

Regional and global elevational patterns of microbial species richness and evenness

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Although elevational gradients in microbial biodiversity have attracted increasing attention recently, the generality in the patterns and underlying mechanisms are still poorly resolved. Further, previous studies focused mostly on species richness, while left understudied evenness, another important aspect of biodiversity. Here, we studied the elevational patterns in species richness and evenness of stream biofilm bacteria and diatoms in six mountains in Asia and Europe. We also reviewed published results for elevational richness patterns for soil and stream microbes in a literature analysis. Our results revealed that even within the same ecosystem type (that is, stream) or geographical region, bacteria and diatoms showed contrasting patterns in diversity. Stream microbes, including present stream data, tend to show significantly increasing or decreasing elevational patterns in richness, contrasting the findings for soil microbes that typically showed nonsignificant or significantly decreasing patterns. In all six mountains for bacteria and in four mountains for diatoms, species richness and evenness were positively correlated. The variation in bacteria and diatom richness and evenness were substantially explained by anthropogenic driven factors, such as total phosphorus (TP). However, diatom richness and evenness were also related to different main drivers as richness was mostly related to pH, while evenness was most explained by TP. Our results highlight the lack of consistent elevational biodiversity patterns of microbes and further indicate that the two facets of biodiversity may respond differently to environmental gradients.

Elevational patterns in biodiversity are one of the oldest research topics in ecology, which can be dated back to ~270 yr ago (Linnaeus 1781, Lomolino 2001, Rahbek 2005). Studies have shown that elevational patterns in diversity mainly occur in one of the two forms: species richness shows a unimodal pattern or decreases monotonically with elevation (Rahbek 2005). While the literature on elevational diversity is relatively extensive and rich for macroorganisms (Rahbek 2005), microorganisms (e.g. bacteria), important to many ecosystem processes, have been less studied until no more than a decade ago (Bryant et al. 2008, Wang et al. 2011). Compared with the increasing number of elevational studies on soil microbes (Bryant et al. 2008, Fierer et al. 2011, Singh et al. 2012, Shen et al. 2013), the aquatic microbes along elevational gradients are still relatively understudied (but see Wang et al. 2011). Therefore, our current understanding about the elevational patterns in aquatic biodiversity

appears to be still immature. For instance, one could ask, are there general elevational patterns in biodiversity for aquatic microbes, especially within regional scales where scale effects of elevational patterns are minimized (Nogués-Bravo et al. 2008)? And what are the main drivers behind elevational patterns in biodiversity for aquatic microbes?

Elevational biodiversity patterns are affected by multiple drivers, such as climatic gradients (Lomolino 2001) and human activities (Nogués-Bravo et al. 2008). A 100-m rise in elevation lowers the air temperature by 0.6–1.0°C – this would render elevational gradients a highly useful ‘natural laboratory’ for examining the potential effects of climatic changes on biodiversity patterns. Furthermore, mountain regions are becoming more urbanized with increasing settlements and transport networks (Price 2006, Nogués-Bravo et al. 2008). Ecosystems at high elevations, i.e. freshwater systems (Sala et al. 2000, Messerli et al. 2004), are expected to be amongst the most vulnerable systems to climatic change and human disturbance, such as forestry practices, agricultural activities, and eutrophication. Such human disturbances are expected

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to influence ecosystems also at higher elevations more in the future given the warming climate. Anthropogenic nutrient inputs, for instance, enhance the productivity of streams, which in turn influences the species richness of benthic algae (Allan and Castillo 2007, Cardinale et al. 2009), and potentially also the community structure of decomposing bacteria. Furthermore, the fast development of small hydropower in mountainous regions, especially in China (Huang and Yan 2009), are threatening the biodiversity and ecosystem services in streams. Thus, there is an urgent need to understand the current spatial patterns in aquatic microbial biodiversity on mountainsides and how such patterns are shaped by local environmental variables (Elmendorf et al. 2012). Moreover, a fruitful approach would be to compare microbial patterns in aquatic ecosystems with those documented for microbes in terrestrial ecosystems.

The majority of elevational studies focused on species richness, and left community evenness along elevational gradients less intensively explored (but see Graham 1983, Fauth et al. 1989, Wang et al. 2011). Evenness is an important aspect of community biodiversity, which measures how similar species are in their abundances (Magurran 2013). Based on the findings from terrestrial plants (Hillebrand et al. 2008), evenness may also be important in governing multiple ecosystem functions, such as primary productivity. Often the relationship of species richness and evenness (RRE) may be positive such that species-poor communities are generally dominated by a few dominant species, as shown by Veech et al. (2003) for arthropods. However, negative RREs have also been found in observational studies, especially among plants, and they may often be explained by ecological processes, such as competitive exclusion (Stirling and Wilsey 2001, Ma 2005, Soininen et al. 2012). Thus, evenness may represent an important component of diversity not captured well by the variation in richness, and different processes may shape the two facets of biodiversity. We expected different spatial patterns in species richness and evenness along elevation, possibly driven by different environmental factors.

Further insights into the elevational patterns in biodiversity and underlying mechanisms can be obtained by covering multiple study regions and microbial taxonomic groups with consistent methods in the same ecosystems (e.g. in mountain streams) across regional and global scales. In this study, we examined elevational patterns of richness and evenness of biofilm bacteria and diatoms in streams in China, Norway, and Spain and investigated the underlying drivers for the observed patterns. Streams represent important aquatic ecosystems in mountain regions and their elevational patterns are relatively easy to be examined using equal elevational sampling intervals. Our main questions were: 1) are there general elevational patterns in species richness and evenness for bacteria and diatoms in streams? And do these patterns differ from the earlier findings from terrestrial and other stream ecosystems? 2) Are richness and evenness related positively or negatively? 3) Are there shared underlying drivers for elevational richness and evenness patterns across studied regions? We show here that there were no consistent elevational patterns in biodiversity for stream bacteria and diatoms. In the literature analysis, stream microbes showed more frequently significant elevational patterns than microorganisms in the

soil environments, where nonsignificant elevational trends and significantly decreasing patterns were dominant.

Material and methods

Study area and field sampling

We sampled six streams for bacteria and diatoms along mountainsides in three regions: one stream in 1) the Balggesvarri Mountain in Norway in 2012, one stream in 2) the Pyrenees Mountain in Spain in 2012, and four streams in 3) the Hengdian Mountain region in China (Supplementary material Appendix 1, Table A1). For the latter, we used the same stream samples from the Laojun Mountain collected in 2009 (Wang et al. 2011), complemented by the three streams sampled in the Haba, Meili, and Yulong Mountains in 2013.

We followed the same protocols as in Wang et al. (2011). Briefly, we sampled the whole elevational gradient starting from the accessible top of the mountain and ending in a valley, river or ocean where elevation did not substantially decrease. Each study site was divided into five or ten cross-sections, depending on the stream width. Twenty stones were selected randomly from riffle/run habitats along these transects, and biofilm was scraped off the stones for sub-samples from a predefined area (9 cm²) using a toothbrush (for diatoms) or a sterilized sponge (for bacteria). The sub-samples were subsequently pooled into a composite sample at each site. We considered stream biofilm microbes, instead of free-living ones, because the latter are easily affected by water currents and would be less predictable by local environments than biofilm communities resulting in lower amount of explained variation in the models. The samples for bacteria were frozen at -18°C immediately after the sampling. Water samples were preserved at -18°C until the chemical analyses.

Several environmental characteristics important for stream organisms were measured at each site. The latitude, longitude and elevation of the sampling sites were logged by using a GPS unit. Shading (% canopy cover) was measured at 10 locations in evenly spaced cross-channel transects covering the whole study section. Depth, current velocity, width and substratum particle size were measured at 10 random locations along the same transects. Water conductivity, pH and temperature were measured at each site.

