

# Testing the theory of island biogeography for microorganisms—patterns for spring diatoms

Anette Teittinen\*, Janne Soininen

Department of Geosciences and Geography, University of Helsinki, PO Box 64, Helsinki 00014, Finland

**ABSTRACT:** According to the theory of island biogeography, species richness in island-like systems is determined by ecosystem size and isolation. However, there is only very little information about how this theory applies to microorganisms. The aim of this study was to examine the effects of local physicochemical variables, land use and geographical factors on diatom species richness and community composition in 50 boreal springs in Finland, and to test for species–area and species–isolation relationships. There were nonsignificant relationships between species richness and spring area and isolation. The best approximating generalized linear model suggested that variation in species richness was related to several local resource and stress factors such as water conductivity, pH, colour, temperature and nutrient concentrations. Based on Mantel tests, diatom community similarity was significantly correlated with environmental distance, but not with geographical distance. Analysis of similarities further showed that community compositions did not differ significantly between ecoregions. These results suggest that spatial factors are of minor importance for spring diatoms compared with environmental filtering. Patterns in spring diatoms do not concur with the theory of island biogeography as we found no support for species–area or species–isolation relationships. At these spatial scales, dispersal limitation does not seem to influence diatom communities.

**KEY WORDS:** Dispersal · Spatial variation · Macroecology · Boreal

*Resale or republication not permitted without written consent of the publisher*

## INTRODUCTION

The causes and consequences of spatial variation in biodiversity have been at the heart of ecology and biogeography for several decades (Gaston 2000). One of the most universal patterns of species richness is the species–area relationship (SAR), which describes the relationship between sampling area and the number of species found within the area (Arrhenius 1921, Gleason 1922, Rosenzweig 1995). The SAR was a fundamental component in the development of MacArthur & Wilson's (1967) island biogeography theory, which holds that in island-like biological systems species richness is determined by ecosystem size and isolation. Area and isolation influence the rates of species immigration and extinc-

tion so that the number of species in any given insular ecosystem should increase with area and decrease with isolation.

Patterns of species richness and composition originating from biogeographical processes are, however, confounded by background variation in species richness and composition caused by effects of, e.g., local environmental conditions within a community (Hobæk et al. 2002). Therefore, the mechanisms that structure local communities comprise large-scale processes such as dispersal limitation, climatic factors and historical effects, as well as local abiotic and biotic factors (MacArthur & Wilson 1967, Ricklefs 1987). While it is accepted that processes operating at multiple scales shape the distribution patterns of larger organisms

\*Corresponding author: anette.teittinen@helsinki.fi

(Rosenzweig 1995), there is an ongoing debate about their relative importance for microbial organisms (Soininen 2012). It has been suggested that microorganisms lack biogeographical patterns due to their small size and high population densities, thus being freely passively dispersed (Finlay 2002, Fenchel & Finlay 2004). In contrast, some recent studies have indicated that microbial organisms seem to exhibit spatial patterns similar to those of larger organisms, such as species–area relationships (Reche et al. 2005), restricted distributions (Vyverman et al. 2007) and the spatial structure of communities (Soininen et al. 2004, Heino et al. 2010).

Small aquatic habitats embedded in terrestrial landscape offer suitable environments for studying the spatial structure of biological communities in an island-like setting (Soininen et al. 2011). In isolated freshwater systems, aquatic habitats are separated by geographical and physicochemical barriers, which can lead to dispersal limitation and restricted distributions of aquatic biota (Cantonati et al. 2012a). Springs, which are formed by emerging groundwater, are interesting study environments as they are spatially isolated habitat patches and their characteristics affect the composition of aquatic biota. Compared with many other aquatic ecosystems, springs offer highly stable growing conditions with relatively little variation in water temperature and discharge regime (van der Kamp 1995). They are often characterized by high habitat heterogeneity, which increases biodiversity (Ilmonen & Paasivirta 2005, Cantonati et al. 2012a). They are often the least-affected freshwater habitats in regions where human effects are widespread, and serve as refuges for rare and sensitive species and are thus aquatic biodiversity hot spots (Cantonati et al. 2012a, Ilmonen et al. 2012). Springs suffer from anthropogenic disturbances, however, and spring biota has become regionally threatened (Heino et al. 2005, Barquín & Scarsbrook 2008). Yet, springs are much less studied than many other aquatic systems. In the boreal region, research on spring habitats has centered on larger organisms, such as bryophytes (Heino et al. 2005) and macroinvertebrates (Ilmonen et al. 2009), while smaller unicellular organisms, such as diatoms, have been neglected.

Diatoms are microscopic algae abundant in all aquatic environments with sufficient light (Dixit et al. 1992). As photosynthetic organisms and primary producers, they play an important role in ecosystem functioning and several global biogeochemical cycles. They are used as bioindicators, since many

species have narrow tolerances for several environmental factors and community structure responds to changing environmental conditions with a brief delay (Stoermer & Smol 1999). The occurrence of benthic diatoms is regulated by multiple factors prevailing at several scales (Biggs et al. 1990). ‘Ultimate’ factors like climate, geology and land use operate at regional spatial scales, i.e. across catchments or ecoregions and control local ‘proximate’ factors which may influence communities. ‘Proximate’ factors comprise resource factors such as nutrients, light and space, and stress factors such as pH, conductivity, temperature, current velocity and grazing by herbivores. Additionally, large-scale processes unrelated to environmental conditions, e.g. historical constraints and dispersal limitation, can affect the structure of local communities (Soininen et al. 2004, Vyverman et al. 2007, Verleyen et al. 2009, Heino et al. 2010). Spring diatom communities have been studied especially in the Alps (Cantonati 1998, Cantonati & Spitale 2009, Cantonati & Lange-Bertalot 2010, Gesierich & Kofler 2010, Cantonati et al. 2012b), but also at lower altitudes, mainly in southern and central Europe (Aboal et al. 1998, Angeli et al. 2010, Wojtal & Sobczyk 2012). Based on earlier studies, spring diatom communities respond to various local-scale drivers such as pH, conductivity and nutrient concentrations, but knowledge of their occurrence along large-scale spatial gradients is still lacking.

The aim of this study was to test the 2 main predictions of the theory of island biogeography, i.e. species–area and species–isolation relationships using data from 50 boreal springs spanning 3 ecoregions of Finland. As aquatic ecosystems can be envisioned as islands in a terrestrial landscape (Dodson 1992, Stomp et al. 2011), boreal springs appear to be suitable model systems for testing the theory. We hypothesized ( $H_1$ ) that larger springs support higher diatom species richness than smaller springs, due to greater habitat heterogeneity, i.e. a higher number of available microhabitats. Although generally considered strong dispersers, the dispersal of freshwater algae is constrained by distance between habitats suitable for colonization, connectivity and dispersal barriers (Kristiansen 1996). We therefore also hypothesized ( $H_2$ ) that isolated springs have lower species richness than more connected springs, due to lower immigration rates. Additionally, we examined the effects of other environmental variables (i.e. water chemistry, shading and land use) and geographical variables on diatom richness and community composition.

