds. The negative LCBD–richness correlation observed here has been reported previously for larger organisms, such as freshwater fish (Legendre & De Cáceres, 2013) and urban pond insects (Heino et al., 2017). However, we also observed a positive relationship between LCBD and richness for non-cyanobacteria, which clearly suggested that the negative LCBD–richness correlation is not a general rule when multiple taxa are considered.

Although pond variables captured most of the explained variation in diatom and cyanobacteria LCBD, the results were more complex for non-cyanobacteria. Variation partitioning indicated that variability in LCBD was best explained by the joint effects of pond variables and elevation. In SEMs, non-cyanobacteria LCBD was linked to elevation, pH and NDVI, all of which had negative coefficients in the models, implying that ponds with high uniqueness of species composition were located at lower elevations or had low NDVI values or low pH. Conductivity was also included in the final SEM, even though it did not have an independent effect on LCBD, presumably owing to its correlation with elevation. These results imply that more unique communities emerged in ponds at lower and higher elevations primarily as a response to the joint effects of elevation and pond variables. The exact mechanisms affecting LCBD remain uncertain, however, as the amount of unexplained variation in the multivariate analyses was relatively high. In fact, for all microbial groups, the proportion of unexplained variation was notably higher for LCBD compared with richness, suggesting that richness responded more strongly to environmental gradients in the present study. It may also be that LCBD is a difficult metric to predict (Tonkin, Heino, Sundermann, Haase, & Jähnig, 2016).

In general, the key factors shaping diversity patterns here differed between the autotrophs and the heterotrophs. Given the strong interdependence between richness and LCBD of diatoms and cyanobacteria, and the primacy of pond variables in driving these patterns, it seems that environmental filtering governed the elevational diversity of aquatic autotrophs here. Compared with pond variables, terrestrial productivity was a poor predictor of diatom and cyanobacteria diversity. NDVI did not have direct effects on richness or LCBD in SEMs, and it failed to explain their variability in variation partitioning. This outcome disagrees with Soininen and Luoto (2012), who reported that the richness of planktonic organisms in small boreal lakes was related to NDVI. One plausible reason for the weak linkages between terrestrial productivity and the diversity of aquatic primary producers in the present study is that NDVI was only weakly associated with pH, which in turn greatly affected diatoms and cyanobacteria. For example, several low-elevation ponds embedded in a relatively productive terrestrial landscape were species poor, presumably because of low pH. As hypothesized, terrestrial productivity was more related to the diversity of non-cyanobacteria, which represent decomposers, although it accounted for relatively small amounts of the explained variation in the multivariate analyses. This may reflect the fact that terrestrial productivity was controlled by elevation, hence lacking a notable independent effect on aquatic diversity.

In summary, microbial diversity in subarctic ponds varies with elevation in response to multifaceted, causally related factors. Climate-induced changes in terrestrial vegetation and pond variables along elevational gradients are reflected by the changes in aquatic microbial communities. Despite some common features, there were also clear differences in the patterns and potential drivers among the three microbial groups. The overall results were highly similar for the two groups of aquatic autotrophs, with pH as the major determinant of species richness and uniqueness of species composition. In contrast, heterotrophic bacteria responded to pond variables but also to elevation and terrestrial productivity. Direct effects of elevation may indicate climatic effects, dispersal-related factors or the importance of unmeasured environmental variables that covary with elevation. We conclude that microbial diversity along pond elevational gradients is likely to be structured by interacting local and regional forces. Given that both aspects of diversity of all three microbial groups were directly or indirectly linked to elevation, climatic changes may have profound impacts on aquatic microbial assemblages, especially in subarctic regions.

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DATA ACCESSIBILITY

Data are available from authors by request.

REFERENCES

Akaike, H. (1974). A new look at the statistical model identification. *IEEE Transactions on Automatic Control*, *19*, 716–724.

Bartels, P., Cucherousset, J., Steger, K., Eklöv, P., Tranvik, L. J., & Hillebrand, H. (2012). Reciprocal subsidies between freshwater and terrestrial ecosystems structure consumer resource dynamics. *Ecology*, *93*, 1173–1182.

Bastidas Navarro, M., Balseiro, E., & Modenutti, B. (2014). Bacterial community structure in Patagonian Andean Lakes above and below timberline: From community composition to community function. *Microbial Ecology*, *68*, 528–541.