Physicochemical analyses

We measured the chromophoric dissolved organic matter abundance (cDOM), represented by the absorption coefficient of cDOM at wavelength 355 nm (m⁻¹) (Zhang et al. 2009). Total nitrogen (TN) and total phosphorus (TP) were analyzed by peroxodisulphate oxidation and spectrophotometric method (Jin and Tu 1990).

Diatom identification

The diatom samples from all sites were treated identically in the laboratory. We used wet combustion with hydrogen

peroxide to clean diatom frustules of organic material. Cleaned diatoms were mounted in Naphrax. A total of 500 frustules per sample were identified and counted, using phase-contrast light microscopy (magnification 1000 \times). Diatoms were identified to species level according to Krammer and Lange-Bertalot (1986–1991), Lavoie (2008) and Metzeltin et al. (2009).

Bacterial community analysis

Genomic DNA was extracted from biofilm using a phenol chloroform method (Zhou et al. 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 8 forward and reverse primer sets. To ensure that the total length of the amplified sequences do 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 have exactly 7 extra bases as the spacer for sequencing phase shift. Barcodes were added to the reverse primer between the sequencing primer and the adaptor.

Positive PCR products were confirmed by agarose gel electrophoresis. PCR products from triplicate reactions were combined and quantified with PicoGreen (Eugene, OR, USA). PCR products from samples to be sequenced in the same MiSeq run were pooled at equal molality to maximize the even-sequencing efforts for all samples. The pooled mixture was purified with a QIAquick Gel Extraction Kit (QIAGEN Sciences, Germantown, MD, USA) and re-quantified with PicoGreen. Sample libraries for sequencing were prepared according to the MiSeq Reagent Kit Preparation Guide (Illumina, San Diego, CA, USA). The sequences were deposited in MG-RAST database under the accession number 17666.

Overlapped paired-end sequences from Miseq were assembled using FLASH (Magoč and 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. Further, the sequences were clustered into 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). Taxonomic identity of each representative sequence was determined using the RDP Classifier (Wang et al. 2007) and chloroplast and archaeal sequences were removed.

Literature analysis

We searched the data in the Web of Science (1990–June 2015) using combined keywords ('elevatio*' or 'altitud')

and ('richness' or 'diversit*') and ('gradien*' or 'patter*' or 'transec*' or 'varian*'). The results were refined to only include the studies on soil and stream microbes, such as bacteria, fungi, and diatoms. We only included studies with ≥ 5 sampling sites for each elevational gradient (Supplementary material Appendix 1, Table A2). We further compiled the elevational patterns in microbial richness, and then classified the reported patterns as significantly increasing, decreasing, hump-shaped, U-shaped, and nonsignificant following the results of original publications.

Statistical analyses

Although decomposition of diversity into truly independent richness and evenness components is mathematically impossible, Pielou's evenness [$J = H/\log(S)$, where H is the Shannon–Weaver diversity index and S is the number of species] (Pielou 1966) is a good measure of distribution of relative abundance in a community (Jost 2010). We chose species richness and Pielou's evenness as biodiversity metrics reflecting the two aspects of community biodiversity (Magurran 2013). There are also other evenness metrics available, such as E_{var} , which is suggested to be a general-purpose equitability measure (Smith and Wilson 1996). However, the meaning of E_{var} in an ecological context is not immediately obvious (Tuomisto 2012), and E_{var} also showed significant correlation with species richness in our data set (Supplementary material Appendix 1, Fig. A1). Because Pielou's evenness is the most commonly applied evenness index and measures the amount of evenness relative to the maximum amount possible for the given richness, we prefer to use Pielou's evenness here so that we can directly compare current results to previous meta-analysis (Soininen et al. 2012). The diatoms and bacteria were rarefied at 500 individuals and 10 000 sequences, respectively, to ensure that the empirical biodiversity was not biased or confounded by variation in abundance or sampling intensity.

The relationships between richness and elevation, evenness and elevation, as well as RREs were explored with linear and quadratic models. The better model was selected based on lower value of Akaike's information criterion (Yamaoka et al. 1978). We sampled over 16 elevations for each mountain to explore elevational patterns with statistical regression with comparative data for each mountain. In this way, robust conclusions on elevational patterns can be obtained with statistical regressions (Lennon 2011).

The relationships between biodiversity metrics and potential explanatory variables were further analyzed separately for bacteria and diatoms using boosted regression trees (BRT) for the whole data sets of 117 samples covering six elevational gradients. BRT is an ensemble method for fitting statistical models that differs fundamentally from conventional techniques that aim to fit a single parsimonious model (Elith et al. 2008). BRT is based on the combination of the strengths of two algorithms: regression trees (models that relate a response to their predictors by recursive binary splits) and boosting (an adaptive method for combining many simple models to give improved predictive performance). The final BRT model can be understood as an additive regression model in which individual terms are simple trees,

fitted in a forward, stagewise fashion (Elith et al. 2008). The following explanatory variables were considered: latitude, longitude, mountain (as a categorical variable), streamwater temperature, pH, conductivity, TN, TP, cDOM, stream width, stream depth, streamwater velocity, substratum size, and stream shading. All explanatory environmental variables (except for mountain) and the biodiversity metrics were standardized at mean = 0 and SD = 1. We did not find any correlation between explanatory variables that was higher than Spearman's $\rho^2 = 0.40$, and we thus kept all variables in the models. Given the relatively low sample size, we 1) kept the size of trees, and consequent interactions' order, low (tree complexity parameter = 2), and 2) chose a low shrinkage parameter (learning rate parameter = 0.002), which controls the contribution of each individual tree to the final model. We produced an optimal number of trees of at least 1000 using cross-validation (Elith et al. 2008). The importance of a predictor variable was determined by its frequency of selection (for splitting) weighted by a measure of improvement of the model given each split and averaged across all trees (contributions were scaled to sum to 100). All BRT results (variable importance and predictions) were averaged across the 'm-imputed' datasets. BRT analyses were implemented with the R package 'gbm' (ver. 2.1).

Results

Patterns in richness

For bacteria, we found significant relationships ($p < 0.05$) between richness and elevation for all mountains, yet the outcomes were contrasting: increasing (2 mountains), decreasing (1), hump-shaped (1) and U-shaped (2) patterns (Fig. 1, Supplementary material Appendix 1, Table A3). In the Hengduan Mountain region alone, we observed three contrasting patterns, that is, increasing (1), decreasing (1)

and U-shaped (2). For diatoms, only two patterns were significant ($p < 0.05$), a decreasing pattern for Laojun Mountain and a hump-shaped pattern for Haba Mountain (Fig. 1, Supplementary material Appendix 1, Table A3).

Literature analysis on richness

Supporting our contradicting results, there were no general elevational patterns in richness for microbes reported in the literature we reviewed (Fig. 2). For soil environments, 35.0% of the cases showed nonsignificant elevational patterns, followed by significantly decreasing (30.0%) and hump-shaped (20.0%) patterns. For stream environments, however, the most frequently observed relationships were significantly decreasing (36.8%) and increasing (26.3%) patterns, followed by nonsignificant trends (21.1%) and significantly hump-shaped (10.5%) patterns. Frequency of different patterns varied significantly between the soil and stream environments (Fig. 2; $\chi^2(4) = 23.09$, $p < 0.001$).

Patterns in evenness

For bacteria, three out of six mountains showed significant elevational patterns, which were hump-shaped for Haba Mountain, decreasing for Meili Mountain and increasing for Yulong Mountain (Fig. 3, Supplementary material Appendix 1, Table A3). For diatoms, however, all six mountains showed significant patterns, which were hump-shaped (3), U-shaped (1), increasing (1) and decreasing (1) (Fig. 3, Supplementary material Appendix 1, Table A3).