## MATERIALS AND METHODS

### Study area

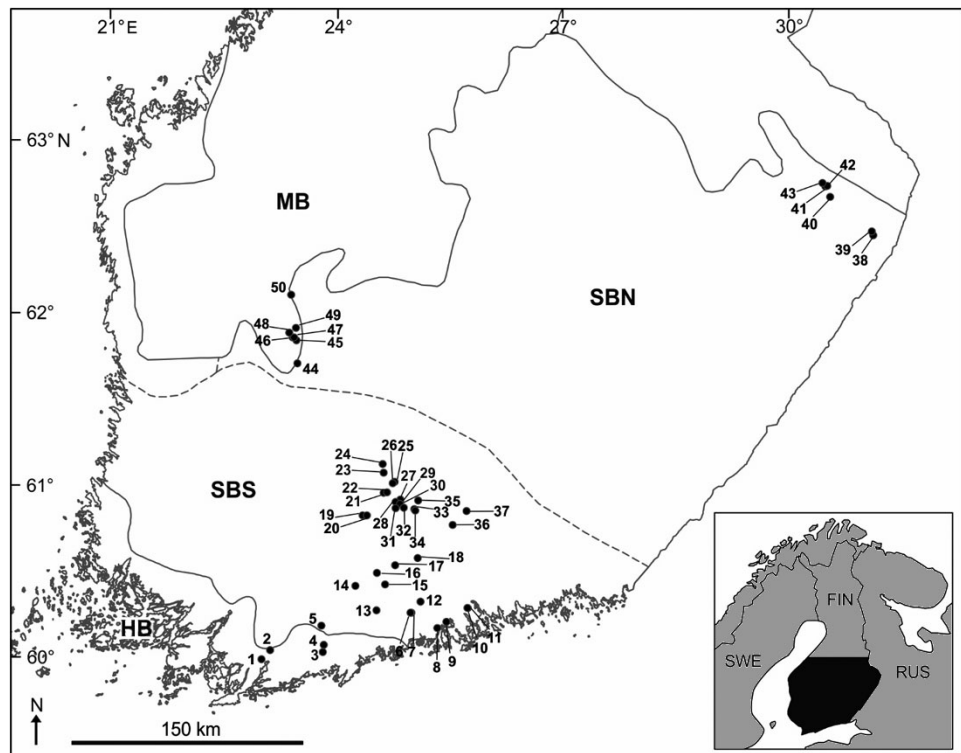
The study area comprised 50 springs located in Finland ( $60^{\circ} 5.77' - 62^{\circ} 53.34' \text{ N}$ ,  $23^{\circ} 34.85' - 30^{\circ} 55.72' \text{ E}$ ) and spanned 3 ecoregions, i.e. hemiboreal, south boreal and middle boreal (Fig. 1). The springs were chosen to represent varying groundwater recharge areas especially in relation to geology, land cover and land use. The following ecoregion descriptions are based on Kalliola (1973), Alalammi (1986, 1990) and Pirinen et al. (2012): The hemiboreal region (HB) covers the southern and southwestern coastal areas of Finland. The ecoregion contains sporadic calcareous bedrock formations favoring diverse types of vegetation, large ice-marginal formations and glaciofluvial eskers. Mixed and coniferous forests are common, but deciduous forests are also typical. Mean annual air temperature ranges from  $+5$  to  $+6^{\circ}\text{C}$ . The south boreal ecoregion includes southeastern Finland and most of southern Finland. Mean annual air temperature ranges from  $+5^{\circ}\text{C}$  in the southwest to  $+2^{\circ}\text{C}$  in the northeast. This ecoregion is further divided into southern and northern subcoregions. In the southern subcoregion (SBS) forests are primarily mixed and coniferous, but deciduous forests are also common. The subcore-

gion is characterized by large ice-marginal formations, glaciofluvial eskers and vast plains of fine sediment, which have been widely taken into agricultural use. In the northern subcoregion (SBN) coniferous and mixed forests prevail. Eskers, ice-marginal formations and various moraine formations are typical in the landscape. The middle boreal (MB) ecoregion encompasses the central parts of Finland. The studied springs are located in the southern part of the ecoregion where mean annual air temperature varies between  $+3$  and  $+4^{\circ}\text{C}$  and vegetation consists mainly of coniferous forests and peatlands.

### Data collection

Water samples were collected during the summers of 2011 and 2012. In 2011, samples of 37 springs were collected between 26 May and 20 July. In 2012, samples of 13 springs were collected between 25 and 31 July. Conductivity, pH, water temperature and spring width and length were measured in the field, and shading by the canopy was estimated. In the laboratory, nutrient concentrations and water colour were analyzed according to the standards SFS-EN ISO 11905-1 (total nitrogen), SFS-EN ISO 6878 (total phosphorus) and SFS-EN ISO 7887 (water colour).

Fig. 1. Locations of the sampled springs ( $n = 50$ ) within the ecoregions and subcoregions of Finland. HB: hemiboreal; MB: middle boreal; SBS: south boreal southern; SBN: south boreal northern (Reproduced from SYKE 2013)



Diatom samples were collected simultaneously during water sampling. At each spring, subsamples from as many different substrata (e.g. bryophytes, branches, stones, wood) as possible were collected in order to cover all microhabitats found in each spring. Samples were preserved in ethanol in the field. In the laboratory, organic material was removed from the diatom samples by boiling with hydrogen peroxide (30%  $\text{H}_2\text{O}_2$ ). Cleaned diatoms were mounted on slides using Naphrax. A total of 300 frustules per sample were identified to the lowest possible taxonomic level (typically to the species level) using a light microscope (magnification 1000 $\times$  oil immersion objective) following Krammer & Lange-Bertalot (1986–1991) and Lange-Bertalot & Metzeltin (1996). For each sample, 300 frustules were identified because we were not able to count more frustules due to the low cell density in many samples. Then, the relative abundance (%) of each species was calculated and the species richness of a site was expressed as the sum of all taxa encountered within the 300 frustules. We emphasize that our estimation of species richness is based on samples and the true number of diatom species in each spring is likely to be higher as more species would presumably be found if more frustules were identified (Gotelli & Colwell 2001). This presumption was verified when we examined the species accumulation curves of the springs: the curve of the spring with the lowest number of species was saturated, but the curve of the most diverse spring did not reach an asymptote (Fig. S1 in the Supplement at [www.int-res.com/articles/suppl/a075p239\\_supp.pdf](http://www.int-res.com/articles/suppl/a075p239_supp.pdf)). Subsamples of 300 frustules are thus likely to somewhat underestimate the species richness of the most diverse diatom assemblages.

Catchment variables were derived from the European land cover and land use classification CORINE (coordination of the information on the environment) database. In Finland, the national CORINE Land Cover 2006 database is available in raster format with a spatial resolution of 25 m (SYKE 2009). Using ArcGIS 10.1 software (ESRI 2013), the proportion of the area covered by 5 different land-cover and land-use types (artificial surfaces, agricultural land, forests and seminatural areas, wetlands and water bodies) was calculated from the CORINE database. The calculation was done using 300 and 1000 m radii around each spring.

