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**Substrate-dependent responses of ciliate assemblages to temperature: a natural experiment in Icelandic streams**

**Keywords:** ciliates; protists; community ecology; diversity; biomass; geothermal; temperature gradient; natural experiment; Iceland.

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## ABSTRACT

1. Ciliate assemblages play a significant role in the microbial food web, and the effects of environmental temperature on their composition can be influenced by abiotic factors such as seasonality and disturbance. The effects of temperature on ciliate assemblages found on different substrates, however, have not been explored. Different substrate types such as sandy bottoms and submerged rocks harbour dissimilar ciliate assemblages; thus we could expect ciliate assemblages from these two substrates to respond differently to temperature.

2. We studied how alpha diversity, beta diversity, and total biomass of ciliate protist assemblages found on sandy bottoms and submerged rocks changed in 13 geothermally heated streams in Iceland, ranging from 5–20 °C. We recorded number of operational taxonomic units (OTUs) and measured the size of cells in ciliate assemblages from both substrates.

3. Effects of temperature on natural ciliate assemblages were substrate-dependent. On rock surfaces, both total ciliate biomass and alpha diversity declined with increasing temperature, and beta diversity increased with increasing temperature difference due to OTU nestedness (assemblages from warm streams being composed chiefly of subsets of the OTUs found in colder streams). In sandy substrates, however, ciliate assemblage composition was independent of temperature.

4. Substrate-specific responses may be due to differences in mechanical disturbance, nutrient availability, or exposure to invertebrate grazers. Rock-surface assemblages may be more exposed to the flow and retain less nutrients than those of sandy substrates, thus they may be more strongly resource-limited and more responsive to direct effects of temperature on their metabolism. Alternatively, rock surface assemblages may be more exposed to grazing by invertebrates, which intensifies with temperature.

5. Our study underpins the need to account for environmental context such as substrate type to fully understand the effect of temperature on microbial assemblages in streams. Future increases in global temperatures may affect fresh waters differently depending on their prevalent substrate. Those dominated by hard substrates may have their ciliate assemblages, and thus food web structures and ecosystem functioning, more strongly affected by warming relative to systems dominated by soft substrates.

## INTRODUCTION

Temperature plays a fundamental role in driving ecological processes (Kordas, Harley & O'Connor 2011; Sibly, Brown & Kodric-Brown 2012) and its effects on one or a few species have received considerable attention in recent years (Montagnes, Kimmance & Atkinson 2003; Vasseur & McCann 2005; Vucic-Pestic *et al.* 2011; Lang, Rall & Brose 2012). Community-level effects such as changes in alpha and beta diversity, however, are still largely unknown (Woodward, Perkins & Brown 2010b), and most studies have focused on large-scale observations over latitudinal or altitudinal gradients, or smaller-scale warming experiments (Stewart *et al.* 2013). Latitudinal gradients of alpha and beta diversity have been documented for many taxa, and temperature is often invoked as the major explanatory variable (Buckley & Jetz 2007). Yet, contrary to the observational studies, experiments have revealed contrasting and contingent community-level effects for grasses (Post & Pedersen 2008; Yang *et al.* 2011; Lamb *et al.* 2011; Sherry *et al.* 2012; Wahren *et al.* 2013; Post 2013), fungi and bacteria (Lamb *et al.* 2011), and larger eukaryotes (Villalpando *et al.*, 2009; Bakonyi *et al.*, 2007; Strecker *et al.*, 2004; Meerhoff *et al.*, 2012). Given the criticisms that can be leveled at either approach – confounding variables in surveys, and a lack of realism in experiments – research that incorporates both the realism of observational studies and the lack of confounding variables in experimental studies

is needed to reconcile these apparently contrasting findings at the community level (O’Gorman *et al.* 2014).

Here, we focus on the effect of temperature on freshwater benthic assemblages of ciliate protists (hereafter “ciliates”). Ciliates respond promptly to environmental change (Jiang & Morin 2004) and can be extremely diverse within small areas (Finlay & Esteban 1998; Esteban & Finlay 2007), which makes them an ideal model system for studying community-level relationships between environmental temperature and biodiversity. Moreover, ciliates play significant roles in the microbial food web and the cycling of organic material. For example, by feeding on bacteria and increasing decomposition rate (Ribblett, Palmer & Coats 2005; Kathol *et al.* 2009), contributing to the import of organic material from the water column into the benthos (Eisenmann *et al.* 2001; Weitere, Schmidt-Denter & Arndt 2003; Kathol *et al.* 2009), and being consumed by invertebrate meiofauna (Norf, Arndt & Weitere 2007).

Previous studies have shown that temperature can increase the total biomass and the species richness of ciliate assemblages, and induce changes in their beta diversity (Norf & Weitere 2010; Vidussi *et al.* 2011; Domaizon *et al.* 2012). Furthermore, the direction and magnitude of temperature effects can change depending on environmental factors such as seasonality (Norf *et al.* 2007), nutrient availability (Norf & Weitere 2010), and mechanical disturbance (Marcus *et al.* 2014). The nature of the bottom substrate is also known to affect the abundance and the species composition of ciliate assemblages (Gucker & Fischer 2003). Yet, to our knowledge, no-one has assessed whether ciliate assemblages found on dissimilar substrates respond differently to a temperature gradient. Given the significant ecological role played by ciliates, understanding the potentially interacting effects of temperature and substrate type on their biomass and diversity is important to predict the effects of climate change on a local scale. In our study, we assessed the effect of temperature on the total biomass, alpha diversity, and beta diversity of freshwater ciliate assemblages found on two contrasting substrates, namely submerged rock surfaces and sandy sediment.

The study was carried out in a network of small streams characterized by a natural and stable geothermal gradient spanning ~15 °C (Friberg *et al.* 2009). This system allows us to evaluate long-term effects of temperature on community composition and organism abundance in a “natural experiment”, without the confounding effects of biogeography associated with altitudinal or latitudinal gradients (O’Gorman *et al.* 2014). Previous studies carried out in the same system have found some clear effects of temperature: e.g. macrofaunal community similarity decreases with increasing pairwise temperature difference between streams, and primary productivity and decomposition rates increase with temperature, whereas effects on the non-algal microbial community are still largely unknown (Friberg *et al.* 2009; Woodward *et al.* 2010a).