The relationship between richness and evenness

For bacteria, the relationships between richness and evenness always showed significantly ($p < 0.05$) positive linear or qua-

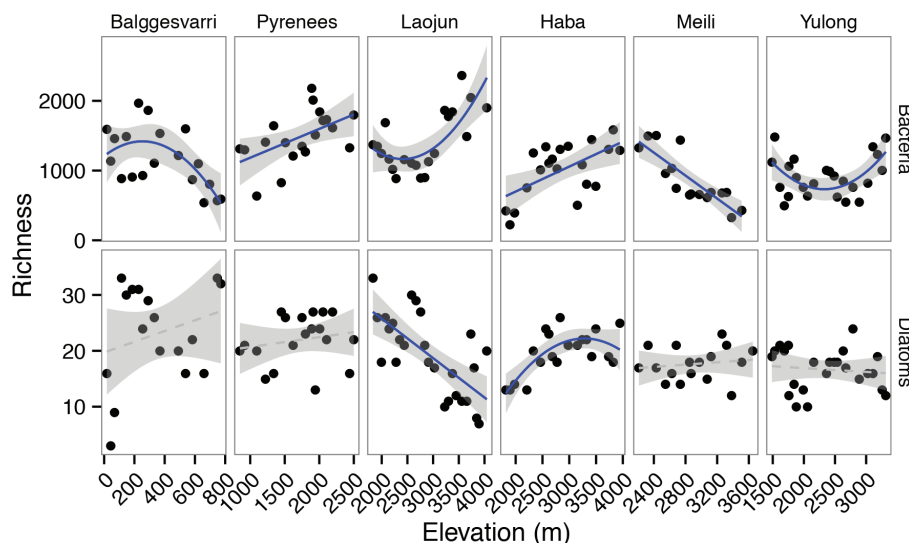


Figure 1. Species richness of stream bacteria and diatoms on the six mountainsides. The trends along elevations were modeled with both linear and quadratic models. The better model was selected based on the lower value of Akaike's information criterion, and is shown as solid line. Non-significant trends of both models are shown as dotted lines. More details on the models can be found in Supplementary material Appendix 1, Table A1. Bacteria and diatom richness are shown in the upper and lower panels, respectively. The mountain names are marked on the top of the upper panels. We used the same diatom data as in Wang et al. (2011) for the Laojun Mountain.

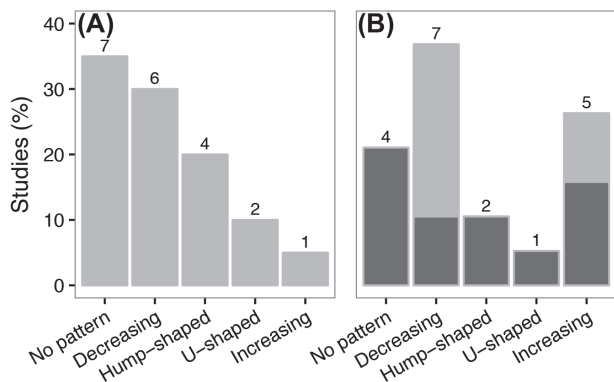


Figure 2. The distribution of elevational patterns for soil (A) and stream (B) environments based on literature analysis and present data. The patterns were classified as monotonically decreasing, hump-shaped, U-shaped, and monotonically increasing patterns according to the literature reports. The other patterns were classified as 'no pattern'. In the panel (B), the results from our six mountains are shown as dark gray, and the other results from literature are shown as light gray. In total, there were 20 and 19 elevational gradients for soil and stream environments, respectively. The numbers of elevational gradients for each pattern are shown at the top of the bars.

dratic patterns (Fig. 4). However, for diatoms, the relationships were significantly positive ($p < 0.05$) only in four out of six mountains (Fig. 4).

Underlying drivers for biodiversity

Water chemistry varied substantially in these alpine streams. For instance, TP varied from 0.077 to 2.477 $\mu\text{mol l}^{-1}$, with median and mean values of 0.472 and 0.568 $\mu\text{mol l}^{-1}$, respectively. Water pH varied from 5.20 to 8.92, with median and mean values of 8.20 and 7.66, respectively.

According to BRT, TP was the most important variable in explaining the variations in bacterial richness (relative influence of 22.2%) and evenness (23.2%). Richness and evenness were highest at intermediate TP and showed decreasing patterns toward higher TP (Fig. 5A, B, Supplementary material Appendix 1, Fig. A2–A3). Bacterial richness varied significantly among mountains and correlated also with other environmental variables, such as pH, shading, conductivity and cDOM (Fig. 5A, Supplementary material Appendix 1, Fig. A2). Water pH, shading and conductivity were also important in explaining bacterial evenness (Fig. 5B, Supplementary material Appendix 1, Fig. A3).

For diatoms, richness was best explained by pH (41.5%), followed by shading (23.3%), TP, depth, cDOM and temperature (Fig. 5C, Supplementary material Appendix 1, Fig. A4), while evenness mostly correlated with TP (23.9%), followed by shading (23.6%) and pH (20.0%) (Fig. 5D, Supplementary material Appendix 1, Fig. A5).

Among the environmental variables, streamwater temperature was less important in explaining biodiversity and only contributed to bacterial evenness with a relative influence of 9.0%.

Discussion

To our knowledge, this is the first study exploring both species richness and evenness of stream bacteria and diatoms along multiple mountainsides across Asia (China) and Europe (Spain and Norway). We employed consistent field methods for sampling throughout the study and the same morphological and molecular methods for diatom and bacterial species identification, respectively, to reduce artifacts and biases. Unexpectedly, even within the same ecosystem type, we did not find consistent elevational patterns in the two facets of biodiversity for the studied groups within or across

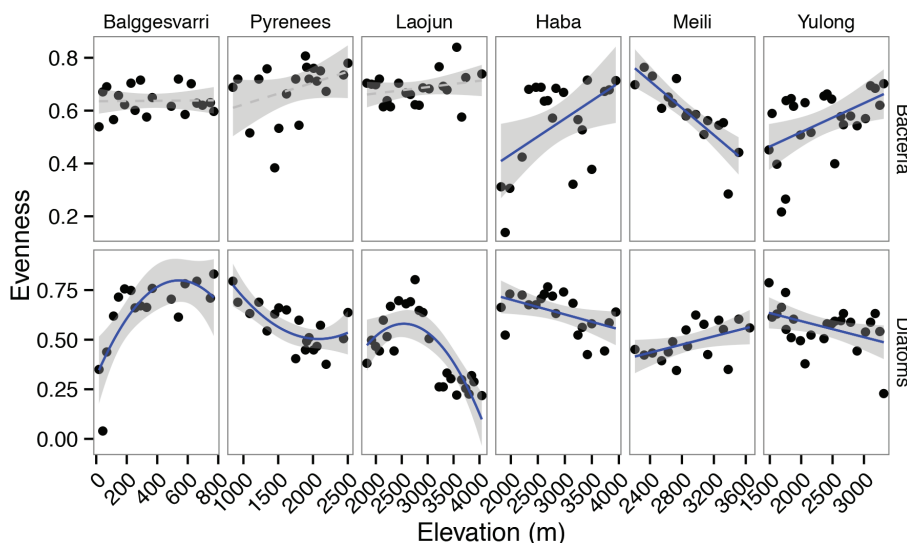


Figure 3. Community evenness of bacteria and diatoms on the six mountainsides. The trends along elevations were modeled with linear and quadratic models. The better model was selected based on the lower value of Akaike's information criterion, and is shown as solid line. Non-significant trends ($p > 0.05$) for both models are shown as dotted lines. More details about the models can be found in Supplementary material Appendix 1, Table A1. Bacteria and diatom evenness are shown in the upper and lower panels, respectively. The mountain names are marked on the top of the upper panels. We used the same diatom data as in Wang et al. (2011) for Laojun Mountain.