In addition, the proportion of the area covered by inland waters (lakes and streams) around each spring was calculated using a 3000 m radius in order to examine the relationship between diatom species

richness and habitat isolation. Such a radius was chosen to minimize overlapping of the buffer zones. We also ran similar analyses with shorter radii around each spring, but as overall results were similar, we show the results using a 3000 m radius here. If dispersal limitation was of importance, springs that were surrounded by a lower proportion of other inland waters were expected to have fewer species than springs that were surrounded by a higher proportion of potential immigration sources (Dodson 1992). This approach was used as it was not feasible to calculate the area covered by other springs around each focal spring, as boreal springs are often small, scattered in the landscape and typically not found on maps or in GIS (geoinformation system) data. Hence, we initially expected springs to harbor the same freshwater diatom taxa as, e.g., boreal streams (Soininen et al. 2004, Virtanen & Soininen 2012) and subarctic lakes (Weckström et al. 1997), and indeed no species were found to be unique to boreal springs in these data. We thus assumed that different freshwater systems presumably share the regional species pool and are connected by dispersal.

### Data analyses

Regional diatom data sets are typically characterized by a high proportion of rare species (Soininen et al. 2009, Heino et al. 2010); therefore, extrapolated richness values should be more reliable than observed ones, given that information on the frequency of rare species is used (Soininen et al. 2009). To better ensure that our sampling/counting effort was sufficient for providing reliable data on species richness (Gotelli & Colwell 2001), we corrected our richness estimates for the number of species that were likely to have remained regionally undetected. We used a nonparametric asymptotic richness estimator Chao1 (Chao 1984) based on individual counts to obtain a lower bound for the expected species richness. All subsequent species-richness analyses were conducted using the extrapolated richness values.

Local environmental variables, except for pH and shading, were  $\log_{10}$ -transformed prior to statistical analyses to reduce their skewed distributions. For variables expressed as relative abundances (%) (shading, catchment variables), arcsine-transformation was used. We assessed statistical dependence between the explanatory variables using Spearman's rank correlation coefficients and considered multicollinearity among the variables by evaluating variance inflation factors (VIF). Then, we constructed

correlograms with Moran's  $I$  (Moran 1950) to examine the degree of spatial autocorrelation in the local environmental variables and diatom species richness. Autocorrelation coefficients were calculated for distance classes with 50 km intervals generating a spatial correlogram. Overall significance of the correlogram (at a significance level of  $\alpha \leq 0.05$ ) was tested by checking whether at least 1 autocorrelation coefficient was significant at  $\alpha/k$ , where  $k$  is the number of distance classes used following the Bonferroni criterion (Legendre & Legendre 1998). The statistical significance of each coefficient was assessed with 1000 permutations.

We used linear regression to test whether species richness was related with spring area (width  $\times$  length) and isolation. Then, generalized linear models (GLM) with Poisson error distribution (McGullagh & Nelder 1989) were used to study the relationship between species richness and local environmental variables (total N, total P, pH, conductivity, temperature, colour and shading) and spatial variables (spring coordinates). In the models, both linear and quadratic terms were included as this also allows one to consider the possible unimodal relationships between response and explanatory variables. The selection of the best approximating model was based on Akaike's information criterion (AIC) (Akaike 1974).

We then conducted Mantel tests to examine community turnover along environmental and geographical gradients (Mantel 1967, Legendre & Legendre 1998). The Mantel test is a method for modelling pairwise community dissimilarities as a function of pairwise environmental or spatial distances (Anderson et al. 2011). Simply put, the Mantel statistic ( $r$ ) is a correlation between 2 dissimilarity or distance matrices. The Mantel test is often used as a distance-based approach to examine the relationship between diatom community dissimilarities (or similarities) and distance matrices (Astorga et al. 2012, Virtanen & Soininen 2012, Wang et al. 2012). We first constructed a similarity matrix for biological data, and distance matrices for environmental and geographical data. We used similarities instead of dissimilarities as we visualized how community similarity decreased with distance (distance decay). Euclidean distances were calculated for all site pairs separately for the data representing environmental variables and spring coordinates. Environmental data comprised of total N, total P, pH, conductivity, temperature, colour, shading, spring area and catchment variables were calculated using a 1000 m radius. We used a Bray-Curtis similarity coefficient

based on the diatom abundance data to calculate pairwise similarities between all sites. Additionally, we ran partial Mantel tests using all 3 matrices to disentangle the pure effects of environmental and geographical variables on diatom community similarity. A partial Mantel test allows one to assess the influence of environmental distance on diatom community similarity while controlling for the geographical distance, and vice versa. The significance of the relationships between community similarity and distance matrices was assessed using 9999 permutations.

Next, we used analysis of similarities (ANOSIM; Clarke 1993) to test whether there were significant differences in diatom species compositions between the ecoregions in the study area. The ANOSIM statistic ( $R$ ) is based on the difference of mean ranks between groups and within groups.  $R$  varies between  $-1$  and  $+1$ ; a value of 0 indicates random grouping.

All analyses were conducted with appropriately transformed data, except for GLM, which uses untransformed data. Statistical analyses were conducted with R Version 3.0.2 (R Development Core Team 2013), using the packages *ncf* (Bjornstad 2012), *MASS* (Venables & Ripley 2002) and *vegan* (Oksanen et al. 2013).

## RESULTS

Measured environmental variables showed considerable variation among the springs (Table S1 in the Supplement at [www.int-res.com/articles/suppl/a075p239\\_supp.pdf](http://www.int-res.com/articles/suppl/a075p239_supp.pdf)). Nutrient concentrations ranged from oligotrophic to eutrophic conditions, pH from acid to slightly alkaline and colour from clear to humic. Conductivity varied between 14 and 294  $\mu\text{S cm}^{-1}$ , and temperature, between  $+4.0$  and  $+14.8^\circ\text{C}$ . The study area comprised very small ( $0.04 \text{ m}^2$ ) as well as large springs ( $120 \text{ m}^2$ ). Catchments of the springs were, on average, primarily forested. Agricultural lands and artificial surfaces were also common, whereas wetlands and water bodies were rare. Pairwise correlations between the variables were generally not strong (Table S2 in the Supplement at [www.int-res.com/articles/suppl/a075p239\\_supp.pdf](http://www.int-res.com/articles/suppl/a075p239_supp.pdf)).