We specifically tested the hypotheses that biomass and alpha diversity of ciliate assemblages are related to environmental temperature as well as substrate. We expect both biomass and alpha diversity to decrease with increasing temperature, because the high metabolic costs linked to high temperature would lead to stronger competition (Brown *et al.* 2004; Savage *et al.* 2004; Binzer *et al.* 2012). We also tested the hypothesis that beta diversity among assemblages increases with increasing temperature difference, as previously observed in mesocosm experiments (Norf & Weitere 2010; Domaizon *et al.* 2012). Furthermore, we assessed the relative contributions of taxa turnover and nestedness to compositional variation. Finally, we tested the hypothesis that substrate type affects the responses of ciliate assemblages to environmental temperature. We expect different substrate types to have different levels of resource availability and to be exposed to different levels of mechanical disturbance. These factors affect the structure of the ciliate assemblages (e.g. Foissner & Berger 1996), and could influence how ciliate assemblages respond to temperature differences (Norf & Weitere 2010; Marcus *et al.* 2014). We expect ciliate assemblages on submerged rock surfaces to be more resource-limited than on sandy substrates, and we thus here predict a stronger reduction in

biomass and alpha diversity with increasing temperature, compared to ciliate assemblages from sandy substrates.

## MATERIALS AND METHODS

### Study site

Our study was conducted in 13 streams within a single geothermal catchment in Hengill, about 30 km east of Reykjavík, Iceland (64°.03'N; 021°.18'W; 350-420 m a.s.l.) (Fig. S1), displaying a gradient of mean water temperatures from 5.4 to 20.2°C. Although there are some minor chemical differences among the streams, these are independent of temperature, which is the major environmental gradient in the system (Table S1) (Woodward *et al.* 2010a; Demars *et al.* 2011; O'Gorman *et al.* 2012; Adams *et al.* 2013).

### Ciliate sampling

During August 2012 we surveyed ciliate assemblages in 13 streams (Fig. S1), from two different substrates in each stream: submerged rock surfaces and coarse sandy sediment (hereafter "sandy substrate"). Three to four samples from each substrate were taken from each stream. All samples in a stream were collected from locations at known coordinates, and analyzed within 24 hours after collection. Sandy substrate samples were collected by scooping ~15 ml of sediment into a 50-ml sterile plastic tube, and topping up the rest of the tube volume with natal stream water. Samples from submerged rock surfaces were collected by haphazardly selecting a rock and removing it from the stream; the size of all rocks was approximately 350 cm<sup>3</sup> (the size of a fist). The upper surface of each rock was brushed with a clean toothbrush, photographed, and measured to allow standardization of the cell abundances per unit area. Brushed material was washed off the toothbrush into a 50-ml sterile plastic tube using a squirt bottle filled with natal stream water, until the tube contained 50 ml.

In the laboratory, the contents of each vial were homogenized by gently shaking the tubes, and 1 ml of water was withdrawn (following O'Gorman *et al.* 2012). This procedure was chosen because it homogenizes the samples without damaging the cells of free-living ciliates in it. The withdrawn volume was poured into a Sedgewick-Rafter cell counter and searched using a Nikon Eclipse E200 compound microscope at 100–400x magnification. Ciliates were identified *in vivo* to the lowest possible taxonomic level, termed operational taxonomic units (hereafter OTUs), using identification keys (Foissner *et al.* 1991; Foissner & Berger 1996; Patterson & Hedley 2009); in most cases, OTU corresponds to genera. The number of individuals of each OTU per milliliter was recorded, and the length and width of each cell were measured *in vivo* to the nearest ten micrometers using ocular micrometers.

Length and width measurements of each individual were used to estimate the corresponding volume. This was performed by approximating the cell shape of each OTU to standard geometrical forms (O'Gorman *et al.* 2012, after Hillebrand *et al.* 1999) and estimating the corresponding volume. Individual cell masses were obtained by multiplying their estimated volumes with their physical density. The density of each cell was taken to be 1 g/cm<sup>3</sup>, the same as distilled water, as water is the major component of eukaryotic cells (Omori & Ikeda 1984, Kageyama *et al.* 1989). Total ciliate biomass per milliliter was calculated as the sum of the masses of all ciliate cells found per milliliter of surveyed.

## Temperature data

Stream temperature was recorded during 7<sup>th</sup>–21<sup>st</sup> August 2012. Streams 2–4, 10, and 12 were surveyed using “iButton” data loggers (Maxim Integrated, San Jose, California, USA), which recorded the temperature once every 20 minutes (precision  $\pm 0.1^\circ\text{C}$ ). Streams 5–9, 11, 13, and 14 were surveyed using “HOBO Pro v2” and “HOBO Pendant” temperature loggers (Onset Computer Corporation, Cape Cod, Massachusetts, USA), which recorded the temperature at 15-minute intervals (precision  $\pm 0.2^\circ\text{C}$ ). All statistical analyses were performed using the mean of the temperatures recorded over the sampling period as the explanatory variable.

## Statistical analyses

Statistical analyses were performed in R version 3.0.3 (2014). Multivariate analyses were performed with the R packages *vegan* version 2.0-10 (Oknesen et al. 2013) and *betapart* version 1.3 (Baselga et al. 2013). Rock- and sand-communities required different sampling techniques and methods of standardizing ciliate densities (individuals per unit area versus individuals per unit volume), thus they were also analyzed separately, as differences in sampling would have confounded the results of joint analyses (e.g. ANCOVA).

### *Alpha diversity*

Alpha diversity was measured as OTU richness, i.e. the number of OTUs found per sample. The relationship between temperature and ciliate OTU richness was assessed separately for each substrate type, using generalized linear mixed effect models (hereafter GLMMs) with stream temperature as fixed effect and stream identity as random-intercept effect to account for variability among streams due to factors other than temperature. We used a Poisson error distribution to account for the nature of the data (integer count data). The models were implemented with function “*glmer*” from package *lme4* version 1.1-7 (Bates *et al.* 2014), and the relative contributions of stream temperature and stream identity to  $R^2$  were estimated with function “*r.squaredGLMM*” from package *MuMIn* version 1.10.0 (Barton 2014).

