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ORIGINAL ARTICLE

Contrasting patterns in elevational diversity between microorganisms and macroorganisms

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ABSTRACT

Aim Data and analyses of elevational gradients in diversity have been central to the development and evaluation of a range of general theories of biodiversity. Elevational diversity patterns have, however, been severely understudied for microbes, which often represent decomposer subsystems. Consequently, generalities in the patterns of elevational diversity across different trophic levels remain poorly understood. Our aim was to examine elevational gradients in the diversity of macroinvertebrates, diatoms and bacteria along a stony stream that covered a large elevational gradient.

Location Laojun Mountain, Yunnan province, China.

Methods The sampling scheme included 26 sites spaced at elevational intervals of 89 m from 1820 to 4050 m elevation along a stony stream. Macroinvertebrate and diatom richness were determined based on the morphology of the specimens. Taxonomic richness for bacteria was quantified using a molecular fingerprinting method. Over 50 environmental variables were measured at each site to quantify environmental variables that could correlate with the patterns of diversity. We used eigenvector-based spatial filters with multiple regressions to account for spatial autocorrelation.

Results The bacterial richness followed an unexpected monotonic increase with elevation. Diatoms decreased monotonically, and macroinvertebrate richness showed a clear unimodal pattern with elevation. The unimodal richness pattern for macroinvertebrates was best explained by the mid-domain effect ($r^2 = 0.72$). The diatom richness was best explained by the variation in nutrient supply, and the increase in bacterial richness with elevation may be related to an increased carbon supply.

Main conclusions We found contrasting patterns in elevational diversity among the three studied multi-trophic groups comprising unicellular and multicellular aquatic taxa. We also found that there may be fundamental differences in the mechanisms underlying these species diversity patterns.

Keywords
Bacteria, biogeography, China, diatoms, elevational gradient, macroecology, macroinvertebrates, mid-domain effect, species diversity patterns, streams.

INTRODUCTION

Data and analyses of elevational gradients in diversity have been central to the development and evaluation of a range of general theories of biodiversity (Lomolino, 2001; Rahbek, 2005; Grytnes & McCain, 2007). The examination of elevational patterns in diversity is important for gaining an understanding of the influence of global change, for determining broad-scale distributions of species, and for differentiating between alternative diversity hypotheses (Grytnes & McCain, 2007). Previous studies have shown that elevational patterns in diversity usually occur in one of two forms: a monotonic decrease with elevation

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or a unimodal pattern (Rahbek, 2005). The proposed hypotheses for explaining the elevational patterns in diversity can be classified into four major categories: climatic, spatial, historical and biotic (Lomolino, 2001; Grytnes & McCain, 2007). Climatic hypotheses are based on the idea that current abiotic conditions shape the distributions of species (for example, harsh conditions decrease species richness at high elevations). Spatial hypotheses suggest that species distributions are constrained by area or by geometrical hard boundaries. Historical hypotheses suggest that species distributions have some historical imprint (for example, there are differences in evolutionary history along the gradient). Finally, biotic hypotheses emphasize interactions between species, and examine, for example, the degree to which members of a local community show overlap in niche space (Grytnes & McCain, 2007).

However, such patterns and hypotheses are based on studies of larger plants and animals, and microorganisms have been left understudied (but see Bryant *et al.*, 2008). Given the important roles of microorganisms in ecosystems as decomposers and primary producers, filling this gap is urgent. Two primary questions regarding microorganisms remain unanswered, namely (1) whether microorganisms show an elevational gradient in diversity, and (2) whether such a pattern, if it exists, resembles the patterns observed for macroorganisms. A promising approach towards understanding the putative differences in the macroecology of microorganisms and macroorganisms is to conduct intertaxonomic comparisons across elevational gradients (Lomolino, 2001). Recent studies have shown that diversity decreases monotonically with elevation for Acidobacteria in the Bacteria kingdom (Bryant *et al.*, 2008). This pattern is thus different from the elevational pattern that was documented for angiosperms in the same study. However, previous studies that have addressed elevational patterns in diversity have often examined gradients at a single trophic level or guild, rather than examining patterns for a suite of interacting communities across all trophic levels. Although there are studies documenting elevational patterns across trophic levels (e.g. Ormerod *et al.*, 1994; Grytnes *et al.*, 2006), no previous study has quantified diversity patterns along elevational gradients for the entire multi-trophic community, including microorganisms.