Moran's correlograms for total P, conductivity, temperature and water colour were significant according to the Bonferroni corrected level of significance ( $p = 0.05/9 = 0.0056$ ) (Fig. 2). For these variables, significant positive autocorrelations were detected in the first distance class, suggesting that the pairs of springs that are located at a distance of up to 50 km

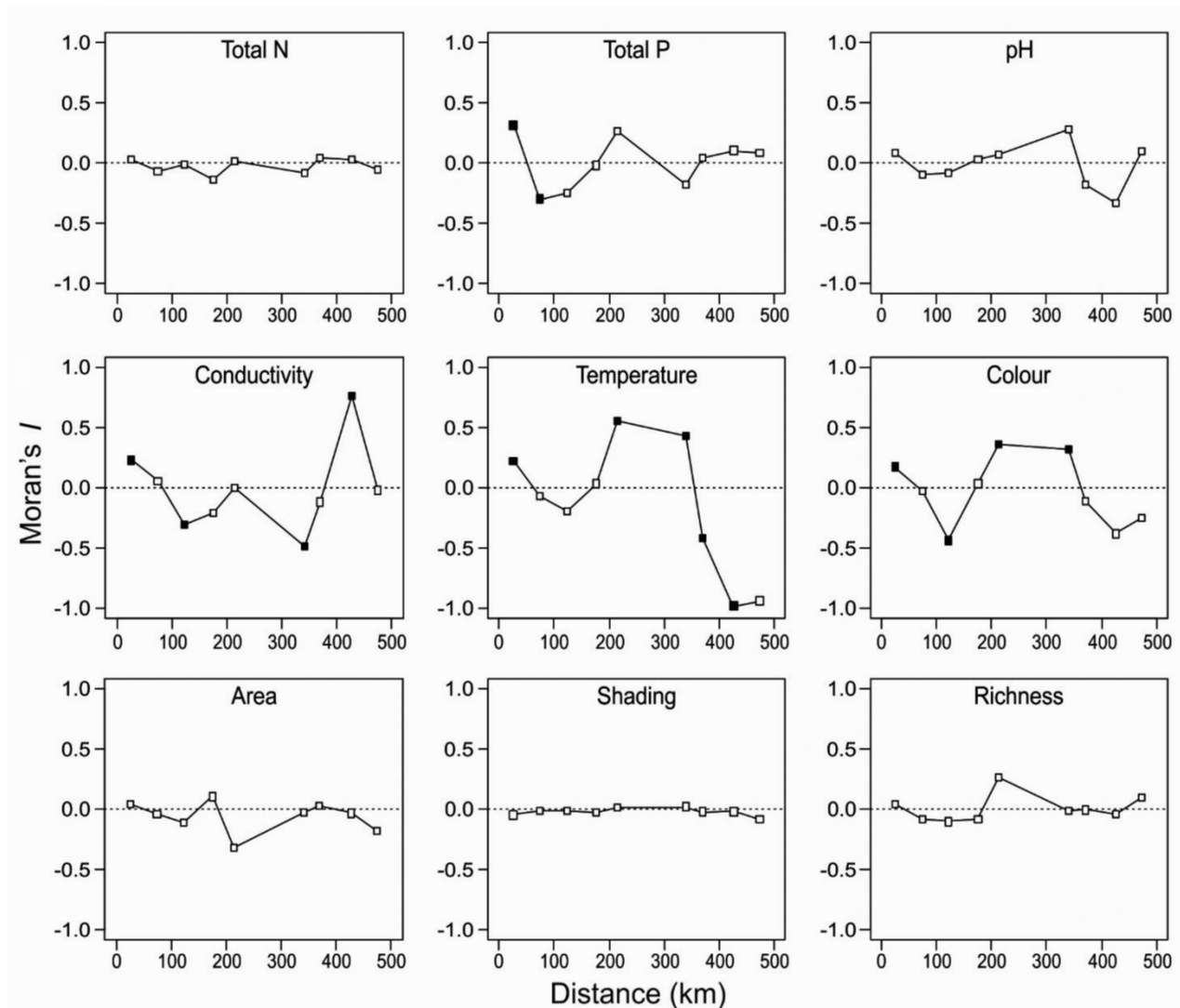


Fig. 2. Spatial autocorrelation in environmental variables and species richness based on Moran's *I*. Filled squares indicate autocorrelation coefficients which remain significant after Bonferroni correction at  $p = 0.05/9 = 0.0056$

apart tended to have similar values for environmental variables. However, correlograms did not show monotonically decreasing trends with increasing distance, implying gradient-like structures. Rather, the alternation of significant positive and negative values along the correlograms suggested patch-like structures. No significant spatial autocorrelation was observed in species richness.

Diatom species richness was not related to spring area ( $R^2 = 0.0006$ ,  $p = 0.870$ , slope  $-0.008$ ) or isolation ( $R^2 = 0.0006$ ,  $p = 0.869$ ) (Fig. 3). There were no notable correlations between the number of species and other local environmental variables either (Fig. S2 in the Supplement at [www.int-res.com/articles/suppl/a075p239\\_supp.pdf](http://www.int-res.com/articles/suppl/a075p239_supp.pdf)). Based on the best approximating GLM, variation in species rich-

ness was related to several variables. The model included linear terms of temperature and latitude, and linear and quadratic terms of total N, total P, pH, conductivity, colour and shading (Table 1). The model explained up to 70.1% of the variation in species richness.

Mantel tests indicated that the community similarity of diatoms was significantly correlated with environmental distance, but not with geographical distance (Table 2, Fig. 4). The negative correlation between community similarity and environmental distance remained significant even when the geographical distance was controlled for (Table 2). According to the ANOSIM, community compositions did not differ significantly between ecoregions ( $R = 0.077$ ,  $p = 0.178$ ).

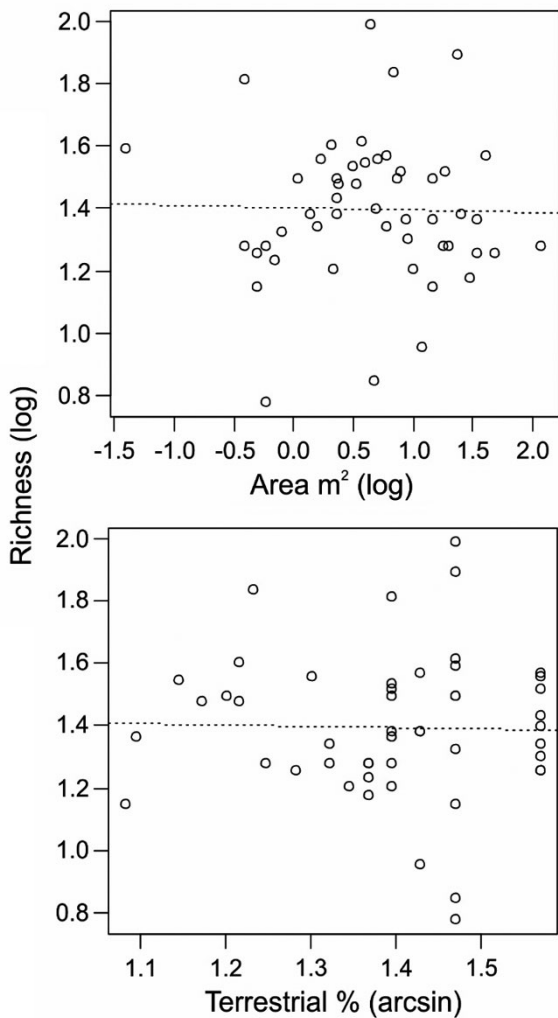


Fig. 3. Relationship between Chao1-estimated diatom species richness and spring area and isolation. Species richness was not related to spring area ( $R^2 = 0.0006$ ,  $p = 0.870$ ) or spring isolation ( $R^2 = 0.0006$ ,  $p = 0.869$ ). 'Terrestrial %' indicates the proportion of terrestrial area in the catchment from the entire catchment area and thus refers to isolation of the spring. The regression lines based on linear models are shown