### *Beta diversity*

Total pairwise beta diversity was measured using the Sørensen index, an effective tool to highlight broad compositional patterns (Gotelli & Ellison 2004). The Sørensen index is based on OTU presence/absence, and represents a more robust approach than abundance-based dissimilarity indices when the assumption of species abundances being measured without bias may not be met (Baselga *et al.* 2013), as is the case in our study. It measures beta diversity due to both OTU nestedness and OTU turnover. We followed the approach proposed by Baselga et al. (2013) to disentangle these two components. For each pair of ciliate assemblages, the beta diversity due to OTU turnover was measured with the Simpson index, while the beta diversity due to OTU nestedness was computed as the difference between the Sørensen index and the Simpson index (Baselga 2010). This was performed using function “*beta.pair*” of the R package *betapart* version 1.3 (Baselga *et al.* 2013).

Mantel tests were used to assess the correlation between beta diversity components (total, due to OTU nestedness, and due to OTU turnover) and temperature differences among streams. Although effective and widely used, the Mantel test has been suggested to be at high risk of incurring type I errors (incorrect rejection of a true null hypothesis) (Guillot & Rousset 2013); moreover, Mantel tests

cannot detect non-linear correlations. To account for these limitations, we also assessed the correlation between each beta diversity component and temperature by means of non-metric multidimensional scaling ordinations (nMDS), arguably the most robust unconstrained ordination method in community ecology (Oksanen *et al.* 2013), combined with two-dimensional generalized additive models (GAM) (Simpson 2011; Battarbee *et al.* 2012). This approach consists of two steps. First, the relative beta diversity distances among assemblages are displayed in two dimensions using ordinations. The outcome of the ordination is then used as a response variable in a GAM, with mean stream temperatures as the explanatory variable. The analysis is performed with the function “ordisurf” in R package *vegan* version 2.0-10, which computes the significance of the GAM and allows the model fit to be visualized as an overlay on the nMDS plot (as smoothing isoclines).

### *Total biomass*

The relationship between temperature and ciliate biomass was assessed separately for each substrate type using linear mixed effect models (hereafter LMMs), with stream temperature as fixed effect and stream identity as random-intercept effect. We used natural-log-transformed biomass as response variable to account for the positive-only nature of biomass values. The models were implemented with function “lmer” from package *lmerTest* 2.0-6 (Kuznetsova, Brockhoff & Bojesen-Christensen 2014), and the contribution of stream temperature and stream identity to  $R^2$  was estimated with function “r.squaredGLMM” from package *MuMIn* 1.10.0 (Barton 2014).

## **RESULTS**

In total we identified 6,018 live individuals from 83 OTUs. Of these, 2,705 individuals of 48 OTUs were found in the samples from rock surfaces and 3,313 individuals from 72 OTUs were found in the samples from sandy substrates; 38 OTUs were common to both substrates (Fig. S2–S3).

### **Effects of temperature on alpha diversity**

The number of OTUs declined significantly with increasing temperature on the rock surfaces (exponent: -0.068 per degree C°,  $z$ -test=-4.808,  $p < 0.0001$ ) and temperature explained 41.6% of this variability (Fig. 1a). The number of OTUs found in assemblages from sandy substrates was not correlated with temperature (exponent: 0.011 per degree C°,  $z$ -test=0.627,  $p=0.53$ ,  $R^2_{\text{temperature}}=0.008$ ) (Fig. 1b). For both substrates, the amount of variability among streams explained by temperature was larger than that explained by stream identity (i.e. reasons other than temperature *per se*), but smaller than the residual variability due to either natural heterogeneity or the observation process (Table S2).

### **Effects of temperature on beta diversity**

Total beta diversity in ciliate assemblages from either substrate was not correlated with changes in environmental temperature (Fig. S4a,b; Fig. S5a,b; Table 1). Similarly, the component of beta diversity due to OTU turnover was unrelated to temperature in either substrate (Fig. S4e,f; Fig. S5e,f; Table 1). The Mantel test (Fig. 2a) and the GAM model run on a nMDS ordination (Fig. S5c) showed that nestedness-related beta diversity of rock-surface assemblages increased linearly with increasing pairwise temperature difference (Table 1). Conversely, ciliate assemblages from sandy substrates did not differ due to taxonomic nestedness across the temperature gradient (Fig. 2b; Fig. S5d; Table 1). In

the light of the observed negative relationship between OTU richness and temperature (see previous section), this result suggests that, on rock surfaces, the OTU composition of ciliate assemblages from warm streams is nested within the OTU composition at lower temperatures; i.e. ciliate assemblages from warm streams are subsets of those from the colder streams.

### **Total ciliate biomass**

The total ciliate biomass found on rock surfaces significantly declined with increasing temperature (slope=-0.177, t-test=-2.891, p=0.0209) (Fig. 3a). Temperature explained 22.7% of the observed variability among streams. Total ciliate biomass found in sandy substrates did not show a significant correlation with temperature (slope=-0.0150, t-test=-0.265, p=0.793) (Fig. 3b). Most of the variability not explained by temperature was due to within-stream variability (Table S3); i.e., either to natural heterogeneity or the observation process.

## **DISCUSSION**

Our goal was to assess the relationship of temperature with the alpha diversity, beta diversity, and total biomass of natural ciliate assemblages found on different substrates. To the best of our knowledge, ours is the first study to do so. The role of temperature depended on the nature of the substrate. In assemblages from submerged rock surfaces, alpha diversity, beta diversity due to nestedness, and total biomass were all significantly affected by temperature, while none of these relationships were observed in sandy substrates.

The negative correlation of ciliate alpha diversity and total biomass with temperature on submerged rock surfaces was in line with our expectation that high temperature increases metabolic demands (Brown *et al.* 2004; Savage *et al.* 2004; Binzer *et al.* 2012), thus increasing competition and potentially reducing carrying capacity. However, on sandy substrates these measures were not correlated with temperature. This could have several explanations. Sandy substrates may retain more nutrients than rock surfaces do because of more interstitial space (Hossain *et al.* 2014), and the two substrate types may be exposed to different regimes of mechanical disturbance due to water flow. Both resource availability and disturbance can change the direction and magnitude of temperature effects on assemblages (Norf & Weitere 2010; Marcus *et al.* 2014). Moreover, sand can be a different substrate depending on its grain size or composition, and retain fine organic material differently. Thus, the diversity and abundance of ciliate communities from sandy substrates may be more strongly correlated with sand grain size or composition than it is to temperature. Sand substrate in the Hengill system, mostly coarsely-grained lava sediment, shows some differences in grain size among streams, but we lack quantitative measurements to assess the relationship between sediment granulometry and the diversity and abundance of ciliate communities it harbours.