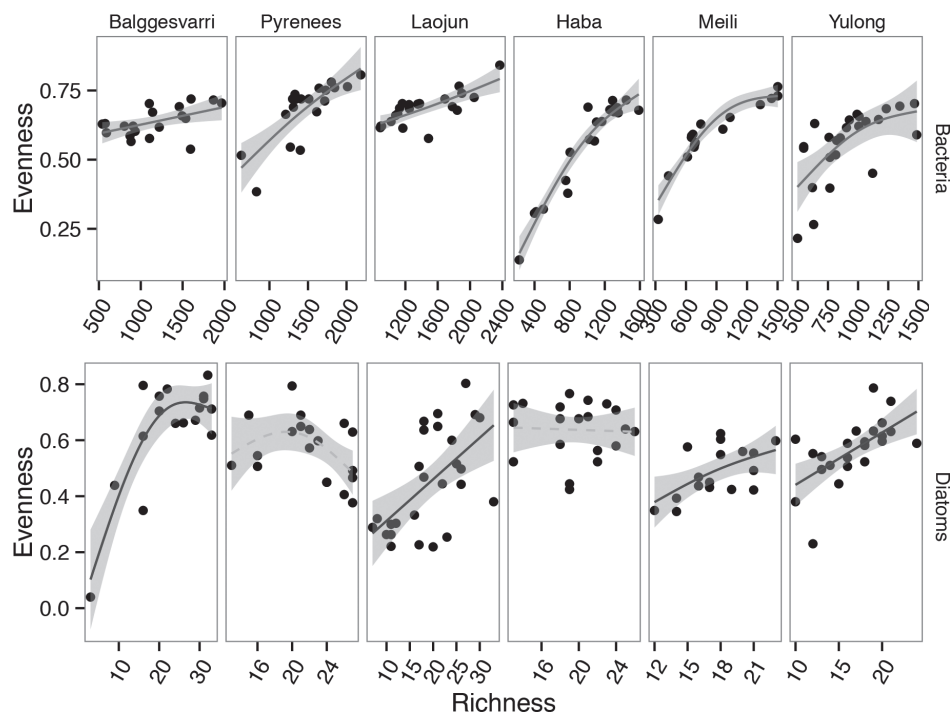


Figure 4. The relationships between richness and evenness for bacteria and diatoms. The trends along elevations were modeled with linear and quadratic models. The better model was selected based on the lower value of Akaike's information criterion, and is shown as solid line. Non-significant trends for both models are shown with dotted lines. More details on the models are in Supplementary material Appendix 1, Table A3. The mountain names are marked on the top of the panels. The upper and lower panels are for bacteria and diatoms, respectively.

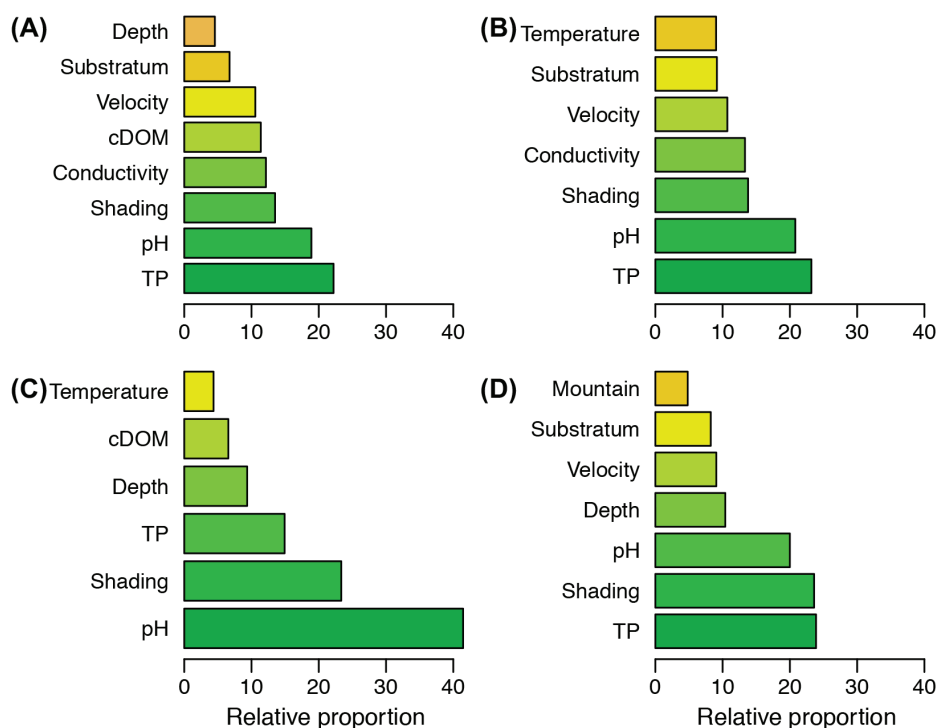


Figure 5. The environmental factors related to the richness and evenness of bacteria and diatoms, identified with Boosted Regression Trees. (A) Bacteria richness. (B) Bacteria evenness. (C) Diatom richness. (D) Diatom evenness. The values of the relative contribution (%) of each variable for each biodiversity metric can be found in parentheses on the x-axes of Supplementary material Appendix 1, Fig. A1–A4. TP: total phosphorus. Mountain: the mountains as a categorical variable. Shading: riparian shading (%). Substratum: median of the substratum particle size. Velocity: current velocity. Depth: streamwater depth. cDOM: chromophoric dissolved organic matter. Temperature: streamwater temperature.

study regions. This indicates that the response of bacterial and diatom biodiversity to elevation-driven changes in temperature and in other associated environmental variables along elevations was not uniform across mountains. Thus, elevational patterns are more likely to reflect the influence of some other local environmental factors and their interactions rather than only the direct effects of linearly decreasing temperature along elevation.

Elevational patterns in richness across regions

Our results highlight the contrasting elevational patterns in bacterial and diatom richness across alpine stream ecosystems, among which some patterns have been rarely reported so far. For instance, we found increasing bacterial richness towards high elevations in the Pyrenees Mountain in Spain, and the Haba Mountain in China, and the U-shaped patterns in the Laojun and Yulong Mountains in China. In the streams of the Laojun Mountains, the increasing and U-shaped patterns in richness were previously revealed with fingerprinting and pyrosequencing methods, respectively (Wang et al. 2011, 2012a), and were accounted for by increasing carbon supply at higher elevations. These findings were similar to our current finding of a U-shaped pattern detected using another high throughput sequencing method – Illumina Miseq. The fact that similar patterns emerged for different high-throughput sequencing methods adds reliability to our findings on rarely reported elevational patterns. It should be warranted that the results obtained from molecular analyses, as opposed to results based on visual identification of morphospecies, may differ slightly. However, the patterns for bacteria and diatoms were so strikingly different that this distinction is less likely to be attributed to the methodological differences.