A total of 169 diatom taxa belonging to 27 genera were identified. Most of the species occurred sporadically and were regionally rare. Species found in only 1 spring comprised 28%, and species found in only 2 springs, 42% of all the species encountered. Seven species occurred in more than half of the springs (Table 3). The species having the greatest average abundance were *Achnanthydium minutissimum* (23.2%), *Eunotia muscicola* (17.4%), *Eunotia bilunaris* (12.2%) and *Gomphonema angustatum* (9.6%). The mean number of observed species in a spring was 21 (range: 6–51). The Chao1-estimated species richness ranged from 6 to 97; the mean was 29.

Table 1. Results of the best approximating generalized linear model, with Poisson error distribution to explain the variation in Chao1-estimated diatom species richness. Estimates, standard errors (SE) and z-values for linear and quadratic terms are given; p-values show whether the term was significant or not (\*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ ). The best approximating model was selected using Akaike's information criterion

Variable	Estimate	SE	z-value	p-value
Total N	0.0007	0.0003	2.474	0.013*
Total N <sup>2</sup>	-2.40E-07	1.59E-07	-1.511	0.131
Total P	0.0314	0.0068	4.650	<0.001***
Total P <sup>2</sup>	-0.0006	9.28E-05	-6.112	<0.001***
pH	4.3990	1.3440	3.274	0.001**
pH <sup>2</sup>	-0.3568	0.1119	-3.188	0.001**
Conductivity	0.0085	0.0021	4.099	<0.001***
Conductivity <sup>2</sup>	-2.59E-05	7.48E-06	-3.461	<0.001***
Temperature	-0.0648	0.0130	-4.988	<0.001***
Colour	0.0117	0.0025	4.747	<0.001***
Colour <sup>2</sup>	-5.59E-05	1.55E-05	-3.607	<0.001***
Shading	0.0191	0.0055	3.471	<0.001***
Shading <sup>2</sup>	-0.0002	5.66E-05	-3.538	<0.001***
Latitude	-1.80E-06	4.59E-07	-3.923	<0.001***

Table 2. Results of Mantel and partial Mantel tests for the correlation between community similarity (Bray-Curtis) and environmental distance (Euclidean), and between community similarity and geographical distance for diatoms in 50 boreal springs

Test	r	p
<b>Mantel</b>		
Environmental	-0.370	<0.001
Geographical	-0.007	0.446
<b>Partial mantel</b>		
Environmental	-0.371	<0.001
Geographical	-0.037	0.289

## DISCUSSION

We found no clear species richness patterns consistent with the main predictions according to the theory of island biogeography. The outcome showing no significant positive SARs disagrees with the results of Reche et al. (2005), who reported a positive relationship between bacterial richness and lake area among alpine lake communities, and with those of Stomp et al. (2011), who reported a SAR for lake phytoplankton communities across the continental United States. However, our results are in line with those of Soininen & Meier (2014), who did not find a significant relationship between pool size and phytoplankton richness in a subarctic rock pool system. The non-significant SAR in this study may be explained using

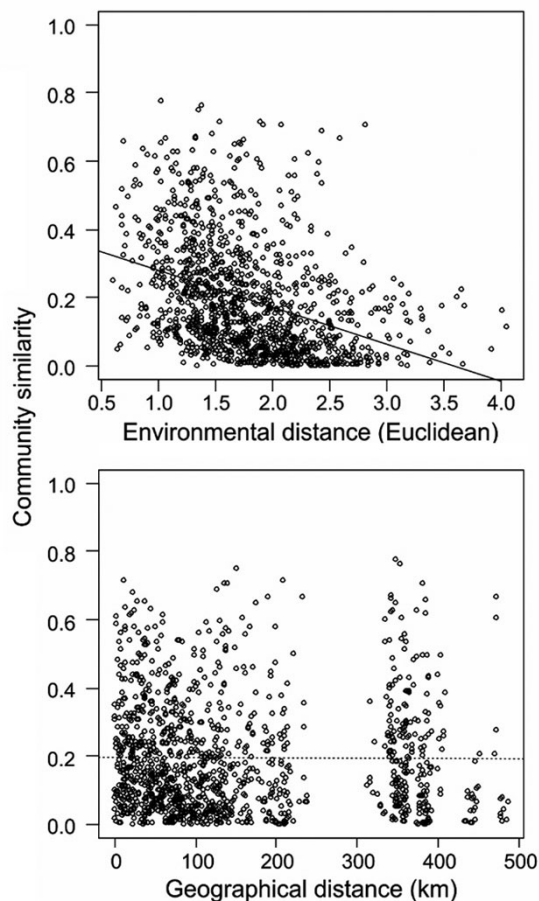


Fig. 4. Relationship between diatom community similarity (Bray-Curtis) and environmental and geographical distance. Community similarity was significantly correlated with environmental distance ( $r = -0.370$ ,  $p < 0.001$ ), but not with geographical distance ( $r = -0.007$ ,  $p = 0.446$ ) based on Mantel tests. Regression lines based on linear models are shown by solid (significant) or dashed (nonsignificant) lines

several avenues of reasoning. Environmental heterogeneity, which often increases along with area, has been invoked as a plausible mechanism underlying positive microbial SARs (Horner-Devine et al. 2004, Reche et al. 2005). However, environmental heterogeneity did not necessarily directly correlate with spring area in these data, as multiple factors (e.g. riparian shading, water depth, microhabitat complexity) influence the heterogeneity of a spring regardless of its size (Cantonati et al. 2012a). As many of the studied springs were internally complex, an increase in area did not necessarily directly lead to an increase in habitat heterogene-

ity, thereby weakening the relationship between species richness and area. Moreover, the nonsignificant SAR can partially be attributed to sampling methods. Because the diatom samples were collected from multiple substrates in each spring, in order to obtain the most representative samples possible, the number of microhabitats sampled varied between sites, possibly affecting the number of species encountered in the samples. Previous studies have highlighted the importance of habitat and substrate type for spring diatom communities, as epiphytic assemblages are often more species-rich than epilithic assemblages (Cantonati 1998, Cantonati & Spitale 2009, Cantonati et al. 2012b). Studying stream diatoms, Smucker & Vis (2011) observed that species richness was significantly higher when multiple habitats were sampled in comparison with sampling only a single habitat. Hence, as the number of microhabitats sampled did not necessarily increase monotonically with spring area, the expected positive SAR may have been confounded by other factors.