Temperature may also affect protist assemblages indirectly, affecting the strength of interactions among species. Temperature increases the metabolic requirements of individuals (Brown *et al.* 2004) and, consequently, it can increase their feeding rates (e.g. Sanford 1999, Yee & Murray 2004). Changes in the intensity of trophic interactions can, in turn, alter the dynamics and composition of whole communities (Paine 1974). Biofilm-grazing gastropods represent common consumers or competitors of ciliates, and temperature is known to increase both the feeding rate and the activity rate of gastropods (Yee & Murray 2004). In our study system, *Radix balthica* snails play a key role in the food web, being the dominant large grazer in Hengill streams (O'Gorman *et al.* 2012). *Radix balthica* consumes the same resources (bacteria and diatoms) as many protists, and it is more abundant and exerts higher grazing pressure on the biofilm at high temperatures (O'Gorman *et al.* 2012). The

increased abundance and grazing pressure of *R. balthica* at high temperatures may reduce the species richness and total biomass in ciliate assemblages, either by direct competition for resources, by physically displacing ciliates while grazing the biofilm, or by consuming them directly. Moreover, an effect of grazing might be more substantial on a firm substrate like rock surfaces than on shifting sandy substrates. Unfortunately, there is no experimental evidence in the literature either supporting or rejecting these possibilities. We did not and could not assess or distinguish between these possible explanations within the scope of this study, and further work is needed to detect the ecological processes underlying the patterns we observed. For example, an exclusion experiment would make it possible to determine the relative importance of temperature and biofilm grazers such as *R. balthica* on the ciliate assemblages on different substrates (Trochine *et al.* 2011).

On submerged rock surfaces, temperature differences caused an increase in ciliate beta diversity due to taxonomic nestedness, while ciliate beta diversity due to taxonomic turnover was not affected. This suggests that, for submerged rock surfaces, changes in the beta diversity of ciliate communities are due to environmental filtering exerted by temperature, more than to niche partitioning of ciliate taxa along the temperature gradient (Baselga 2010). As temperature increases, it may act as an "environmental filter", progressively excluding more and more ciliate taxa from a submerged rock surfaces. This role may be a direct reflection of the ciliates' niche breadth, or it may be mediated by trophic interactions. In the former case, the taxonomic nestedness of ciliate assemblages found on submerged rock surfaces at different temperatures suggests ciliates found on rock surfaces are similarly tolerant to low but not to high temperatures. Conversely, sandy-substrate assemblages may include more "temperature-generalist" ciliates, explaining why temperature did not drive changes in their beta diversity. In the latter case, resource competition with invertebrate grazers such as *R. balthica* may also explain the progressive exclusion of species from low to high temperatures in rock-surface assemblages, but not in the sandy-substrate ones. None of the taxa we identified are classified as being psychrophilic (cold-specific) or thermophilic (Foissner *et al.* 1991). Further studies are required to disentangle whether the "environmental filtering" role of temperature is directly related to the niche breadth of the taxa or trophically mediated, and whether ciliates from sandy substrates share any traits, such as large cell size or phenotypic plasticity, that would enable them to cope with such broad temperature gradients.

The different effect of temperature on the components of beta diversity is a novel and important finding, because previous studies have only tested its influence on total beta diversity, without evaluating whether the observed patterns were due to changes in taxonomic turnover or taxonomic nestedness.

We acknowledge some limitations and possible sources of bias in our study. Because samples need to be identified live, such studies are forced to employ a relatively low sampling intensity (three to four samples for each combination of stream and substrate type), which may not have been enough to reach taxonomic saturation; i.e., rare species may be under-represented in our dataset. A higher sampling effort in future studies would allow to confirm that our results also apply when all rare species are accounted for. A second possible source of bias is represented by the procedure for sampling sandy substrates. While the gentle stirring of the samples allowed homogenization while minimizing cell damage, it may have led to underestimation of the abundance of some ciliate groups, such as those living attached to the substrate (e.g. stalked peritrichs). Future studies should use the same technique we used for sampling delicate, free-swimming ciliates, paired with a more vigorous shaking of the samples to ensure the detachment of ciliates attached to the substrate.

Our study highlights that differences in environmental context, such as substrate type, must be taken into account if we are to understand and predict the effect of temperature on microbial diversity and biomass. In the context of ongoing climate change, predicted increases in global temperatures may produce locally different effects on ciliate assemblages depending on their substrate. Specifically,

ciliate assemblages found on submerged rock surfaces may be more prone to undergo changes in their diversity and total biomass than ciliate assemblages from sandy substrates. We might expect to see larger changes in systems dominated by hard substrates (e.g., upland ecosystems), than in those dominated by soft or sandy substrates (e.g., lowland ecosystems). In turn, this may produce substrate-dependent changes in the microbial food web structure and ultimately in the ecosystem functioning. Thus, substrate-dependent changes in the response to temperature at a microscopic level may ultimately be manifested at far larger spatial scales.

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## TABLES AND FIGURES

Table 1. Results of Mantel tests and GAM models, aimed at detecting linear and non-linear relationships between beta diversity and temperature, respectively. Mantel's  $r$  is based on Pearson's product-moment correlation, and  $p$  is the corresponding  $p$ -value computed over 999 permutations. GAM's  $R^2$  is the adjusted  $r$ -square;  $p$  is the approximate significance of smooth terms based on an F-test.