We studied the elevational gradients in diversity for macroinvertebrates, diatoms and bacteria along a stony stream in China that covers a large elevational gradient. Here, we show that the richness of bacterial communities followed an unexpected monotonic increase with elevation. Furthermore, the elevational patterns of diversity were different among macroorganisms and the two groups of microorganisms (diatoms and bacteria).

MATERIALS AND METHODS

Field sampling

In October–November of 2009, we sampled macroinvertebrates, diatoms and bacteria from 26 sites spaced at elevational intervals of *c.* 89 m along a stony stream located on Laojun Mountain, Yunnan province, China (26°44″ N, 99°48″ E). The sampling sites ranged in elevation from 1820 to 4050 m and spanned a distance of 33.5 km. As Laojun Mountain is at the junction of the Tibetan Plateau and the Yungui Plateau, the elevation of this region is high. The stream section begins at the top of the mountain and ends in a valley with an elevation of *c.* 1800 m (the elevation does not substantially decrease beyond this point). Below the elevation of 1800 m, the stony stream turns into a deep, slow-running large river (Jinsha River, Upper Yangtze River), where suitable stony riffle habitats for benthic organisms that live on stones are lacking. Therefore, we considered that the stream section sampled was delimited by 'hard' boundaries.

Each study site was divided into five or 10 cross-sections, depending on the stream width. For diatoms and bacteria, 10 stones were selected randomly from riffle/run habitats along these sections. Biofilm was scraped off the stones for subsamples from a predefined area (9 cm^2) using a toothbrush (for diatoms) or a sterilized sponge (for bacteria). The subsamples were subsequently pooled into a composite sample from each site. The samples for bacteria were frozen at −18 °C immediately after sampling. We collected four kicknet samples of macroinvertebrates from stony riffle/run habitats. These samples were pooled at each site and stored in 70% ethanol in the field. Water samples and filtered water samples (0.45 μm) were preserved at −18 °C until the chemical analyses were conducted.

Several environmental characteristics that are important for stream organisms were measured at each site. The latitude, longitude and elevation of the sampling sites were logged using a GPS unit. Shading (percentage 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 also measured at each site.

Physicochemical analyses

Dissolved organic carbon (DOC), dissolved inorganic carbon (DIC) and total organic carbon (TOC) were measured by high-temperature oxidation with a Shimadzu TOC analyser (model 5000; Tokyo, Japan). Ammonium (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-), silicon (Si) and dissolved inorganic phosphorus (DIP) were measured using a continuous flow analyser (Skalar SA 1000, Breda, The Netherlands). We measured the chromophoric dissolved organic matter (cDOM) concentration, represented by the absorption coefficients of cDOM (m^{-1}) at wavelength 355 nm. We also measured the exponential spectral slope of cDOM (S, nm^{-1}, 280–500 nm) as a measure of how rapidly the absorption decreased with increasing wavelength (Bricaud *et al.*, 1981). Total nitrogen (TN) and total phosphorus (TP) were analysed by peroxodisulphate oxidation and the spectrophotometric method. Alkalinity, CO_3^{2-} and HCO_3^- were measured using

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Gran titrations via a Metrohm auto-titrator (702 STAT-Titrino, Herisau, Switzerland). Other dissolved anions were analysed by ion chromatography, inductively coupled plasma atomic emission spectroscopy or inductively coupled plasma mass spectrometry.

Biofilm characteristics

Biofilm community respiration (CR) was measured in triplicate in the laboratory in the dark at 25 °C. The incubation chamber had a volume of 2 mL and was bathed in water at a constant temperature. The biofilm mixture (100 μL) was carefully injected into the incubation system, which was filled with 0.2-μm-filtered stream water and then sealed airtight and wrapped in aluminium foil. During the incubation, the samples were stirred gently, and dissolved oxygen levels in the chamber were measured every 10 s with a fibre-optic oxygen meter (Presens, Regensburg, Germany). The biofilm oxygen consumption rate was determined by the linear decrease of the oxygen level within 1–2 h of the chamber being closed. Similar to the measurement of CR, the net photosynthesis production (NPP) was calculated by the difference in the oxygen consumption rate between the samples in the dark and the samples in the light. Two fluorescent lamps (cool white, 23 W each) were placed 10 cm away from the chamber as a light source. Chlorophyll *a* was extracted using 90% acetone (Dalsgaard *et al.*, 2000).

Macroinvertebrate identification

Macroinvertebrates were identified to species level when possible using the standard keys (Morse *et al.*, 1994). Most Ephemeroptera, Plecoptera, Trichoptera, Coleoptera, Diptera, Hemiptera, Lepidoptera and Odonata were identified to species level. The identification level of all other taxonomic groups varied from species to family. Oligochaeta were identified to class level only. We recorded all macroinvertebrate taxa, and the numbers of individuals were recorded at each site.