Disturbance regime may also have regulated the number of species among the springs, and one would expect that species richness peaks at intermediate disturbance intensity and frequency (Connell 1978). For example, flow velocity is known to influence the occurrence of spring diatoms as springs with fast currents generally have a lower diversity compared with slow-flowing springs (Sabater & Roca 1992, Gesierich & Kofler 2010). Because of the limited amount of available data, it was not possible to assess the role of disturbances here. As disturbances should be more frequent and intense in smaller ecosystems

Table 3. The most frequent taxa in the studied springs. The percentage of springs where the taxon was present and the mean and range of its relative abundance across these sites are shown

Taxon	Springs (%)	Mean (range) (%)
<i>Eunotia bilunaris</i> (Ehrenberg) Schaarschmidt	92	12.2 (0.3–83.9)
<i>Achnantheidium minutissimum</i> (Kützing) Czarnecki	78	23.2 (0.3–79.4)
<i>Gomphonema angustatum</i> (Kützing) Rabenhorst	78	9.6 (0.3–71.0)
<i>Eunotia minor</i> (Kützing) Grunow in Van Heurck	58	2.6 (0.3–15.0)
<i>Eunotia muscicola</i> Krasske	58	17.4 (0.3–65.3)
<i>Fragilaria capucina</i> Desmazières	54	4.5 (0.3–31.3)
<i>Tabellaria flocculosa</i> (Roth) Kützing	54	3.2 (0.3–29.1)
<i>Eunotia steineckii</i> Petersen	48	3.4 (0.3–26.8)
<i>Nitzschia perminuta</i> (Grunow) M. Peragallo	48	1.1 (0.3–7.5)
<i>Planothidium lanceolatum</i> (Bréb. ex Kütz.) Lange-Bertalot	46	8.0 (0.3–69.8)
<i>Nitzschia hantzschiana</i> Rabenhorst	46	2.0 (0.3–7.0)
<i>Pinnularia subcapitata</i> Gregory	46	2.7 (0.3–15.1)



(Adler et al. 2005), however, it seems that disturbance regime did not play a major role in maintaining species richness in these springs.

Many small springs harbored a relatively high number of species. This seems to contradict the perception that small insular systems should have smaller species populations than large systems and, consequently, a lower number of species due to smaller populations being more prone to local extinctions (MacArthur & Wilson 1967). It may be that permanent algal seed banks in the springs have prevented local extinctions by acting as an internal buffer to reduce the rate of species extinctions through rescue effects (Smith et al. 2005). Another explanation for the high species richness in small springs might be that small springs have a greater surface area to volume ratio, providing niches for benthic microorganisms. Moreover, the data used comprised only abiotic explanatory variables; hence, the potential influences of biotic interactions on species richness were missed. For example, the number of microinvertebrates has been found to be significantly correlated with lake area (Dodson 1992), so it may be that lower grazing pressure has allowed more diatom species to be present in small springs. Nølby et al. (2015) also studied shallow lakes in the light of island biogeography theory and found that, while fish richness and biomass both increased with lake size and basin connectivity, the diversity of aquatic macrophytes and invertebrates did not show similar patterns. Instead, they were driven by biotic interactions and fish-associated impacts, suggesting that the positive influences of larger lake size and higher immigration rates might have been counteracted by more severe negative biotic interactions (Nølby et al. 2015).

As diatom species richness was not related to spring isolation, it appears that among-site dispersal limitation was not likely to take place at the small spatial scales considered here. This indicates that efficient passive dispersal via air or by, e.g., waterbirds (Kristiansen 1996) probably facilitates species occurrence across the springs. Overall, connectivity of the sites is presumably less important for strong dispersers, especially when spatial distances between suitable habitats are small (Soininen et al. 2011). On the contrary, Vyverman et al. (2007) found that, on a global scale, variability in both the regional and local genus richness of lacustrine diatoms was best explained by spatial variables, e.g. the degree of isolation of lake districts and the connectivity between habitats among the lake districts, thus indicating that dispersal limitation is of great importance

for freshwater diatom diversity at global scales. Furthermore, the negligible importance of isolation in this study may partially stem from the fact that the degree of isolation did not vary much among the springs, as inland waters generally covered a small proportion of the catchments.

The failure of species richness to scale with area or isolation was perhaps also because of an overriding control by local environmental factors. The best approximating GLM included a number of variables which indicated that species richness was regulated jointly by multiple interacting factors. Diatom richness seemed to be related to proximate resource and stress factors such as pH, conductivity, nutrient concentrations, colour and temperature. Species richness was also related to latitude in GLM; however, rather than having a strong independent contribution to diatom richness, it may have reflected geographical variation in the environmental variables, as latitude was correlated with, e.g., water temperature and colour. Generally, species-poor communities were found in springs where growing conditions were harsh, based on one or more of the measured environmental variables. Species-rich assemblages appeared in springs that were characterized by average environmental conditions with regard to many variables. This outcome is in line with the findings of Soininen & Heino (2007), who reported that in boreal streams highly diverse diatom communities were found at sites where neither stress nor resource factors limited the occurrence of species.

The primacy of environmental variables in structuring spring diatom communities was further emphasized in Mantel tests. Community similarity was significantly related to environmental distance, but not to geographical distance, which implies that spatial factors are of minor importance for spring diatoms compared with environmental filtering. The fact that ecoregions did not have significantly different community compositions further highlighted the weak spatial structure of the communities, suggesting that dispersal limitation was not strong. This outcome agrees with the findings of Van der Gucht et al. (2007), who showed that local environmental factors drive lacustrine bacterial community composition over a wide range of spatial scales and that dispersal limitation does not strongly impact microbial communities. However, this result contradicts studies in which both spatial and local environmental factors have been found to contribute to the structure of freshwater diatom communities (Soininen et al. 2004, Verleyen et al. 2009). It may be that the spatial extent of this study was too small for dispersal limitation to

take place, as the relative importance of environmental versus spatial control of freshwater algal communities is known to vary with study scale. At large scales, dispersal limitation may act more strongly and environmental gradients are longer, resulting in a stronger spatial component in community compositions (Soininen 2007, Verleyen et al. 2009). For example, Verleyen et al. (2009) showed that lake diatom community structure was not generally influenced by dispersal-related factors, with the exception of datasets spanning large geographical extents (>2000 km). The scale dependence of environmental versus spatial control was similarly emphasized by Soininen et al. (2011), who reported that boreal lake phytoplankton communities were overall weakly spatially structured within drainage systems, yet across drainage systems, the spatial structure was clear.

Besides being affected by study scale, our results are likely to reflect the taxonomic resolution used. Higher taxonomic resolution (i.e. splitting of some species into varieties) would probably have increased the relative importance of spatial factors by revealing stronger biogeographical patterns (Van de Vijver et al. 2005, Potapova & Hamilton 2007, Vanormelingen et al. 2008).