Substrate	Beta diversity component	Mantel test		GAM		
		$r$	$p$	$R^2$	F	$p$
Rock	Total (Sørensen)	-0.03	0.591	0.287	0.492	0.225
	Nestedness	0.66	0.001 **	0.768	4.042	0.0003 ***
	Turnover	-0.44	0.998	0	0	0.793
Sand	Total (Sørensen)	-0.1	0.804	0.165	0.263	0.291
	Nestedness	-0.06	0.652	0.371	0.786	0.0871
	Turnover	-0.04	0.632	0	0	0.491

Figure 1. Relationship between temperature and alpha diversity (taxon richness) in ciliate assemblages found on submerged rock surfaces (a) and sandy substrates (b). Solid and dashed lines represent statistically significant relationships and non-significant trends, respectively.

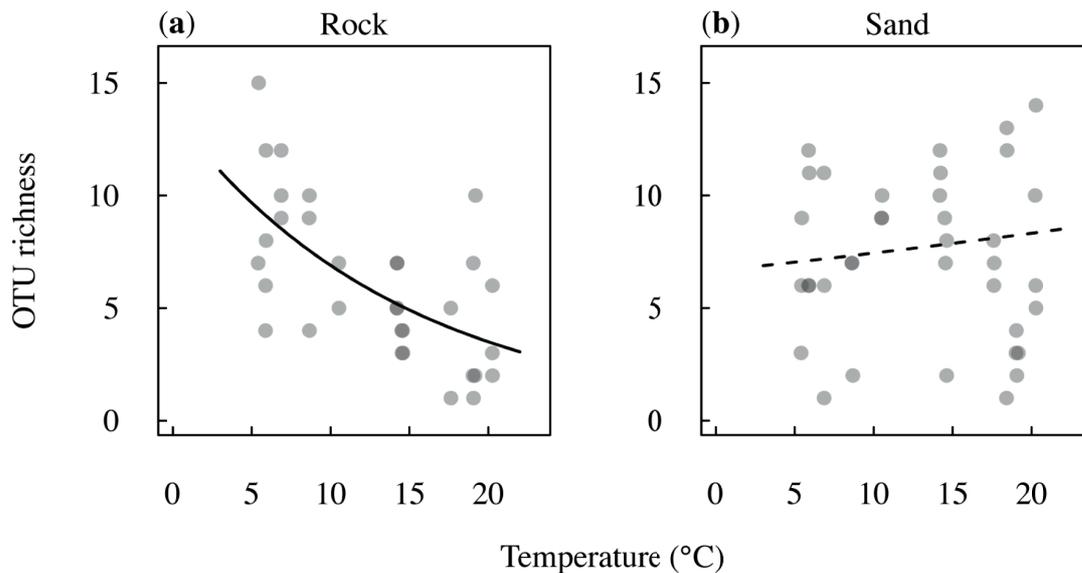


Figure 2. Correlations between the pairwise temperature difference between streams ( $\Delta T$ ) and the corresponding pairwise differences in beta diversity due to nestedness, for ciliate assemblages found on submerged rock surfaces (a) and sandy substrates (b).

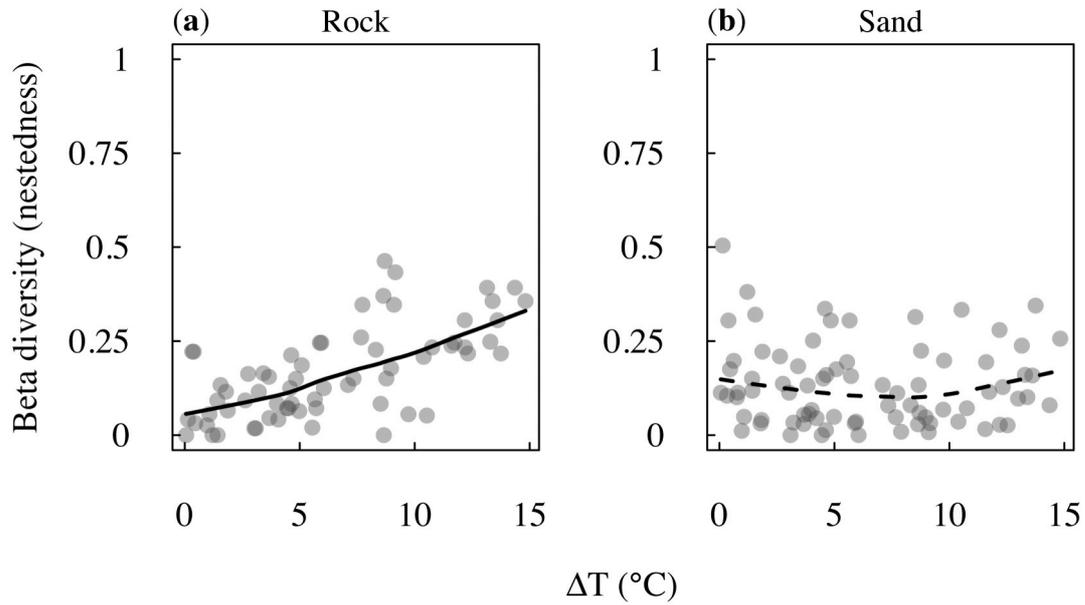
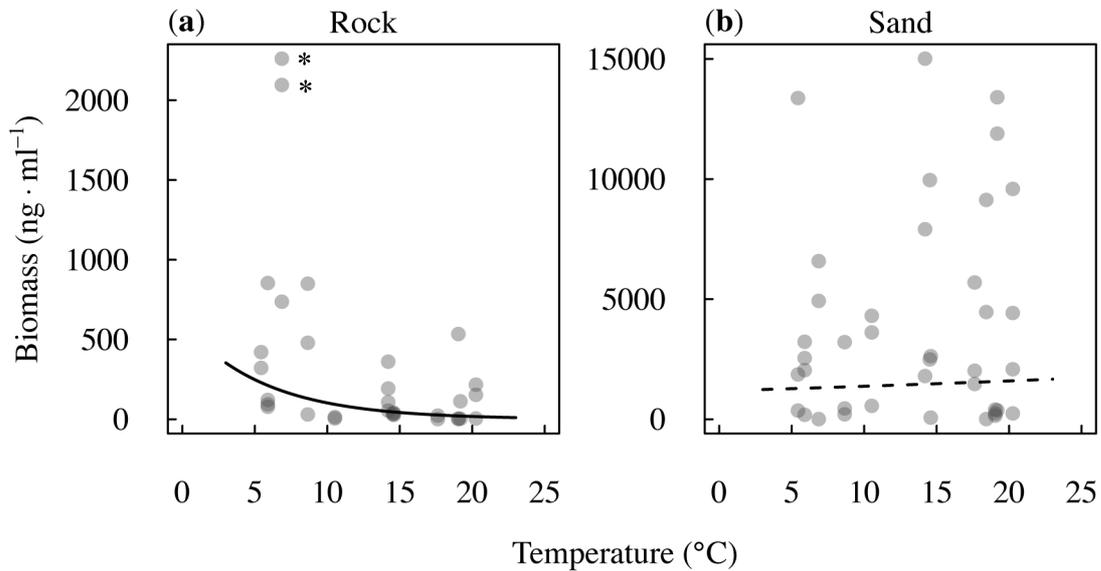