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×). Diatoms were identified to species level according to Krammer and Lange-Bertalot (1986–1991) and Metzeltin *et al.* (2009).

Bacterial community analysis

Genomic DNA was extracted from freeze-dried biofilm using the phenol chloroform method as described by Zhou *et al.* (1996). Genomic DNA was then concentrated to a volume of 100 μL. The quality and quantity of the DNA was determined using a spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Polymerase chain reactions (PCRs), with the primers Bac27F (5′-AGA GTT TGG ATC MTG GCT CAG-3′) and Univ1492R (5′-CGG TTA CCT TGT TAC GAC TT-3′), were performed in a 50-μL solution composed of 0.2 μM dNTP, 1.5 mM $MgCl_2$, 0.2 μM of each primer, 5 μL of buffer, 1.5 U of *Taq* DNA polymerase (Fermentas, Shenzhen, China) and 0.5 μL of template DNA. The mixture was incubated in a thermal cycler for 25 cycles under conditions of 95 °C for 30 s, 58 °C for 30 s and 72 °C for 2 min, followed by a final elongation step at 72 °C for 10 min. For the denaturing gradient gel electrophoresis (DGGE), 16S rRNA genes of bacteria were amplified in a thermal cycler with the forward primer 338f-GC and the reverse primer 907r from the purified PCR products with the primers Bac27F and Univ1492R (Muyzer *et al.*, 1993). Each 50-μL PCR mixture contained 1× PCR buffer, 200 μM nucleotide mixture, 1.5 mM $MgCl_2$, 0.5 μM of each primer, 1.5 U of *Taq* DNA polymerase (Fermentas) and 1 μL of template. The mixture was incubated in a thermal cycler for 30 cycles under conditions of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 45 s, followed by a final elongation step at 72 °C for 10 min. All PCR products were checked by electrophoresis on a 0.8% (w/v) agarose gel.

The DGGE was performed on a CBS-DGGE 2000 system (C.B.S. Scientific, Del Mar, CA, USA) following the method introduced by Muyzer *et al.* (1993). The PCR products (500 ng per lane) were all separated in 6% acrylamide gels with a denaturing gradient from 30% to 55%. Gels were run in 1× TAE buffer (Tris-acetate-EDTA) at 60 °C and 75 V for 800 min, stained for 30 min in 1× TAE containing SYBR Green I (Roche, Mannheim, Germany), and documented by the Gel Doc EQ gel documentation system (Bio-Rad Laboratories, Hercules, CA, USA). Each DGGE band identified by Bionumerics 5.0 (Applied Maths, Sint-Martens-Latem, Belgium) represented an operational taxonomic unit (OTU). The number of DGGE bands was used to define the bacterial taxonomic richness at each site. Although the definitions of OTUs in fingerprints underestimate the true bacterial richness, the consistency of the method allowed us to examine the patterns of bacterial diversity among the samples (Hughes *et al.*, 2001).

Statistical analyses

We used species richness and Pielou's evenness as two measures of community diversity. We first studied whether gradients in macroinvertebrate richness could be explained by pure geographical constraints of species distributions, that is, by the mid-domain effect (MDE) (Colwell & Lees, 2000). We therefore ran a mid-domain null model with 50,000 simulations to retrieve the predicted MDE richness according to McCain (2004). The range size of a species was calculated using the upper and lower elevation limits of its occurrence. In MDE analysis, the ranges were 'filled', as we assumed that a species occurred at a certain elevation if it was detected at both higher

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and lower elevations (Colwell & Lees, 2000; Colwell *et al.*, 2004; McCain, 2004). This was a useful assumption because, for small organisms, one may not detect all species that occur at a site. When a species occurred at only one single sampling site, we set the range size as zero. We conducted the MDE analysis using both continuous and discrete methods (see Dunn *et al.*, 2006). However, as the two methods gave relatively similar results, we will discuss only the results of the continuous method, because this method may be more suitable for our data as the changes in elevation between the sampling sites were not strictly even. The occurrences of the species of macroinvertebrates, diatoms and bacteria along the elevational gradient are shown in Fig. S1 in the Supporting Information.