In conclusion, the patterns in spring diatoms do not concur with the main predictions of the theory of island biogeography. Rather, the results suggest that diatom species richness and community composition are strongly influenced by water chemistry and land use, while no support for species–area or species–isolation relationships was found. Moreover, spatial factors also seem to be less important for community composition. At these study scales, dispersal limitation does not appear to influence diatom community structure or richness. Regardless of location in the study area, species tend to occur in springs where their habitat requirements are fulfilled. Thus, species are filtered into local communities from a regional species pool mainly via environmental factors.

*Acknowledgements.* We thank the Emil Aaltonen Foundation for financial support. We also thank Laura Virtanen and Jyrki Eskelinen for collecting data in the field, and 3 reviewers for their constructive comments on an earlier version of the manuscript.

#### LITERATURE CITED

- Aboal M, Puig MA, Prefasi M (1998) Diatom assemblages in springs in Castellón province, eastern Spain. *Algol Stud* 90:79–95
- Adler PB, White EP, Lauenroth WK, Kaufman DM, Rasweiler A, Rusak JA (2005) Evidence for a general species–time–area relationship. *Ecology* 86:2032–2039
- Akaike H (1974) A new look at the statistical model identification. *IEEE Trans Automat Contr* 19:716–724
- Alalammi P (ed) (1986) Atlas of Finland, Folio 121–122. Relief and landforms. National Board of Survey and Geographical Society of Finland, Helsinki
- Alalammi P (ed) (1990) Atlas of Finland, Folio 123–126. Geology. National Board of Survey and Geographical Society of Finland, Helsinki
- Anderson MJ, Crist TO, Chase JM, Vellend M and others (2011) Navigating the multiple meanings of  $\beta$  diversity: a roadmap for the practicing ecologist. *Ecol Lett* 14:19–28
- Angeli N, Cantonati M, Spitale D, Lange-Bertalot H (2010) A comparison between diatom assemblages in two groups of carbonate, low-altitude springs with different levels of anthropogenic disturbances. *Fottea* 10:115–128
- Arrhenius O (1921) Species and area. *J Ecol* 9:95–99
- Astorga A, Oksanen J, Luoto M, Soininen J, Virtanen R, Muotka T (2012) Distance decay of similarity in freshwater communities: Do macro- and microorganisms follow the same rules? *Glob Ecol Biogeogr* 21:365–375
- Barquín J, Scarsbrook M (2008) Management and conservation strategies for coldwater springs. *Aquat Conserv* 18: 580–591
- Biggs B, Duncan MJ, Jowett IG, Quinn JM, Hickey CW, Davies-Colley RJ, Close ME (1990) Ecological characterisation, classification, and modelling of New Zealand rivers: an introduction and synthesis. *N Z J Mar Freshw Res* 24:277–304
- Bjornstad ON (2012) Ncf: spatial nonparametric covariance functions. R package Version 1.1-4. Available at: <http://CRAN.R-project.org/package=nfc>
- Cantonati M (1998) Diatom communities of springs in the southern Alps. *Diatom Res* 13:201–220
- Cantonati M, Lange-Bertalot H (2010) Diatom biodiversity of springs in the Berchtesgaden National Park (north-eastern Alps, Germany), with the ecological and morphological characterization of two species new to science. *Diatom Res* 25:251–280
- Cantonati M, Spitale D (2009) The role of environmental variables in structuring epiphytic and epilithic diatom assemblages in springs and streams of the Dolomiti Bellunesi National Park (south-eastern Alps). *Fundam Appl Limnol* 174:117–133
- Cantonati M, Füreder L, Gerecke R, Jüttner I, Cox EJ (2012a) Crenic habitats, hotspots for freshwater biodiversity conservation: toward an understanding of their ecology. *Freshw Sci* 31:463–480
- Cantonati M, Angeli N, Bertuzzi E, Spitale D, Lange-Bertalot H (2012b) Diatoms in springs of the Alps: spring types, environmental determinants, and substratum. *Freshw Sci* 31:499–524
- Chao A (1984) Nonparametric estimation of the number of classes in a population. *Scand J Stat* 11:265–270
- Clarke KR (1993) Non-parametric multivariate analysis of changes in community structure. *Aust J Ecol* 18:117–143
- Connell JH (1978) Diversity in tropical rain forests and coral reefs. High diversity of trees and corals is maintained only in a nonequilibrium state. *Science* 199:1302–1310
- Dixit SS, Smol JP, Kingston JC, Charles DF (1992) Diatoms: powerful indicators of environmental change. *Environ Sci Technol* 26:22–33
- Dodson S (1992) Predicting crustacean zooplankton species richness. *Limnol Oceanogr* 37:848–856
- ESRI (Environmental System Research Institute) (2013)