Besemer, K., Singer, G., Quince, C., Bertuzzo, E., Sloan, W., & Battin, T. J. (2013). Headwaters are critical reservoirs of microbial diversity for fluvial networks. *Proceedings of the Royal Society B: Biological Sciences*, *280*, 20131760.

Biggs, J., Williams, P., Whitfield, M., Nicolet, P., & Weatherby, A. (2005). 15 years of pond assessment in Britain: Results and lessons learned from the work of Pond Conservation. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *15*, 693–714.

Borcard, D., Legendre, P., & Drapeau, P. (1992). Partialling out the spatial component of ecological variation. *Ecology*, *73*, 1045–1055.

Bryant, J. A., Lamanna, C., Morlon, H., Kerkhoff, A. J., Enquist, B. J., & Green, J. L. (2008). Microbes on mountainsides: Contrasting elevational patterns of bacterial and plant diversity. *Proceedings of the National Academy of Sciences USA*, *105*, 11505–11511.

Cameron, N. G., Birks, H. J. B., Jones, V. J., Berge, F., Catalan, J., Flower, R. J., … Toro, M. (1999). Surface-sediment and epilithic diatom pH calibration sets for remote European mountain lakes (AL:PE Project) and their comparison with the Surface Waters Acidification Programme (SWAP) calibration set. *Journal of Paleolimnology*, *22*, 291–317.

Caporaso, J. G., Bittinger, K., Bushman, F. D., DeSantis, T. Z., Andersen, G. L., & Knight, R. (2010). PyNAST: A flexible tool for aligning sequences to a template alignment. *Bioinformatics*, *26*, 266–267.

Crump, B. C., Amaral-Zettler, L. A., & Kling, G. W. (2012). Microbial diversity in arctic freshwaters is structured by inoculation of microbes from soils. *The ISME Journal*, *6*, 1629–1639.

DeSantis, T. Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E. L., Keller, K., … Andersen, G. L. (2006). Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Applied and Environmental Microbiology*, *72*, 5069–5072.

Edgar, R. C. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*, *26*, 2460–2461.

ESRI. (2015). *ArcGIS 10.2.1 for desktop*. Redlands, CA: Environmental Systems Research Institute.

Fierer, N., McCain, C. M., Meir, P., Zimmerman, M., Rapp, J. M., Silman, M. R., & Knight, R. (2011). Microbes do not follow the elevational diversity patterns of plants and animals. *Ecology*, *92*, 797–804.

Gaston, K. J. (2000). Global patterns in biodiversity. *Nature*, *405*, 220–227.

Grace, J. B. (2006). *Structural equation modeling and natural systems*. Cambridge, U.K.: Cambridge University Press.

Grace, J. B., & Bollen, K. A. (2005). Interpreting the results from multiple regression and structural equation models. *The Bulletin of the Ecological Society of America*, *86*, 283–295.

Hayden, C. J., & Beman, J. M. (2016). Microbial diversity and community structure along a lake elevation gradient in Yosemite National Park, California, USA. *Environmental Microbiology*, *18*, 1782–1791.

Heino, J., Bini, L. M., Andersson, J., Bergsten, J., Bjelke, U., & Johansson, F. (2017). Unravelling the correlates of species richness and ecological uniqueness in a metacommunity of urban pond insects. *Ecological Indicators*, *73*, 422–431.

Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G., & Jarvis, A. (2005). Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, *25*, 1965–1978.

Karlsson, J., Jonsson, A., & Jansson, M. (2001). Bacterioplankton production in lakes along an altitude gradient in the subarctic north of Sweden. *Microbial Ecology*, *42*, 372–382.

Kerr, J. T., & Ostrovsky, M. (2003). From space to species: Ecological applications for remote sensing. *Trends in Ecology and Evolution*, *18*, 299–305.

Körner, C. (2000). Why are there global gradients in species richness? Mountains might hold the answer. *Trends in Ecology and Evolution*, *15*, 513–514.

Krammer, K., & Lange-Bertalot, H. (1986–1991) Bacillariophyceae. In H. Ettl, J. Gerloff, H. Heynig & D. Mollenhauer (Eds.), *Süßwasserflora von Mitteleuropa* (Vol. *2*, no. 1−4). Stuttgart, Germany: Gustav Fischer Verlag.