Figure 3. Correlation between temperature and total ciliate biomass (in  $\text{ng}/\text{ml}$ ) found on submerged rock surfaces (a) and sandy substrates (b). Solid and dashed lines represent statistically significant relationships and non-significant trends, respectively. Data outliers in the figure are represented by asterisks (\*).



## SUPPORTING INFORMATION

Additional tables and figures:

- Table S1. Table showing the values for abiotic variables for each stream, ordered by mean stream temperature.
- Table S2. Results of the GLMMs assessing the correlation between temperature and alpha diversity (taxon richness) in ciliate assemblages found on submerged rock surfaces and sandy substrates.
- Table S3. Results of the LMMs assessing the correlation between temperature and the natural log of total ciliate biomass (originally in ng/ml) found on submerged rock surfaces and sandy substrates.
- Figure S1. Location of Hengill in Iceland and map of the stream network. Stream 12, not shown, is located about 1.5 km south-east of Stream 15.
- Figure S2. Protist OTUs present in the samples from soft sediment collected at each temperature.
- Figure S3. Protist OTUs present in the samples from submerged rock surfaces collected at each temperature.
- Figure S4. Plots showing pairwise differences in beta diversity against pairwise temperature difference between streams ( $\Delta T$ , in °C); data refer to ciliate assemblages found on submerged rock surfaces (panels a, c, e) and sandy substrates (panels b, d, f). On top of each panel, the corresponding Pearson's r- and p-values are reported.
- Figure S5. Non-linear Multi Dimensional Scaling (nMDS) ordinations based on the differences in ciliate beta diversity for submerged rock surfaces (a, c, e) and sandy substrates (b, d, f). The isoclines come from two-dimensional GAM models having the nMDS ordinations as a response variable and the mean stream temperatures as explanatory variable.

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**Substrate-dependent responses of ciliate assemblages to temperature: a natural experiment in Icelandic streams**

**ADDITIONAL INFORMATION**

Table S1: Table showing the values for abiotic variables for each stream, ordered by mean stream temperature. Temperature data were collected during 7<sup>th</sup>–21<sup>st</sup> August 2012. Abiotic variables other than temperature were collected in August 2008 for all streams but Stream 2, for which the data refer to Summer 2004 (data from Adams et al. 2013 and Woodward et al. 2009, respectively). Temp = mean temperature in °C; pH is unitless; Cond = conductivity in  $\mu\text{S cm}^{-1}$ ; all other environmental parameters are given in  $\text{mg l}^{-1}$ . DOC = dissolved organic carbon; DO = dissolved oxygen; TN = total nitrogen; TP = total phosphorous.

Stream	Temp	pH	Cond	DOC	DO	NH <sub>4</sub>	NO <sub>3</sub>	TN	PO <sub>4</sub>	TP	Ca <sup>2+</sup>	K <sup>+</sup>	Mg <sup>2+</sup>	Na <sup>2+</sup>	Si	Cl <sup>-</sup>	SO <sub>2</sub> <sup>4-</sup>
10	5.43	7.7	129	0.314	11.7	0.006	0.002	0.054	0.010	0.018	20.1	0.3	4.7	11.0	6.0	6.6	3.8
7	5.89	7.6	110	0.208	11.4	0.010	0.001	0.012	0.012	0.025	14.1	0.5	4.1	10.6	7.0	7.7	1.5
13	6.86	7.6	201	0.294	11.1	0.018	0.001	0.114	0.001	0.006	28.4	0.5	8.9	15.1	9.9	7.2	5.9
11	8.65	8.0	624	0.581	9.9	0.009	0.001	0.085	0.001	0.015	32.4	2.5	29.5	123.8	25.4	5.7	0.2
14	10.52	8.1	254	0.403	10.7	0.010	0.003	0.123	0.001	0.010	35.9	0.5	8.5	17.5	11.4	6.4	5.8
9	14.20	8.1	262	0.263	8.5	0.008	0.004	0.036	0.018	0.036	26.9	1.5	6.6	34.6	17.9	6.6	2.7
12	14.53	7.9	223	0.618	8.1	0.012	0.005	0.098	0.001	0.011	25.1	1.4	7.7	19.6	17.6	7.0	2.6
4	14.59	7.7	153	0.465	10.7	0.008	0.006	0.041	0.001	0.008	20.3	0.3	5.1	7.4	9.8	7.6	2.9
5	17.62	8.0	282	0.427	8.0	0.006	0.006	0.032	0.002	0.019	31.1	2.2	6.7	36.8	19.8	6.3	4.8
2	18.42	7.9	236	NA	10.6	NA	NA	NA	0.013	0.019	NA	NA	NA	NA	NA	NA	14.4
3	19.04	7.9	275	0.226	7.1	0.013	0.004	0.019	0.009	0.028	29.5	1.8	6.5	29.9	19.6	6.4	6.4
6	19.18	8.1	283	0.317	8.6	0.011	0.002	0.016	0.007	0.028	29.8	2.2	6.4	37.0	19.7	6.5	4.6
8	20.25	8.1	300	0.330	6.8	0.009	0.006	0.014	0.012	0.031	28.2	2.6	5.5	37.5	20.2	6.3	3.5

Table S2. Results of the GLMMs assessing the correlation between temperature and alpha diversity (OTU richness) in ciliate assemblages found on rock surfaces and sandy substrates.  $R^2_{(temperature)}$  and  $R^2_{(Stream)}$  represent the proportion of total variation explained by temperature and stream identity, respectively.