By performing principal components analysis (PCA), the electronic conductivity, alkalinity, and concentration of dissolved ions (Si, Cl^-, SO_4^{2-}, K, Na, Ca, Mg, Ba, Sr, As, Al, Fe, Mn, Zn, Cr, Cu, Pb, Ni, PO_4^{3-}, NH_4^+, NO_2^-, NO_3^-, DIC, HCO_3^- and CO_3^{2-}) were reduced to the first two principal components (PC1 and PC2) as explanatory variables that reflected the geochemical factors. The two extracted components accounted for 62.5% of the total variance. The remaining measured variables were used as explanatory variables without a PCA step. All explanatory variables predicted a MDE richness for macroinvertebrates, and the empirical richness of each of the three groups was standardized at a mean of 0 and a standard deviation of 1. The PCA was conducted using the statistical software R (http://www.r-project.org).

The relationship between the potential explanatory variables and species richness was analysed using multiple ordinary least squares (OLS) regression. The best models were identified using Akaike's information criterion (AIC) (Fotheringham *et al.*, 2002). Spatial autocorrelation was taken into account by including eigenvector-based spatial filters derived from geographic distances in all the models (Diniz-Filho & Bini, 2005). The OLS was performed using the software SAM 4.0 (Spatial Analysis in Macroecology, Rangel *et al.*, 2010, http://www.ecoevol.ufg.br/sam).

RESULTS

Elevation was the pivotal gradient among the measured environmental variables (Fig. 1), and it was significantly correlated with the physicochemical factors, namely water temperature, conductivity and the TN/TP ratio (Figs 1 & 2). The riparian shading in the upper part of the stream was significantly higher than the shading in the lower part of the stream (Fig. 2a). Water temperature increased from 0 °C at the highest elevation to 13.3 °C at the lowest elevation ($r^2 = 0.75$, $P < 0.001$) (Fig. 2b). At the highest elevation, water temperature showed high variation during the day. The temperature was 0 °C in the early morning and *c.* 4 °C by noon. There were also obvious changes in the stream morphology along the elevational gradient. For example, the width of the stream decreased linearly with increasing elevation ($r^2 = 0.68$, $P < 0.001$). The median for substratum particle size increased from 59 cm^3 at the lowest elevation to 8265 cm^3 at 3560 m, and then decreased above this elevation (Fig. 2c).

The amounts of total phosphorus and nitrogen were low in the stream water (means of 0.375 and 21.071 μmol L^{-1}, respectively). The TP decreased substantially with increasing elevation (Fig. 2d). In contrast, the TN and TN/TP ratio increased significantly with increasing elevation ($r^2 = 0.28$, $P < 0.01$ and $r^2 = 0.49$, $P < 0.001$, respectively) (Fig. 2e). Organic carbon concentration also showed predictable variation with elevation. For example, the DOC increased from 1.19 mg L^{-1} below 1950 m elevation to 3.57 mg L^{-1} over 3000 m elevation. Similarly, the chromophoric dissolved organic matter (cDOM) also increased with elevation, from 2.8 m^{-1} below 1950 m elevation to 6.3 m^{-1} above 3000 m elevation (Fig. 2f). In contrast, the inorganic carbon showed a contrasting pattern, and both DIC and total alkalinity