Lange-Bertalot, H., & Metzeltin, D. (1996). Indicators of oligotrophy: 800 taxa representative of three ecologically distinct lake types: Carbonate buffered, oligodystrophic, weakly buffered soft water. In H. Lange-Bertalot (Ed.), *Iconographia diatomologica* (Vol. *2*, pp. 7–390). Königstein, Germany: Koeltz Scientific Books.

Legendre, P., & De Cáceres, M. (2013). Beta diversity as the variance of community data: Dissimilarity coefficients and partitioning. *Ecology Letters*, *16*, 951–963.

Lomolino, M. V. (2001). Elevation gradients of species-density: Historical and prospective views. *Global Ecology and Biogeography*, *10*, 3–13.

Magoč, T., & Salzberg, S. L. (2011). FLASH: Fast length adjustment of short reads to improve genome assemblies. *Bioinformatics*, *27*, 2957–2963.

Masek, J. G., Vermote, E. F., Saleous, N., Wolfe, R., Hall, F. G., Huemmrich, F., … Lim, T. K. (2006). A Landsat surface reflectance data set for North America, 1990–2000. *IEEE Geoscience and Remote Sensing Letters*, *3*, 68–72.

McCain, C. M., & Grytnes, J.-A. (2010). Elevational gradients in species richness. *Encyclopedia of life sciences (ELS)* (pp. 1–10). Chichester, U. K.: Wiley.

McGullagh, P., & Nelder, J. A. (1989). *Generalized linear models* (2nd ed.). London, U.K.: Chapman & Hall.

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., … Wagner, H. (2015). vegan: Community ecology package. *R Package Version 2.2–1*. Retrieved from https://CRAN.R-project.org/package=vegan

Pettorelli, N., Vik, J. O., Mysterud, A., Gaillard, J.-M., Tucker, C. J., & Stenseth, N. C. (2005). Using the satellite-derived NDVI to assess ecological responses to environmental change. *Trends in Ecology and Evolution*, *20*, 503–510.

Polis, G. A., Anderson, W. B., & Holt, R. D. (1997). Toward an integration of landscape and food web ecology: The dynamics of spatially subsidized food webs. *Annual Review of Ecology and Systematics*, *28*, 289–316.

Rahbek, C. (2005). The role of spatial scale and the perception of large-scale species-richness patterns. *Ecology Letters*, *8*, 224–239.

Rautio, M., Dufresne, F., Laurion, I., Bonilla, S., Vincent, W. F., & Christoffersen, K. S. (2011). Shallow freshwater ecosystems of the circumpolar Arctic. *Écoscience*, *18*, 204–222.

R Development Core Team. (2015). *R: A language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing. Retrieved from http://www.R-project.org

Roiha, T., Tiirola, M., Cazzanelli, M., & Rautio, M. (2012). Carbon quantity defines productivity while its quality defines community composition of bacterioplankton in subarctic ponds. *Aquatic Sciences*, *74*, 513–525.

Rose, K. C., Williamson, C. E., Kissman, C. E. H., & Saros, J. E. (2015). Does allochthony in lakes change across an elevation gradient? *Ecology*, *96*, 3281–3291.

Rosseel, Y. (2012). lavaan: An R package for structural equation modeling. *Journal of Statistical Software*, *48*, 1–36. Retrieved from http://www.jstatsoft.org/v48/i02/

Rühland, K. M., Paterson, A. M., Keller, W., Michelutti, N., & Smol, J. P. (2013). Global warming triggers the loss of a key Arctic refugium. *Proceedings of the Royal Society B: Biological Sciences*, *280*, 20131887.

Schindler, D. W., & Smol, J. P. (2006). Cumulative effects of climate warming and other human activities on freshwaters of Arctic and Subarctic North America. *AMBIO: A Journal of the Human Environment*, *35*, 160–168.

Shen, C., Xiong, J., Zhang, H., Feng, Y., Lin, X., Li, X., … Chu, H. (2013). Soil pH drives the spatial distribution of bacterial communities along elevation on Changbai Mountain. *Soil Biology & Biochemistry*, *57*, 204–211.