Substrate	Temperature effect	SE	Test	p	$R^2_{(temperature)}$	$R^2_{(Stream)}$
Rock	-0.06776	0.01409	z = -4.808	<0.0001***	0.416	0
Sand	0.01061	0.01691	z = 0.627	0.53	0.008	0.004

Table S3. Results of the LMMs assessing the correlation between temperature and the natural log of total ciliate biomass (originally in ng/ml) found on rock surfaces and sandy substrates.  $R^2_{(temperature)}$  and  $R^2_{(Stream)}$  represent the proportion of total variation explained by temperature and stream identity, respectively. (<sup>1</sup>): outliers included.

Substrate	Temperature effect	SE	Test	p	$R^2_{(temperature)}$	$R^2_{(Stream)}$
Rock	-0.2133	0.0685	t = -3.113	0.0128 *	0.2886	0.1131
Rock <sup>1</sup>	-0.1770	0.0612	t = -2.891	0.0209 *	0.2273	0.0086
Sand	0.0150	0.0566	t = 0.265	0.793	0.0018	0

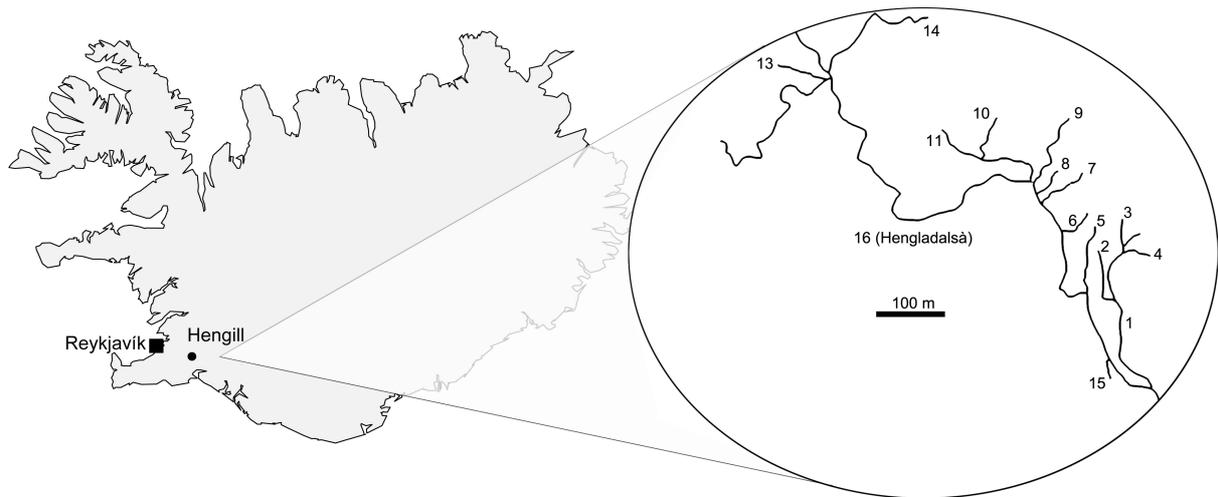
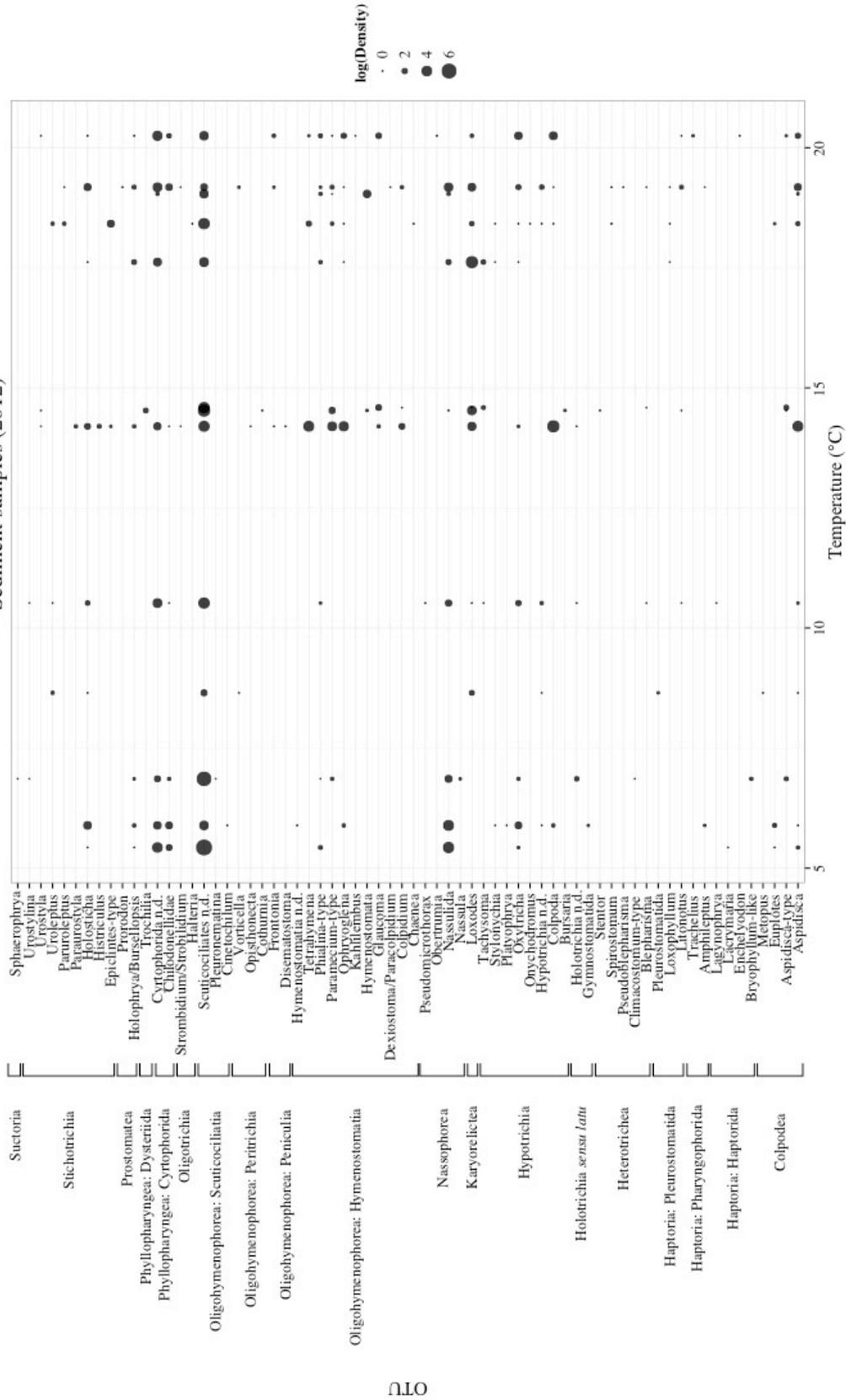