- ArcGIS 10.1 for desktop. ESRI, Redlands, CA
- Fenchel T, Finlay BJ (2004) The ubiquity of small species: patterns of local and global diversity. *BioScience* 54: 777–784
- Finlay BJ (2002) Global dispersal of free-living microbial eukaryote species. *Science* 296:1061–1063
- Gaston KJ (2000) Global patterns in biodiversity. *Nature* 405:220–227
- Gesierich D, Kofler W (2010) Epilithic diatoms from rheocene springs in the eastern Alps (Vorarlberg, Austria). *Diatom Res* 25:43–66
- Gleason HA (1922) On the relation between species and area. *Ecology* 3:158–162
- Gotelli NJ, Colwell RK (2001) Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. *Ecol Lett* 4:379–391
- Heino J, Virtanen R, Vuori KM, Saastamoinen J, Ohtonen A, Muotka T (2005) Spring bryophytes in forested landscapes: land use effects on bryophyte species richness, community structure and persistence. *Biol Conserv* 124: 539–545
- Heino J, Bini LM, Karjalainen SM, Mykrä H, Soininen J, Vieira LCG, Diniz-Filho JAF (2010) Geographical patterns of micro-organismal community structure: Are diatoms ubiquitously distributed across boreal streams? *Oikos* 119:129–137
- Hobæk A, Manca M, Andersen T (2002) Factors influencing species richness in lacustrine zooplankton. *Acta Oecol* 23:155–163
- Horner-Devine MC, Lage M, Hughes JB, Bohannan BJM (2004) A taxa–area relationship for bacteria. *Nature* 432: 750–753
- Ilmonen J, Paasivirta L (2005) Benthic macrocrustacean and insect assemblages in relation to spring habitat characteristics: patterns in abundance and diversity. *Hydrobiologia* 533:99–113
- Ilmonen J, Paasivirta L, Virtanen R, Muotka T (2009) Regional and local drivers of macroinvertebrate assemblages in boreal springs. *J Biogeogr* 36:822–834
- Ilmonen J, Mykrä H, Virtanen R, Paasivirta L, Muotka T (2012) Responses of spring macroinvertebrate and bryophyte communities to habitat modification: community composition, species richness, and red-listed species. *Freshw Sci* 31:657–667
- Kalliola R (1973) Suomen kasvimaantiede. Werner Söderström Osakeyhtiö, Porvoo
- Krammer K, Lange-Bertalot H (1986–1991) Bacillariophyceae. Süßwasserflora von Mitteleuropa 2 (1–4). Gustav Fischer Verlag, Stuttgart
- Kristiansen J (1996) Dispersal of freshwater algae—a review. *Hydrobiologia* 336:151–157
- 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: Lange-Bertalot H (ed) *Iconographia Diatomologica* 2. Koeltz Scientific Books, Königstein
- Legendre P, Legendre L (1998) *Numerical ecology*, 2nd edn. Elsevier, Amsterdam
- MacArthur RH, Wilson EO (1967) *The theory of island biogeography*. Princeton University Press, Princeton, NJ
- Mantel N (1967) The detection of disease clustering and a generalized regression approach. *Cancer Res* 27: 209–220
- McCullagh P, Nelder JA (1989) *Generalized linear models*, 2nd edn. Chapman & Hall, London
- Moran PAP (1950) Notes on continuous stochastic phenomena. *Biometrika* 37:17–23
- Nolby LE, Zimmer KD, Hanson MA, Herwig BR (2015) Is the island biogeography model a poor predictor of biodiversity patterns in shallow lakes? *Freshw Biol* 60:870–880
- Oksanen J, Blanchet FG, Kindt R, Legendre P and others (2013) *Vegan: community ecology package*. R package Version 2.0-9. Available at: <http://CRAN.R-project.org/package=vegan>
- Pirinen P, Simola H, Aalto J, Kaukoranta JP, Karlsson P, Ruuhela R (2012) Climatological statistics of Finland 1981–2010. Finnish Meteorological Institute Reports 2012:1, Finnish Meteorological Institute, Helsinki
- Potapova M, Hamilton PB (2007) Morphological and ecological variation within the *Achnanthydium minutissimum* (Bacillariophyceae) species complex. *J Phycol* 43: 561–575
- R Development Core Team (2013) *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna. Available at: [www.R-project.org](http://www.R-project.org)
- Reche I, Pulido-Villena E, Morales-Baquero R, Casamayor EO (2005) Does ecosystem size determine aquatic bacterial richness? *Ecology* 86:1715–1722
- Ricklefs RE (1987) Community diversity: relative roles of local and regional processes. *Science* 235:167–171
- Rosenzweig ML (1995) *Species diversity in space and time*. Cambridge University Press, Cambridge
- Sabater S, Roca JR (1992) Ecological and biogeographical aspects of diatom distribution in Pyrenean springs. *Br Phycol J* 27:203–213
- Smith VH, Foster BL, Grover JP, Holt RD, Leibold MA, deNoyelles F Jr (2005) Phytoplankton species richness scales consistently from laboratory microcosms to the world's oceans. *Proc Natl Acad Sci USA* 102:4393–4396
- Smucker NJ, Vis ML (2011) Contributions of habitat sampling and alkalinity to diatom diversity and distributional patterns in streams: implications for conservation. *Biodivers Conserv* 20:643–661
- Soininen J (2007) Environmental and spatial control of freshwater diatoms—a review. *Diatom Res* 22:473–490
- Soininen J (2012) Macroecology of unicellular organisms—patterns and processes. *Environ Microbiol Rep* 4:10–22
- Soininen J, Heino J (2007) Variation in niche parameters along the diversity gradient of unicellular eukaryote assemblages. *Protist* 158:181–191
- Soininen J, Meier S (2014) Phytoplankton richness is related to nutrient availability, not to pool size, in a subarctic rock pool system. *Hydrobiologia* 740:137–145
- Soininen J, Paavola R, Muotka T (2004) Benthic diatom communities in boreal streams: community structure in relation to environmental and spatial gradients. *Ecography* 27:330–342
- Soininen J, Heino J, Kokocinski M, Muotka T (2009) Local–regional diversity relationship varies with spatial scale in lotic diatoms. *J Biogeogr* 36:720–727
- Soininen J, Korhonen JJ, Karhu J, Vetterli A (2011) Disentangling the spatial patterns in community composition of prokaryotic and eukaryotic lake plankton. *Limnol Oceanogr* 56:508–520
- Stoermer EF, Smol JP (eds) (1999) *The diatoms: applications for the environmental and earth sciences*. Cambridge University Press, Cambridge
- Stomp M, Huisman J, Mittelbach GG, Litchman E, Klaus-

- meier CA (2011) Large-scale biodiversity patterns in freshwater phytoplankton. *Ecology* 92:2096–2107
- SYKE (Finnish Environment Institute) (2009) CLC2006 Finland: final technical report. SYKE, Helsinki
- SYKE (Finnish Environment Institute) (2013) Metsäkasvillisuusvyöhykkeet (Forest Vegetation Zones). SYKE, Helsinki
- van de Vijver B, Gremmen NJM, Beyens L (2005) The genus *Stauroneis* (Bacillariophyceae) in the Antarctic region. *J Biogeogr* 32:1791–1798
- van der Gucht K, Cottenie K, Muylaert K, Vloemans N and others (2007) The power of species sorting: local factors drive bacterial community composition over a wide range of spatial scales. *Proc Natl Acad Sci USA* 104: 20404–20409
- van der Kamp G (1995) The hydrogeology of springs in relation to the biodiversity of spring fauna: a review. *J Kans Entomol Soc* 68:4–17
- Vanormelingen P, Verleyen E, Vyverman W (2008) The diversity and distribution of diatoms: from cosmopolitanism to narrow endemism. *Biodivers Conserv* 17: 393–405
- Venables WN, Ripley BD (2002) *Modern applied statistics with S*, 4th edn. Springer, New York, NY
- Verleyen E, Vyverman W, Sterken M, Hodgson DA and others (2009) The importance of dispersal related and local factors in shaping the taxonomic structure of diatom metacommunities. *Oikos* 118:1239–1249
- Virtanen L, Soininen J (2012) The roles of environment and space in shaping stream diatom communities. *Eur J Phycol* 47:160–168
- Vyverman W, Verleyen E, Sabbe K, Vanhoutte K and others (2007) Historical processes constrain patterns in global diatom diversity. *Ecology* 88:1924–1931
- Wang J, Soininen J, Zhang Y, Wang B, Yang X, Shen J (2012) Patterns of elevational beta diversity in micro- and macroorganisms. *Glob Ecol Biogeogr* 21:743–750
- Weckström J, Korhola A, Blom T (1997) Diatoms as quantitative indicators of pH and water temperature in subarctic Fennoscandian lakes. *Hydrobiologia* 347:171–184
- Wojtal AZ, Sobczyk Ł (2012) The influence of substrates and physicochemical factors on the composition of diatom assemblages in karst springs and their applicability in water-quality assessment. *Hydrobiologia* 695:97–108

*Editorial responsibility: Tom Battin,  
Vienna, Austria*

*Submitted: February 3, 2015; Accepted: May 5, 2015  
Proofs received from author(s): June 26, 2015*