Singh, D., Takahashi, K., Kim, M., Chun, J., & Adams, J. M. (2012). A hump-backed trend in bacterial diversity with elevation on Mount Fuji, Japan. *Microbial Ecology*, *63*, 429–437.

Soininen, J., & Eloranta, P. (2004). Seasonal persistence and stability of diatom communities in rivers: Are there habitat specific differences? *European Journal of Phycology*, *39*, 153–160.

Soininen, J., Bartels, P., Heino, J., Luoto, M., & Hillebrand, H. (2015). Toward more integrated ecosystem research in aquatic and terrestrial environments. *BioScience*, *65*, 174–182.

Soininen, J., Jamoneau, A., Rosebery, J., & Passy, S. I. (2016). Global patterns and drivers of species and trait composition in diatoms. *Global Ecology and Biogeography*, *25*, 940–950.

Soininen, J., & Luoto, M. (2012). Is catchment productivity a useful predictor of taxa richness in lake plankton communities? *Ecological Applications*, *22*, 624–633.

Stokes, P. M. (1986). Ecological effects of acidification on primary producers in aquatic systems. *Water, Air and Soil Pollution*, *30*, 421–438.

Stomp, M., Huisman, J., Mittelbach, G. G., Litchman, E., & Klausmeier, C. A. (2011). Large-scale biodiversity patterns in freshwater phytoplankton. *Ecology*, *92*, 2096–2107.

Sundqvist, M. K., Sanders, N. J., & Wardle, D. A. (2013). Community and ecosystem responses to elevational gradients: Processes, mechanisms, and insights for global change. *Annual Review of Ecology, Evolution, and Systematics*, *44*, 261–280.

Tonkin, J. D., Heino, J., Sundermann, A., Haase, P., & Jähnig, S. C. (2016). Context dependency in biodiversity patterns of central German stream metacommunities. *Freshwater Biology*, *61*, 607–620.

Venables, W. N., & Ripley, B. D. (2002). *Modern applied statistics with S* (4th ed.). New York: Springer.

Verleyen, E., Vyverman, W., Sterken, M., Hodgson, D. A., De Wever, A., Juggins, S., … Sabbe, K. (2009). The importance of dispersal related and local factors in shaping the taxonomic structure of diatom metacommunities. *Oikos*, *118*, 1239–1249.

Vincent, W. F., Hobbie, J. E., & Laybourn-Parry, J. (2008). Introduction to the limnology of high-latitude lake and river ecosystems. In W. F. Vincent & J. Laybourn-Parry (Eds.), *Polar lakes and rivers: Limnology of arctic and antarctic aquatic ecosystems* (pp. 1–23). Oxford, U.K.: Oxford University Press.

Vinebrooke, R. D., & Leavitt, P. R. (1999). Phytobenthos and phytoplankton as potential indicators of climate change in mountain lakes and ponds: A HPLC-based pigment approach. *Journal of the North American Benthological Society*, *18*, 15–33.

Wang, J., Meier, S., Soininen, J., Casamayor, E. O., Pan, F., Tang, X., … Shen, J. (2017). Regional and global elevational patterns of microbial species richness and evenness. *Ecography*, *40*, 393–402.

Wang, J., Pan, F., Soininen, J., Heino, J., & Shen, J. (2016). Nutrient enrichment modifies temperature-biodiversity relationships in large-scale field experiments. *Nature Communications*, *7*, 13960.

Wang, J., Soininen, J., Zhang, Y., Wang, B., Yang, X., & Shen, J. (2011). Contrasting patterns in elevational diversity between microorganisms and macroorganisms. *Journal of Biogeography*, *38*, 595–603.

Wang, Q., Garrity, G. M., Tiedje, J. M., & Cole, J. R. (2007). Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology*, *73*, 5261–5267.

Weckström, J., & Korhola, A. (2001). Patterns in the distribution, composition and diversity of diatom assemblages in relation to ecoclimatic factors in Arctic Lapland. *Journal of Biogeography*, *28*, 31–45.

Weckström, J., Korhola, A., & Blom, T. (1997). Diatoms as quantitative indicators of pH and water temperature in subarctic Fennoscandian lakes. *Hydrobiologia*, *347*, 171–184.

Zhou, J., Bruns, M. A., & Tiedje, J. M. (1996). DNA recovery from soils of diverse composition. *Applied and Environmental Microbiology*, *62*, 316–322.

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