Literature analysis of elevational patterns in richness

Based on our literature analysis, such increasing or decreasing patterns in stream microbial richness found here are surprisingly commonly observed in the literature too. The stream microbes, however, showed different distributions in elevational patterns from those of soil environments, which indicate the importance of considering the habitat differences for comparative studies of elevational diversity patterns. In the streams across New Zealand, Lear et al. (2013) reported weak decreasing elevational patterns in richness of biofilm bacteria revealed with a fingerprinting method. In glacier-fed streams in Austria, Wilhelm et al. (2013) also reported that the richness of both streamwater and biofilm communities decreased significantly with elevation using pyrosequencing method. In contrast, Lujan and colleagues (2013) found that richness of epilithic algae increased slightly with elevation whereas macroinvertebrate and fish richness decreased. Generally, our literature analysis showed that decreasing (36.8%) and increasing (26.3%) elevational patterns dominated the aquatic environments. Such contrasting patterns suggest that richness is not solely driven by temperature, but predominantly by some local environmental variables like nutrient inputs or carbon supply (see below and Wang et al.

2011). This is supported by the fact that water temperature was less important in explaining the biodiversity of both taxon groups than other local environmental variables, such as TP, pH, and shading.

Our results for stream microbes contrast with corresponding patterns for macroorganisms documented in quantitative analyses of elevational species richness gradients (Rahbek 2005, Guo et al. 2013). For higher plants and animals, most elevational gradients (50–63%) showed unimodal patterns, while only < 25% followed monotonically decreasing patterns (Rahbek 1995, Guo et al. 2013). The lack of generality of elevational patterns in macroorganisms may be due to the varying spatial grains and extent of the elevational gradients among studies (Rahbek 2005, Nogués-Bravo et al. 2008). The scale effects, however, are rarely considered in the comparative studies of elevational diversity gradients (Sanders and Rahbek 2012). Here, we controlled the extent of the elevational gradients sampled by considering four mountains as replicates within one region – the Hengduan Mountain regions, Yunnan, China. This allowed the direct comparison of four similar elevational gradients of ~ 2000 m without any notable variations in scale. Nevertheless, we found the elevational patterns in biodiversity varied substantially among individual mountains at this regional scale. The lack of generality in such regional elevational patterns in bacteria and diatoms further supports the notion that microbial diversity may be more affected by local environmental factors rather than by climatic variables associated with elevation, such as air or water temperature.

Relationship between richness and evenness

For stream biofilm microbes, we found significantly positive RREs in all mountains for bacteria and in four mountains for diatoms. The ratio of significantly positive RREs in our study (83%) is higher than reported in a recent meta-analysis, which showed that the significant RRE only has a ratio of 31% (Soininen et al. 2012). Further, in the stream ecosystems, RREs are typically negative for macroorganisms (Soininen et al. 2012), which also contrasts with our current findings of positive RREs for stream bacteria and diatoms. The differences in RREs between micro- and macroorganisms in the literature reviewed may be caused by the high dispersal ability for microbes. Local microbial species are frequently ‘rescued’ because they have large populations, providing a high number of propagules (Finlay 2002). One may thus envisage that in communities of microorganisms, richness scales more positively with evenness than in communities of macroorganisms because extinctions are rare. Our findings on positive RRE further imply that the variation in species richness and evenness may be explained by similar underlying factors, which is confirmed by BRT analyses across mountains. For instance, TP and pH substantially explained richness and evenness for both taxon groups. Some other environmental variables were also related to the two biodiversity metrics: with increasing shading, richness and evenness of bacteria communities increased, but decreased in diatom communities.

Explaining the elevational patterns in richness and evenness

Phosphorus, an essential nutrient in aquatic ecosystems, was the main environmental driver of biodiversity as richness and evenness of bacteria, and richness of diatoms decreased towards higher TP. This decreasing trend is consistent with the general pattern of decreasing relative species richness for freshwater species with increasing phosphorus content in lakes and streams worldwide (Azevedo et al. 2013). The TP concentrations usually correlate with human influence, which is typically stronger at lower elevations. For instance, TP decreased substantially with increasing elevation in the Laojun Mountain, which corresponded with the decreasing human populations towards high elevations (Wang et al. 2011, 2012b). Anthropogenic nutrient inputs were also observed in a survey study in the Hengduan Mountain regions, Yunnan, China, which shows that the stream TP increased with human populations along Nujiang and Lancang Rivers (unpubl.). In Meili Mountains, the input of domestic sewage from the Yubeng Village (elevation ~ 3100 m) to the streams increased substantially the downstream TP from a mean value of ~ 0.50 $\mu\text{mol l}^{-1}$ to ~ 1.30 $\mu\text{mol l}^{-1}$. Thus, it is likely that the increased human activities decreased aquatic biodiversity via anthropogenic driven variables, such as nutrient inputs.

Riparian shading showed contrasting effects on the biodiversity of diatoms and bacteria. For instance, shading was highly positively correlated with bacterial richness and evenness, while it negatively influenced diatom richness and evenness (Supplementary material Appendix 1, Fig. A2–A5). On one hand, the variations in riparian shading are related to the falling of leaves or removal of riparian vegetation, which can have dramatic effects on stream organic inputs (Young et al. 2008, Bartels et al. 2012) and organic availability will further affect bacterial richness (Wang et al. 2011, Besemer 2016). Such shading effects were supported also here in terms of a positive relationship between cDOM and bacterial biodiversity (Supplementary material Appendix 1, Fig. A2). On the other hand, riparian shading controls the amount of light reaching the streambed, which would be the main factor influencing the primary production of periphyton (Hill et al. 2001). It is most likely that an increased light input widens the niches for diatom species and potentially promotes the diatom biodiversity. Overall, however, the contrasting effects of riparian shading on bacterial and diatom biodiversity indicate that the stream bacterial biodiversity seems to be more affected by the allochthonous terrestrial organic inputs, rather than by the autochthonous primary productivity. This is in line with the inconsistent elevational patterns between periphyton biomass and bacterial biodiversity. For instance, the periphyton biomass and primary production in Laojun Mountains showed unimodal patterns along elevations (Wang et al. 2011), while bacterial richness showed U-shaped patterns.

Water pH also explained a considerable fraction of biodiversity for both taxon groups, although its relative influences varied between richness and evenness, especially for diatoms. Both bacterial species richness and evenness showed a hump-shaped pattern along the pH gradient (Supplementary material Appendix 1, Fig. A2, A3), which

has been rarely observed so far for stream ecosystems (Besemer 2016). This hump-shaped pattern is in line with the findings from other habitats, such as lakes (Ren et al. 2015) and soils (Fierer and Jackson 2006), where species richness usually peaks at neutral pH. For diatoms, pH was the main driver for richness, showing a negative relationship between richness and pH (Supplementary material Appendix 1, Fig. A4), which is congruent with recent findings at global scales (Soininen et al. 2016), but is different from regional scales, which show very weak (Heino et al. 2010) or hump-shaped (Schneider et al. 2013) relationships between richness and pH. However, pH was less important for diatom evenness than for richness. The different relative influences of pH and TP on diatom richness and evenness further highlight the importance of considering both richness and evenness in elevational studies regarding climatic changes and human effects.

Conclusions

There were no consistent patterns in species richness and evenness across the six mountains for the two microbial taxa groups, bacteria and diatoms. The literature analysis revealed different elevational patterns in species richness between stream and soil environments. The latter were dominated by nonsignificant elevational trends in richness, followed by significantly decreasing patterns in richness. However, the significant decreasing and increasing patterns were often revealed in stream environments. Although the number of studies included in the analysis was relatively limited, such findings can be regarded to be the first attempt to synthesize the elevational patterns in microbial diversity. Further, we found that there were significant positive relationships between species richness and evenness, especially for stream bacteria. This is consistent with the fact that the variations in species richness and evenness may be explained by similar underlying factors, such as TP, shading, and pH. For diatoms, however, species richness responded most strongly to pH, while evenness was mostly affected by TP. Regarding the importance of human-driven nutrient inputs to stream ecosystems, investigating both richness and evenness is crucial to explore the climatic changes or human effects on biodiversity. Thus, in addition to species richness, patterns in evenness need also to be studied to fully appreciate consequences of human impacts on aquatic ecosystems that cause extinction and/or changes of species abundance distribution.