Figure S1. Location of Hengill in Iceland and map of the stream network. Streams 1, 15, and 16 were not included in the study. Stream 12, not shown, is located about 1.5 km south-east of Stream 15.

Figure S2 (page 5). Protist OTU present in the samples from soft sediment collected at each temperature.

Figure S3 (page 6). Protist OTU present in the samples from rock surfaces collected at each temperature.

Protist OTU composition  
Sediment samples (2012)



Protist OTU composition  
Rock samples (2012)

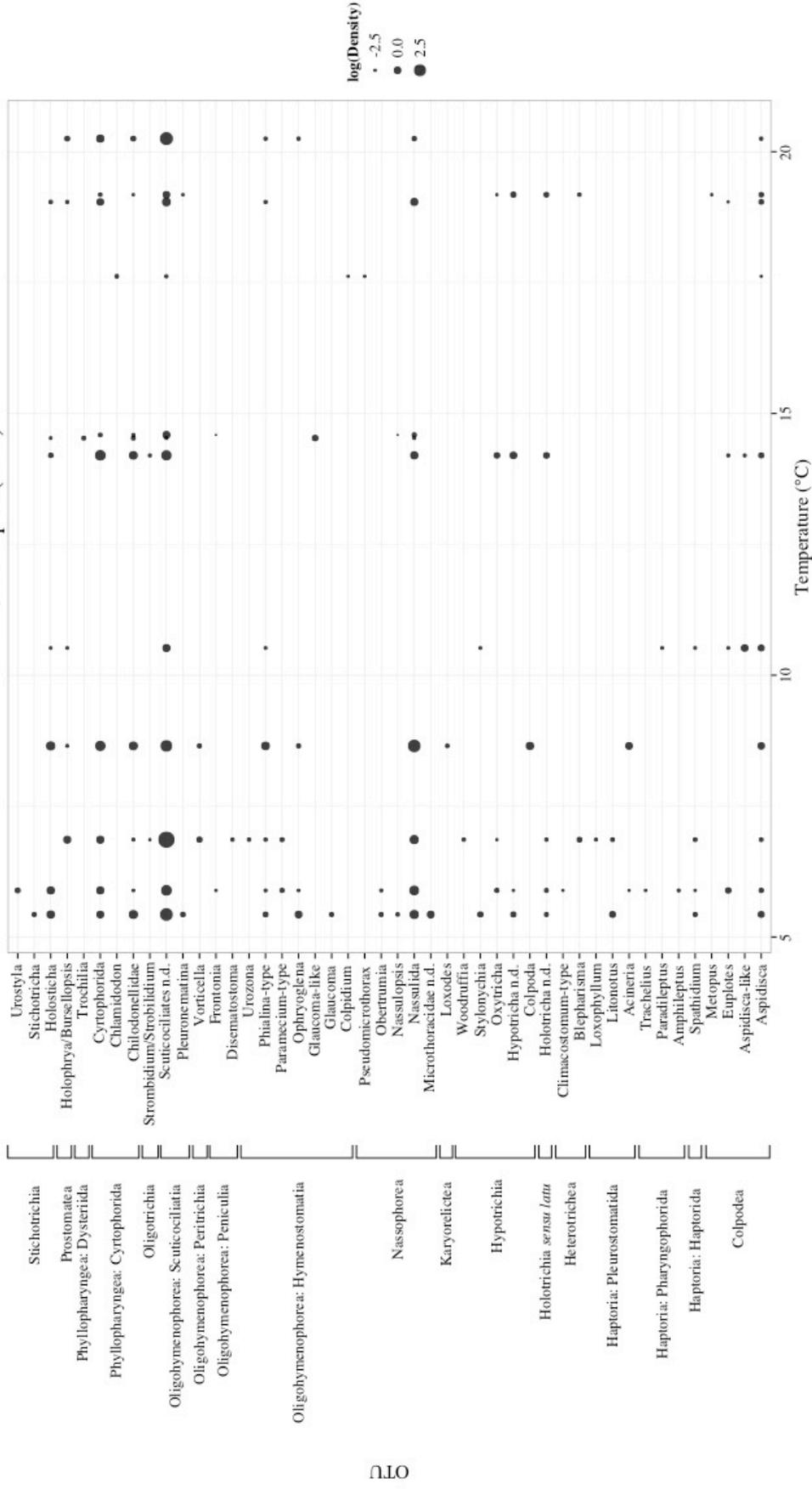


Figure S4 (page 8). Correlations between the pairwise temperature difference between streams ( $\Delta T$ , in °C) and the corresponding pairwise differences in beta diversity for ciliate assemblages found on rocky (panels a, c, e) and sandy substrates (panels b, d, f). On top of each panel, the corresponding outcome of a Mantel test is reported;  $r$  is based on Pearson's product-moment correlation and  $p$  is the corresponding  $p$ -value computed by bootstrapping over 999 permutations.

Figure S5 (page 9). Non-linear Multi Dimensional Scaling (nMDS) ordinations based on the differences in beta diversity for ciliate assemblages found on rocky (panels a, c, e) and sandy substrates (panels b, d, f). The isoclines represent a smooth surface obtained by fitting a two-dimensional GAM model having the nMDS ordinations as a response variable and the mean stream temperatures as explanatory variable. The only significant correlation is that between temperature and the nestedness component of beta diversity in rock assemblages (F test,  $p = 0.0003$ ;  $r^2 = 0.768$ ) (c).