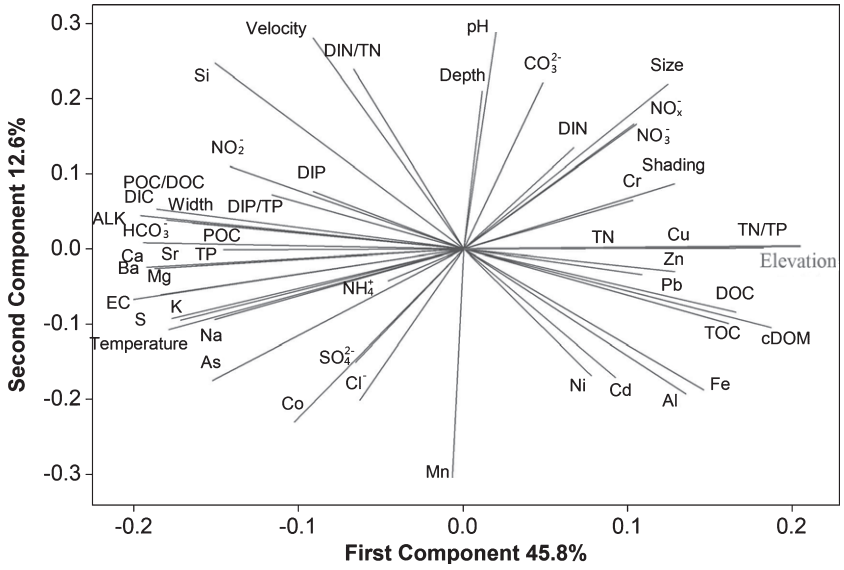

Figure 1 Plot of the principal components analysis for the 26 samples of stream organisms with 50 environmental variables sampled in Laojun Mountain, China. ALK, total alkalinity; cDOM, chromophoric dissolved organic matter; Depth, depth of the stream; DIC, dissolved inorganic carbon; DIN, dissolved inorganic nitrogen; DIP, dissolved inorganic phosphorus; DOC, dissolved organic carbon; EC, electronic conductivity; POC, particulate organic carbon; Shading, canopy cover; Size, substratum particle size; Temperature, water temperature; TN, total nitrogen; TOC, total organic carbon; TP, total phosphorus; Velocity, current velocity; Width, width of the stream.

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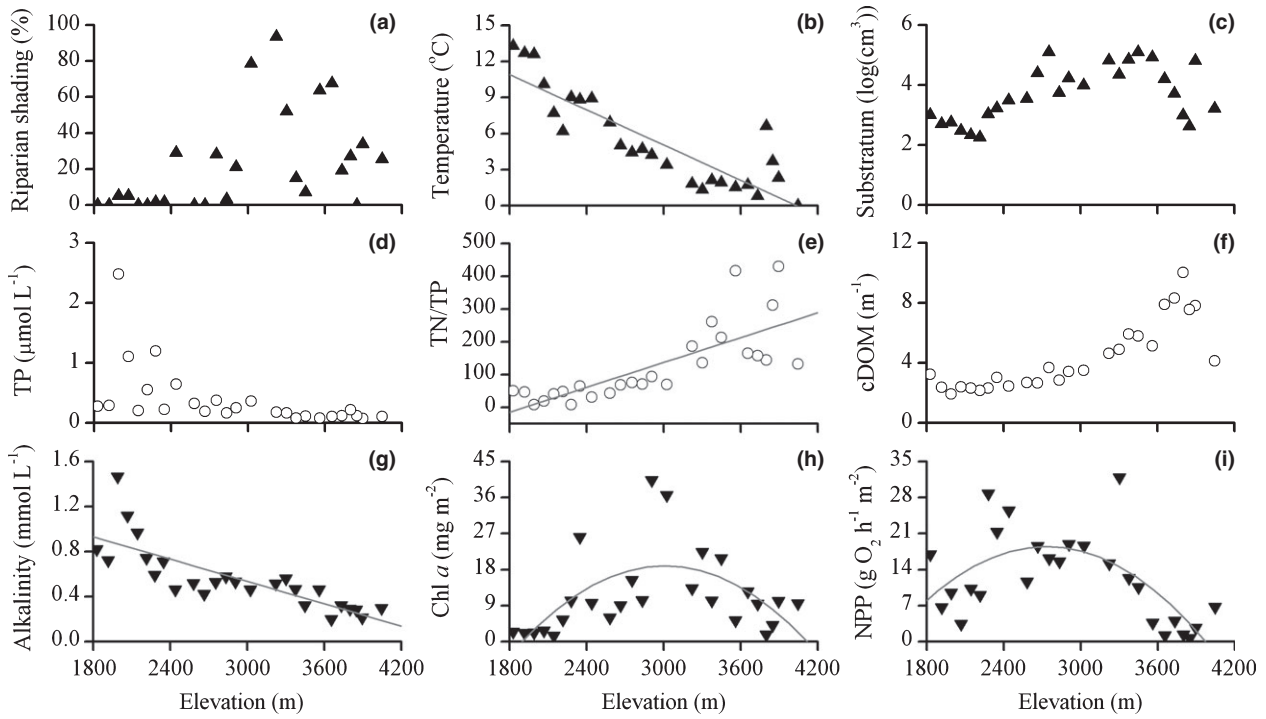

Figure 2 Variation of the nine selected physicochemical variables along the elevational gradient in Laojun Mountain, China. The solid lines indicate the trends from a generalized linear model ($P < 0.05$). (a) Riparian shading, (b) water temperature, (c) median for substratum particle size, (d) total phosphorus (TP), (e) ratio of total nitrogen (TN) to total phosphorus (TP), (f) chromophoric dissolved organic matter (cDOM), (g) alkalinity, (h) chlorophyll *a* (Chl *a*), (i) net photosynthesis production (NPP).

decreased with elevation ($r^2 = 0.72$, $P < 0.001$ and $r^2 = 0.64$, $P < 0.001$, respectively) (see Fig. 2g for alkalinity). Variables associated with the biofilm processes did not show any monotonic patterns along the elevational gradient. For example, chlorophyll *a* and NPP showed unimodal patterns along the gradient (Fig. 2h,i).