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Supplementary material (Appendix ECOG-02216 at <www.ecography.org/appendix/ecog-02216>). Appendix 1.

Ecography

ECOG-02216

Wang, J., Meier, S., Soininen, J., Casamayor, E. O., Pan, F., Tang, X., Yang, X., Zhang, Y., Wu, Q., Zhou, J. and Shen, J. 2016. Regional and global elevational patterns of microbial species richness and evenness. – Ecography doi: 10.1111/ecog.02216

Supplementary material

Appendix 1

Supplemental information for

Regional and global elevational patterns of microbial species richness and evenness

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Running title: Elevational patterns in microbial diversity

Table A1. Brief description of the mountains studied. Lowest-Highest elevations indicate the lowest and highest elevations of the stream samples. Sample numbers for bacteria and diatoms of each mountain may differ due to the failing in sample pretreatments. Geographic distance indicates the longest geographic distance between samples for each mountain.

Mountain	Mountain Latitude & Longitude (°)	Lowest-Highest elevations (m)	Treeline (m)	Visiting year	Samples (bacteria/ diatoms)	Geographic distance (km)
Balgesvarri	69.3809N; 20.3483E	0 - 770	550	Jul, 2012	19 / 17	8.9
Pyrenees	42.5457N; 0.8907E	850 - 2500	2100	Oct, 2012	19 / 19	27.3
Laojun	26.6959N; 99.7759E	1800 - 4200	4200	Oct, 2009	22 / 26	33.5
Haba	27.3758N; 100.1259E	1810 - 3940	3900	Oct, 2013	20 / 20	9.8
Meili	28.3705N; 98.8057E	2210 - 3650	3700	Oct, 2013	16 / 17	8.8
Yulong	27.1508N; 100.2931E	1490 - 3320	3900	Oct, 2013	24 / 24	18.8

Table A2. Summary of Elevational patterns in literature. Citations are shown below the table.

First author	Year	Habitat	Microorganisms	Patterns
Ormerod	1994	Stream biofilm	Diatoms	Decreasing
Bryant	2008	Soil	Acidobacteria	Decreasing
Fierer	2011	Soil	Bacteria	Hump-shaped
Fierer	2011	soil	Bacteria	U-shaped
Bahram	2011	Soil	Ectomycorrhizal fungi	Decreasing
Bahram	2011	Soil	Ectomycorrhizal fungi	Decreasing
Bahram	2011	Soil	Ectomycorrhizal fungi	No pattern
Singh	2011	Soil	Bacteria	Hump-shaped
Wang	2011	Stream biofilm	Diatoms	Decreasing
Wang	2011	Stream biofilm	Bacteria	Increasing
Shen	2012	Soil	Bacteria	No pattern
Singh	2012	Soil	Archaea	Hump-shaped
Wang	2012	Stream biofilm	Proteobacteria	Increasing
Singh	2013	Soil	Bacteria	No pattern
Yuan	2013	Soil	Bacteria	No pattern
Wilhelm	2013	Stream water	Bacteria	Decreasing
Wilhelm	2013	Stream biofilm	Bacteria	Decreasing
Lear	2013	Stream biofilm	Bacteria	Decreasing
Lujan	2013	Stream biofilm	Algae	Increasing
Miyamoto	2014	Soil	Ectomycorrhizal fungi	Hump-shaped
Shen	2014	Soil	Eukaryotes	No pattern
Singh	2014	Soil	Bacteria	U-shaped
Singh	2014	Soil	Bacteria	Decreasing
Zhang	2014	Soil	Acidobacteria	U-shaped
Wang	2014	Soil	Bacteria	Decreasing
Wang	2014	Soil	Archaea	No pattern
Pellissier	2014	Soil	Fungi	Increasing
Zhang	2015	Soil	Bacteria	Decreasing
Wilhelm	2015	Stream biofilm	Bacteria	Decreasing
Jarvis	2015	Soil	Ectomycorrhizal fungi	No pattern

Citations: (Ormerod, et al. 1994); (Bryant, et al. 2012); (Fierer, et al. 2011); (Bahram, et al. 2011); (Dharmesh Singh, et al. 2012, D. Singh, et al. 2012, Singh, et al. 2013, Singh, et al. 2014); (Wang, et al. 2011); (Wang, et al. 2012); (Wilhelm, et al. 2013, Wilhelm, et al. 2015); (Lear, et al. 2013); (Lujan, et al. 2013); (Miyamoto, et al. 2014); (Shen, et al. 2013); (Zhang, et al. 2015); (Zhang, et al. 2014); (Wang, et al. 2014); (Pellissier, et al. 2014); (Jarvis, et al. 2015);

Table A3. The relationships between elevations, richness and evenness. The trends of richness or evenness along elevations, and the relationships between richness and evenness were modeled with both linear (L) and quadratic (Q) models. The better model was selected with the lower value of Akaike's Information Criterion.

Relations	Taxa	Locations	Linear model		Quadratic model		Better Model
			R ²	P	R ²	P	
Richness - Elevation	Bacteria	Balggesvarri	0.301	0.015	0.438	0.010	Q
		Pyrenees	0.277	0.021	0.283	0.070	L
		Laojun	0.378	0.003	0.544	0.001	Q
		Haba	0.353	0.006	0.434	0.008	L
		Meili	0.712	0.000	0.729	0.000	L
		Yulong	0.020	0.508	0.319	0.018	Q
	Diatom	Balggesvarri	0.071	0.300	0.097	0.488	L
		Pyrenees	0.036	0.435	0.118	0.366	L
		Laojun	0.478	0.000	0.482	0.001	L
		Haba	0.365	0.005	0.568	0.001	Q
		Meili	0.022	0.574	0.051	0.695	L
		Yulong	0.012	0.613	0.012	0.882	L
Evenness - Elevation	Bacteria	Balggesvarri	0.000	0.930	0.071	0.555	L
		Pyrenees	0.130	0.129	0.190	0.186	L
		Laojun	0.070	0.246	0.141	0.254	L
		Haba	0.253	0.024	0.381	0.017	L
		Meili	0.704	0.000	0.719	0.000	L
		Yulong	0.252	0.012	0.255	0.045	L
	Diatom	Balggesvarri	0.389	0.007	0.567	0.003	Q
		Pyrenees	0.424	0.003	0.567	0.001	Q
		Laojun	0.405	0.000	0.613	0.000	Q
		Haba	0.230	0.032	0.321	0.037	L
		Meili	0.233	0.049	0.234	0.155	L
		Yulong	0.184	0.036	0.190	0.110	L
Evenness - Richness	Bacteria	Balggesvarri	0.264	0.025	0.301	0.057	L
		Pyrenees	0.624	0.000	0.652	0.000	L
		Laojun	0.575	0.000	0.600	0.000	L
		Haba	0.902	0.000	0.939	0.000	Q
		Meili	0.798	0.000	0.914	0.000	Q
		Yulong	0.389	0.001	0.463	0.001	L
	Diatom	Balggesvarri	0.511	0.001	0.748	0.000	Q
		Pyrenees	0.104	0.179	0.305	0.054	Q
		Laojun	0.343	0.002	0.372	0.005	L
		Haba	0.002	0.848	0.012	0.900	L
		Meili	0.310	0.020	0.362	0.043	L
		Yulong	0.383	0.001	0.388	0.006	L

Figure A1. The relationships of richness-evenness for bacteria (A-B) and diatoms (C-D) with two evenness metrics for the whole data set. These two metrics are Evar (A, C) and Pielou's evenness (B, D). All relationships were significantly ($P < 0.01$) fitted by a linear model.

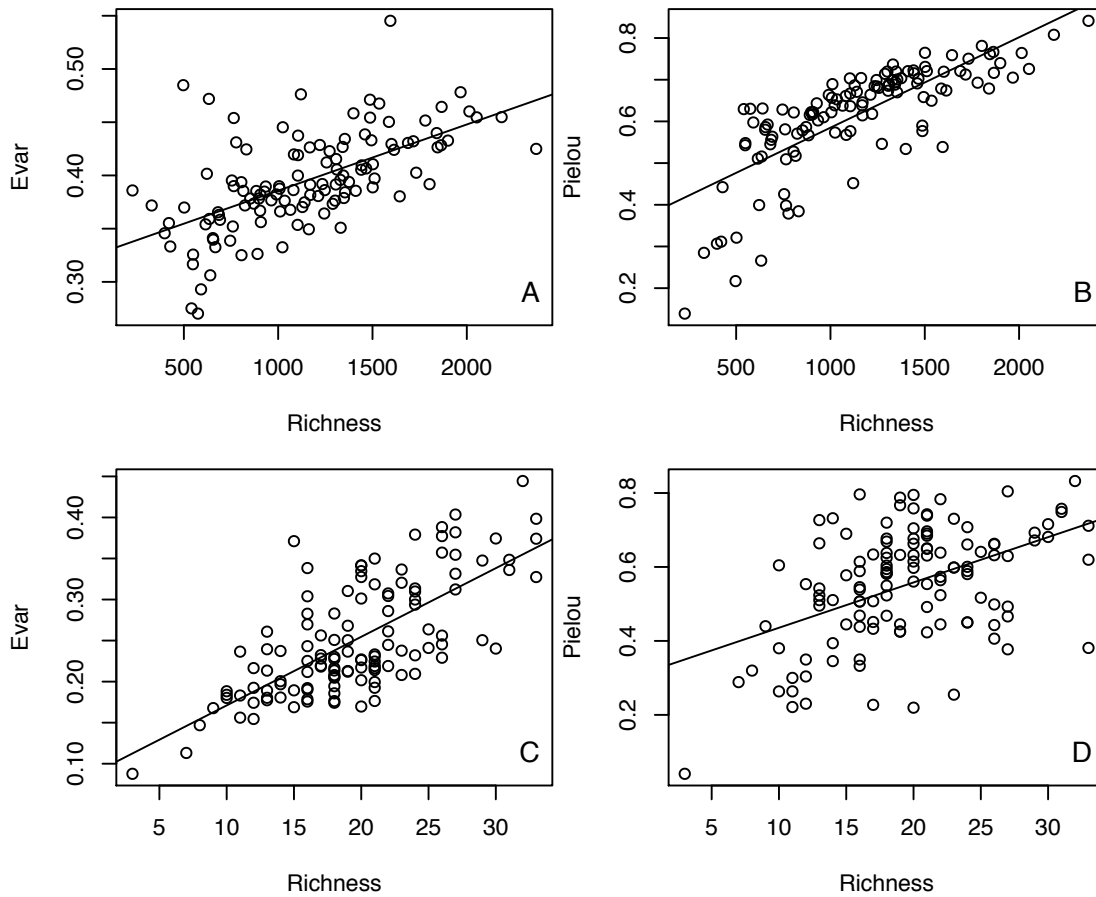


Figure A2. Partial dependence plots for all variables included in the Boosted Regression Trees (Elith, et al. 2008) model of bacterial species richness. The y-axes represent the marginal effect of the respective explanatory variable on the predicted species richness (centred to zero mean). The relative contribution (%) of each variable is shown in parentheses on the x-axes. The relative contributions are visualized in Figure 5A. The solid line is the BRT-modeled response curve to the most important variables. The dashed line is the locally weighted scatterplot smoothing (LOESS) regression (span 0.25) fitted to the response curve. TP: total phosphorus. Mountain: the mountains as a categorical variable. Shading: riparian shading (%). Substratum: median of the substratum particle size. Velocity: current velocity. Depth: streamwater depth. cDOM: chromophoric dissolved organic matter. Temperature: streamwater temperature.

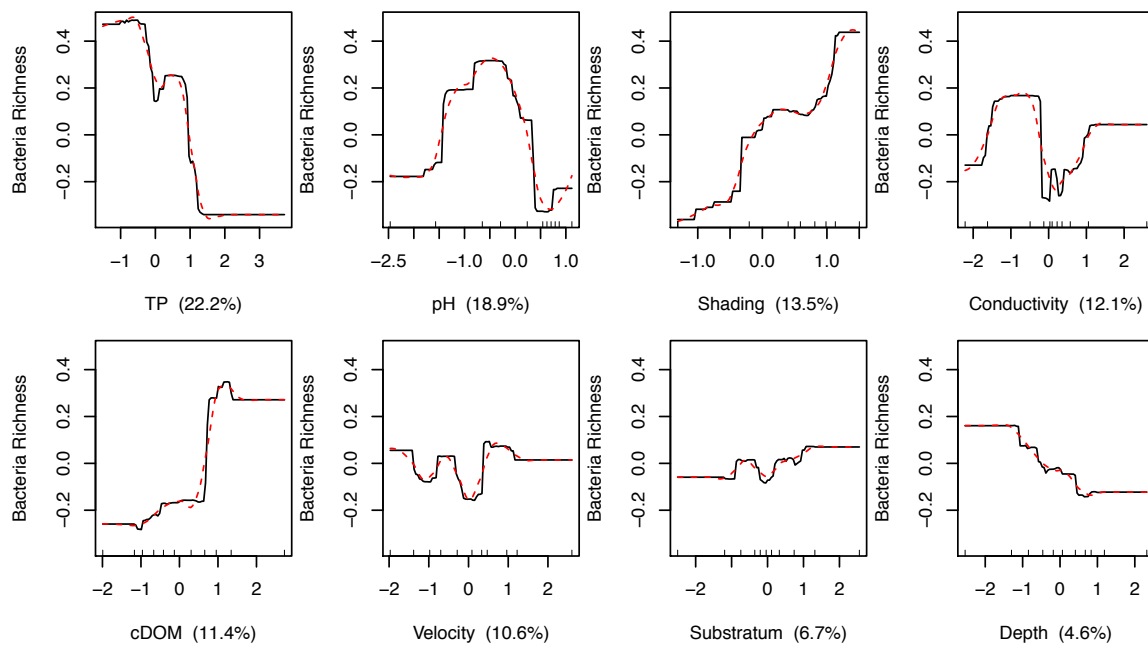


Figure A3. Partial dependence plots for all variables included in the Boosted Regression Tree model of bacterial species evenness. The y-axes represent the marginal effect of the respective explanatory variable on the predicted species richness (centred to zero mean). The relative contribution (%) of each variable is shown in parentheses on the x-axes. The relative contributions are visualized in Figure 5B. The solid line is the BRT-modeled response curve to the most important variables. The dashed line is the LOESS smoother (span 0.25) fitted to the response curve. The abbreviations of environmental variables are shown in Figure A2.