Macroinvertebrate richness followed a clear unimodal pattern, with a steeper decline towards higher elevations (Fig. 3a). In contrast, diatoms monotonically decreased in richness from the lowest elevation to the highest elevation (Fig. 3b). Most interestingly, the richness of bacteria increased with increasing elevation (Fig. 3c). Elevational gradients in community evenness also differed for the focal organisms, as the evenness monotonically increased with elevation for macroinvertebrates, whereas the elevational pattern for diatom evenness was unimodal (Fig. 3d). The bacterial evenness did not vary along the elevational gradient (Fig. 3d).

The relationships between species richness and explanatory variables were examined using multiple OLS with the selection of the best model (Table 1). The variation in macroinvertebrate richness along the elevational gradient was best explained by the MDE (β-weight = 0.83, Table 1). The predicted MDE species richness for macroinvertebrates followed the observed unimodal pattern, and the 95% prediction curves from 50,000 simulations of the mid-domain null indicated a moderate fit for the predictions of the null model (Fig. 4). Among the variables considered, the MDE had the greatest explanatory power for macroinvertebrate richness ($r^2 = 0.72$, $P < 0.001$). For example, NPP showed a clearly lower correlation with macroinvertebrate richness (Pearson correlation $r = 0.484$, $P = 0.012$). For diatom richness, the ratio of TN/TP accounted for the largest part of the total variation by a β-weight of −0.66, and the other variables, such as TP, PC1 and shading, were also significant (Table 1). This was consistent with the Pearson correlation results that showed that TN/TP had a strong correlation with the diatom richness ($r = 0.72$, $P < 0.001$, 999 permutations). For bacteria, the concentration of cDOM was the strongest factor associated with the variation in richness (β-weight = 0.57) (Table 1).

DISCUSSION

Our results highlight the different patterns in elevational diversity occurring among the three focal organism groups comprising unicellular and multicellular aquatic taxa. The unimodal richness pattern that we detected for macroinvertebrates concurs with much of the macroecological literature (Rahbek, 2005; Nogués-Bravo *et al.*, 2008). It also seems that the decreasing pattern in richness we found for diatoms is consistent with earlier studies on river ecosystems (e.g. Ormerod *et al.*, 1994) and on water-saturated mosses (Gremmen *et al.*, 2007). However, the finding that bacteria exhibited a monotonically increasing pattern along the elevational gradient was unexpected. This increasing pattern is extremely rarely observed in nature, considering the large number of elevational gradients studied. Rahbek's (2005)

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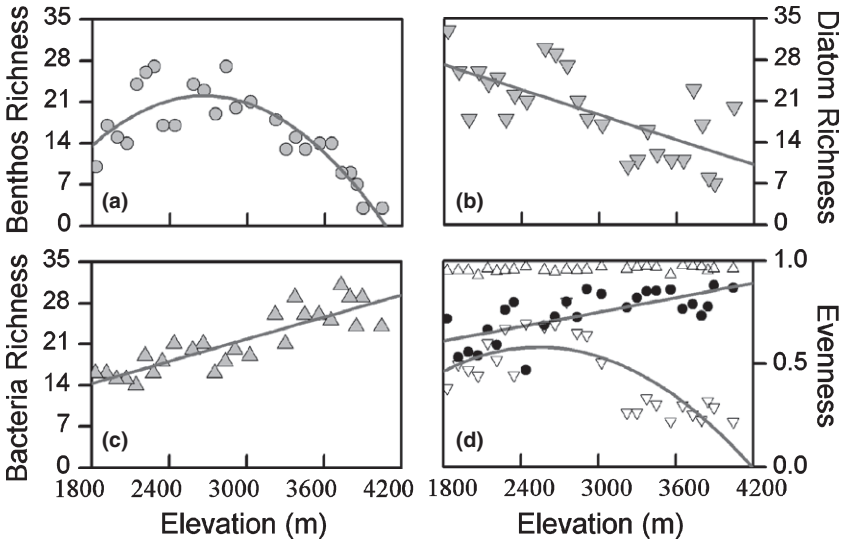

Figure 3 Elevational diversity patterns for various stream organisms sampled in Laojun Mountain, China. The solid lines indicate the prediction of diversity as a function of elevation by a linear or quadratic model with the lowest Akaike's information criterion. (a) The unimodal pattern for macroinvertebrate richness ($r^2 = 0.76$, $P < 0.001$), (b) the monotonically decreasing pattern for diatom richness ($r^2 = 0.48$, $P < 0.001$), (c) the monotonically increasing pattern for bacterial genetic richness ($r^2 = 0.75$, $P < 0.001$), (d) the unimodal pattern for diatom evenness (▽, $r^2 = 0.61$, $P < 0.001$), the monotonically increasing pattern for macroinvertebrate evenness (●, $r^2 = 0.49$, $P < 0.001$), and no trend for bacterial evenness (△).