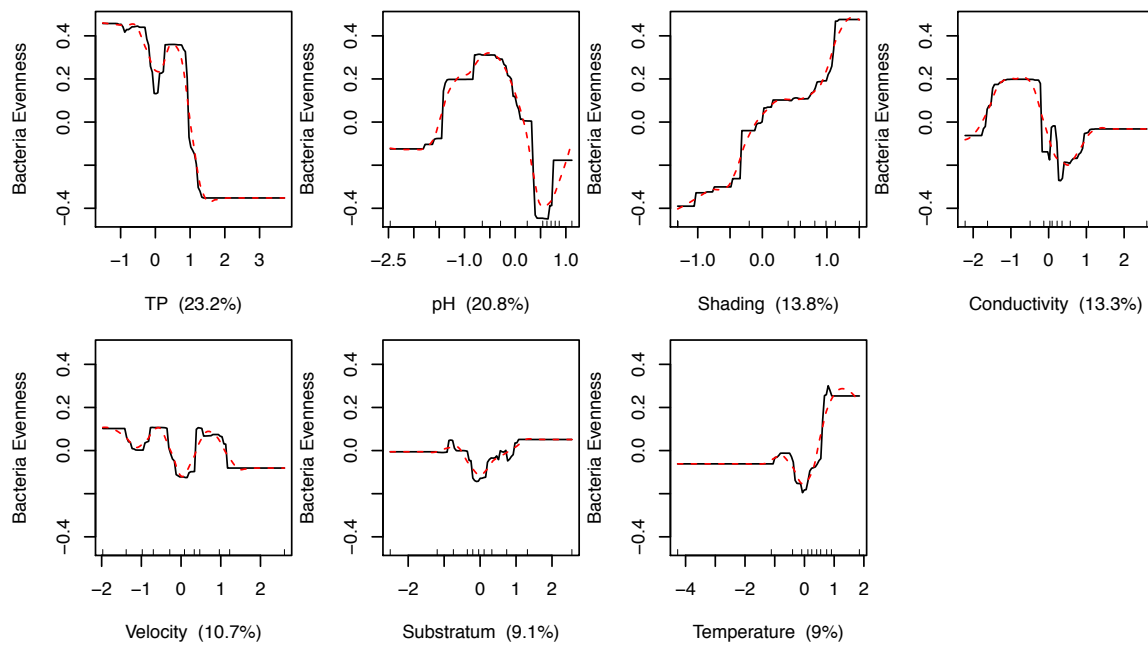


Figure A4. Partial dependence plots for all variables included in the Boosted Regression Tree model of species richness of diatoms. The y-axes represent the marginal effect of the respective explanatory variable on the predicted species richness (centred to zero mean). The relative contribution (%) of each variable is shown in parentheses on the x-axes. The relative contributions are visualized in Figure 5C. The solid line is the BRT-modeled response curve to the most important variables. The dashed line is the LOESS smoother (span 0.25) fitted to the response curve. The abbreviations of environmental variables are shown in Figure A2.

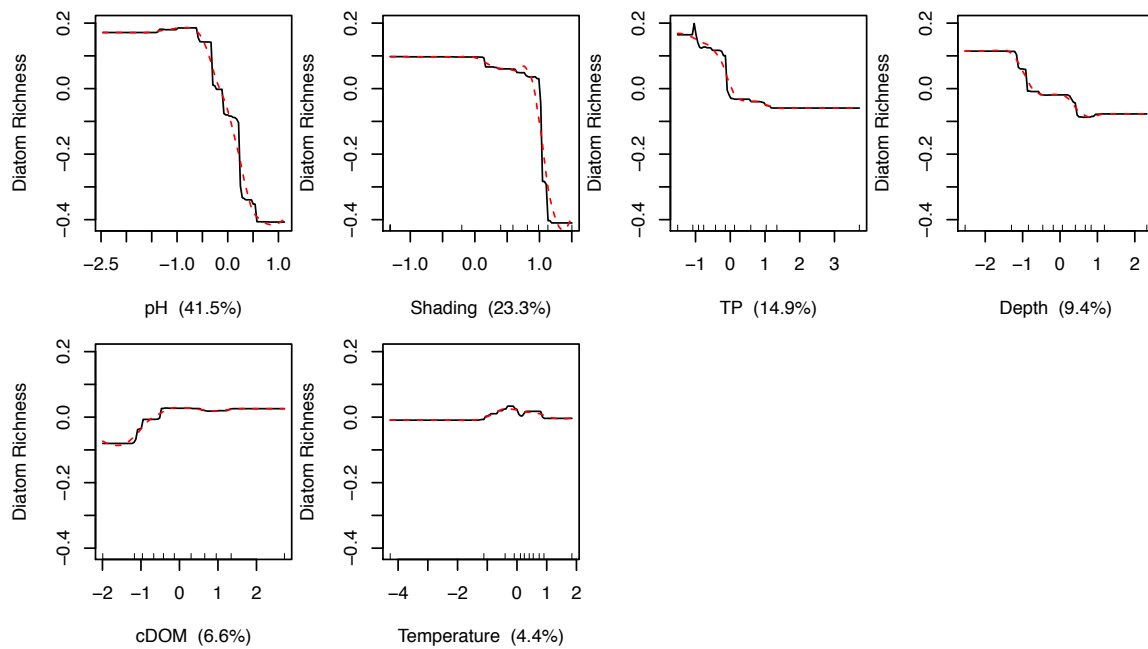
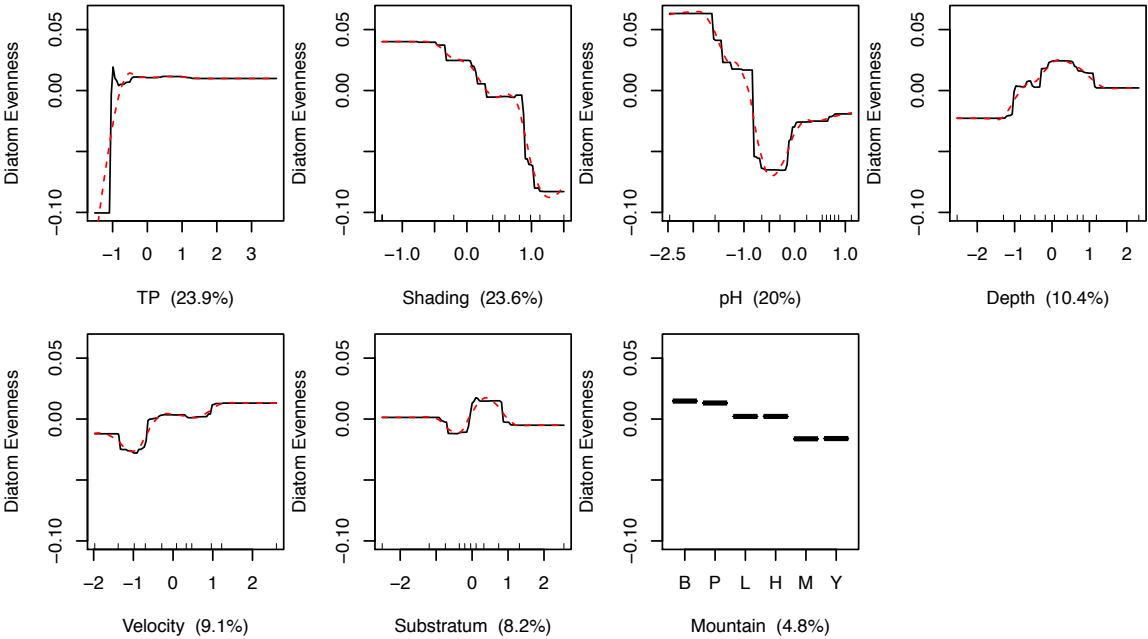


Figure A5. Partial dependence plots for all variables included in the Boosted Regression Tree model of species evenness of diatoms. The y-axes represent the marginal effect of the respective explanatory variable on the predicted species richness (centred to zero mean). The relative contribution (%) of each variable is shown in parentheses on the x-axes. The relative contributions are visualized in Figure 5D. The solid line is the BRT-modeled response curve to the most important variables. The dashed line is the LOESS smoother (span 0.25) fitted to the response curve. The abbreviations of environmental variables are shown in Figure A2.



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