	Model r^2	AIC	Explanatory variables and β-weights†				
Macroinvertebrates			MDE***				
	0.72	49.8	0.83				
Diatoms			TN/TP***	TP***	PC1**	Shading*	Chl *a*
	0.83	51.1	−0.66	−0.59	1.09	−0.31	−0.21
Bacteria			cDOM***				
	0.79	42.0	0.57				

Table 1 Relationships between the species richness of stream organisms and potential explanatory variables modelled using multiple ordinary least squares (OLS) regression. The best models were identified using Akaike's information criterion (AIC). The spatial autocorrelation in the model residuals was taken into account. All of the variables were standardized (mean = 0; SD = 1) and are displayed with increasing *P*-values.

MDE, mid-domain effect; TN, total nitrogen; TP, total phosphorus; Chl *a*, chlorophyll *a*; PC1, the first principal component of the 27 geochemical variables (see text for details); cDOM, chromophoric dissolved organic matter.

†Standardized partial regression coefficients, ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$.

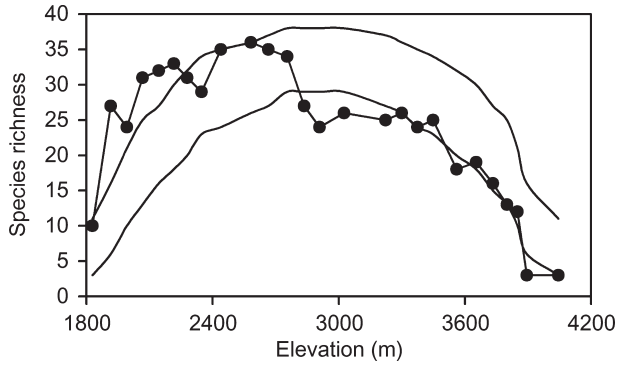

Figure 4 The predicted species richness pattern for stream macroinvertebrates (line and data points) in Laojun Mountain, China, and the 95% prediction curves sampled without replacement, using a mid-domain null model according to McCain (2004) (50,000 simulations).

quantitative analysis of elevational species richness gradients found that *c.* 50% of the elevational patterns were unimodal, *c.* 25% followed a monotonically decreasing pattern, and the remaining 25% of the gradients followed other patterns. Our results contrast with those of a recent study on soil bacteria by Bryant *et al.* (2008), who detected a decreasing elevational pattern in richness for Acidobacteria within an elevation range of 2460–3380 m. Furthermore, a recent study by Zhang *et al.* (2009) showed that the phylogenetic diversity of soil ammonia oxidizers did not exhibit any clear elevational patterns within an elevational range of 4000 to 6550 m.

One of the most interesting findings of our study was that the two groups of microorganisms (bacteria and diatoms) showed exactly opposite patterns in richness along the same environmental gradient. This is surprising because both taxonomic groups are expected to be efficient dispersers across localities (Fenchel & Finlay, 2004). It should be noted that the

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results obtained from molecular fingerprinting analyses, as opposed to results based on visual identification of morphospecies, may differ slightly (Patterson, 2009). However, the patterns for bacteria and diatoms were so strikingly different that this distinction cannot be attributed to the methodological differences. Consistent with other microbial studies using molecular fingerprinting methods (i.e. Bell *et al.*, 2005; Fuhrman *et al.*, 2008), we should emphasize that our estimates of bacterial richness are possibly lower than true values of richness because the method we employed does not detect genotypes below a relative abundance of 0.1%. However, we are confident that the method did not distort the main elevational pattern in richness because this underestimation is likely to be constant among the samples (Bryant *et al.*, 2008).

In addition to species richness, we also studied the distribution of dominance among the communities. This was done because most studies on elevational diversity gradients neglect the evenness component of community diversity. The different patterns in richness and evenness of the three organism groups highlight the fact that these components of diversity vary along large elevational gradients, and not only in local assemblages (Hillebrand *et al.*, 2007). Given the clear patterns of species evenness along the elevational gradient (i.e. the unimodal pattern for diatoms, monotonic increase for macroinvertebrates and non-significant pattern for bacteria), it seems that evenness is an important component of community diversity.

What, then, are the underlying processes behind these patterns? The contrasting patterns in richness that we found for the three organism groups are most likely to be caused by different mechanisms. For macroinvertebrates, the unimodal pattern could be caused by the interplay between environmental conditions and human effects (Nogués-Bravo *et al.*, 2008). The environmental filtering decreases the richness at high elevations, where the environmental conditions are harsh, and only species coping with the harsh climate can occupy the sites at the highest elevation. The human effects in turn decrease the richness at lower elevations (below 2580 m), by means of deteriorating water quality, and the local community consists mainly of species that tolerate low water quality. More generally, this reasoning supports the climatic hypotheses in explaining the richness gradient. Another possible mechanism for producing the unimodal pattern is the MDE. Based solely on geometric constraints on species richness, the MDE predicts that when hard boundaries (such as oceans and mountaintops) limit species ranges, variously sized ranges create a peak in species richness in the middle of the gradient (Colwell & Hurtt, 1994; Colwell & Lees, 2000). Although the MDE has been supported by some observations (Colwell *et al.*, 2004), it has less often been reported to correlate well with the distribution of aquatic macroinvertebrates along such gradients (Jacobsen, 2008). However, our results indicate that the MDE had the best statistical fit among the factors considered in respect of the elevational richness gradient of macroinvertebrates. We emphasize, though, that the assumption that a species occurs continuously along the elevational gradient between the lowest and highest points at which it was observed (even if it was missing from the counts at some of the sites), i.e. range filling, may produce an artefactual MDE.

The variation in TN/TP was most likely to be the strongest factor in controlling the diatom richness, and it was among the most important factors in explaining variation in diatom evenness ($r^2 = 0.41$, $P < 0.001$). The higher elevations were characterized by very low phosphorus supply, and the mean TN/TP was much higher than the standard Redfield ratio of 16:1. Serious phosphorus limitation thereby favoured dominance by only a few diatom taxa. For instance, the relative abundance of the diatom species *Achnanthes minutissima* was higher than 80% of the counted specimens above an elevation of 3050 m, and then decreased linearly to < 5% at the lowest elevations. This is consistent with the fact that *A. minutissima* prefers low nutrient levels and is a pioneer species in harsh and fluctuating conditions (Allan, 1995). Our results thus indicate that the variation in abiotic conditions (especially the variation in TN/TP) may be the best hypothesis to explain the variation of diatom richness along the elevational gradient. More generally, our findings support the effect of climate as underlying the richness gradient in diatoms.

For bacteria, at least two mechanisms contributing to increasing richness with elevation could be put forward. First, the increasing amount of cDOM with elevation provides more energy to bacteria. In fact, the increased activity (indicated by the water temperature and community respiration of the biofilm) and biomass (indicated by the ratio between DNA mass and chlorophyll *a* in the biofilm) of prokaryotes at the lower elevations may have consumed most of the organic carbon in the stream water. This may have resulted in increased alkalinity, increased DIC concentration, and decreased TN concentration down the stream. Second, the number of locally coexisting species may have increased because of the temporal storage effect (Chesson, 2000): the observed large day–night variation in temperature at high elevations probably widens the bacterial niches (Adams *et al.*, 2010) and favours their population growth compared with that of larger organisms. Overall, it seems that the pattern in bacterial richness is best explained by the climatic hypotheses (current abiotic conditions of cDOM and temperature variation).

The hypotheses discussed above do not exclude other possible mechanisms controlling the elevational patterns in species diversity. For instance, the same mechanism may be capable of generating different patterns in different trophic or taxonomic groups, as these groups have different ecosystem roles. However, our results indicate that the underlying processes for microorganisms and macroorganisms are probably different, resulting in variable elevational patterns in species diversity. The present findings call for further studies on microbial macroecology and also highlight the need for an adjustment of ecological theories to be more pertinent to microorganisms.

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SUPPORTING INFORMATION

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

Figure S1 Species occurrences along the elevational gradient.

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BIOSKETCH

Jianjun Wang is interested in the factors influencing aquatic microbial distributions and in the relationship between microbial biodiversity and its biogeochemical function.

Author contributions: J.J. Wang, J. Soininen and J. Shen conceived the ideas; J.J. Wang, J. Soininen and Y. Zhang collected the field samples; J.J. Wang and Y. Zhang provided bacterial data; X.D. Yang helped with diatom pretreatment; J. Soininen provided diatom data; B.X. Wang provided macroinvertebrate data; J.J. Wang and J. Soininen analysed the data and led the writing. All co-authors contributed to writing and commented on the final version of the manuscript.

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