Science of The Total Environment
December 2024, Volume 955 Pages 177304 (12p.)
https://doi.org/10.1016/j.scitotenv.2024.177304
https://archimer.ifremer.fr/doc/00918/103021/

Could landscape ecology principles apply at the microscale? A metabarcoding approach on Trichoptera larvae-associated microbial diversity

Allen Joey [1, 2, *], Sire Marion [1], Belouard Nadège [1], Gorzerino Caroline [4], Coutellec Marie-Agnès [4], Mony Cendrine [1, 2], Pannard Alexandrine [1, 4], Piscart Christophe [1, 2]

[1] ECOBIO, CNRS, University of Rennes, UMR 6553, Rennes, France
[2] LTSER-FR Zone Atelier Armorique, France
[3] Centre de Recherche sur la Biodiversité et l'Environnement (CRBE), Université de Toulouse, CNRS, IRD, Toulouse INP, Université Toulouse 3 – Paul Sabatier (UT3), Toulouse, France
[4] DECOD (Ecosystem Dynamics and Sustainability), INRAE, IFREMER, L'Institut Agro, Rennes, France

* Corresponding author : Joey Allen, email address : joeyallen@orange.fr

Abstract :

Landscape heterogeneity is known as a major factor of community structure and composition. Whether this effect of the landscape extends at different scales and particularly at the relevant scale for microorganisms remained to be determined. We used the cases produced by aquatic larvae of Trichoptera, which assemble organic or mineral particles, as naturally replicated experimental systems representing structured substrates to determine the effect of landscape structuration on microbial communities. A metabarcoding approach was used to characterise fungal, bacterial and diatom communities on cases produced by six Trichoptera species and related unstructured organic and mineral substrates. The structuration of the particles constituting the cases was also determined as a measure of microscale landscape. Structured substrates harboured communities of diatoms, fungi and bacteria that differed from those found on unstructured substrates. Microbial communities also differed between organic and mineral substrates. We found a higher microbial diversity on structured substrates than on unstructured substrates. The heterogeneity of the microscale landscape also affected bacterial and fungal communities within cases. These results highlight the importance of microscale landscape structuration for microbial diversity and demonstrate that approaches of landscape ecology could be downscaled to the microscale.

Graphical abstract

Highlights

► The structuration of substrate by Trichoptera larvae affects microbial communities. ► Trichoptera case hosts higher microbial richness than the unstructured substrates. ► Fine scale heterogeneity of structured substrates promotes bacterial diversity. ► Microscale landscape heterogeneity promotes microbial richness. ► Trichoptera are ecosystem engineer that increase stream microbial diversity.

Keywords : Diatoms, Fungi, Bacteria, Stream microbial ecology, Landscape heterogeneity, Biodiversity

Introduction

Predicting community structure has been formalized using a large conceptual framework including biogeography (Macarthur and Wilson, 1967), niche theory (Hutchinson, 1957),

species selection through environmental filters (Lortie et al., 2004), and neutral theory (Hubbell, 2001). Community assembly is driven by stochastic processes and by deterministic environmental filters that select from a regional species pool, species able to survive and develop in a specific local habitat patch (Mittelbach and Schemske, 2015). Factors operating both at the local scale (abiotic stresses, disturbances, biotic interactions) and at the landscape scale (dispersal) shape species selection (Vellend, 2010). Application of these theories to the microbial world has been slow to develop. However, due to the short generation time and the small-distance dispersal of most microbes (Telford et al., 2006), the need for investigating the relevance of these concepts at scales smaller than those effective in macroorganisms has been highlighted (Bergmann and Leveau, 2022; Mony et al., 2020).

Microbes include an incredible biodiversity colonising all ecosystems worldwide (Marsland et al., 2020). The diversity and composition of microbial communities display a huge spatial heterogeneity recorded at the millimetric and centimetric scales (Besemer, 2016; Fierer, 2008; Li et al., 2023). Downscaling the landscape parameters considered may be necessary to understand this heterogeneity in microbial community. In streams, due to high water flow, microbial communities mainly develop as biofilms attached to any solid, stable surface (Stoodley et al., 2002). Biofilm is known as one of the most successful lifeforms (Stoodley et al., 2002), generating a complex ecosystem within the “microbial landscape” (Battin et al., 2007; Besemer, 2016, 2015). The microorganisms present in biofilms include protozoa, microalgae, archaea, bacteria and fungi, and contribute to all the main geochemical cycles in aquatic ecosystems (Battin et al., 2016). The ability of microorganisms to form biofilm is related to substrate properties, which influence its colonisation (Dang and Lovell, 2015; Kearns and Bärlocher, 2008; Laviale et al., 2019; Zheng et al., 2021), which is likely to result in deterministic assemblages. Microbial communities are likely to be controlled by a number of

other environmental factors influencing small-scale heterogeneity of microbial communities, which is still poorly understood.

Habitat heterogeneity over space has been recorded as impacting biodiversity, through changes in the composition of habitat patches (i.e. landscape element that forms part of or the entire species habitat), but also in terms of patch size and arrangement in space (i.e., landscape configuration) (Fahrig et al., 2011; Riera et al., 2023; Wiens, 1995). Biodiversity is expected to increase with habitat heterogeneity, due to the diversification of habitat types available for colonisation by different species. Furthermore, species dispersal within the landscape is favoured by the reduction of between-patch distances (Fahrig et al., 2011). Applying this rationale to small-scale studies, it is expected that substrate heterogeneity may influence biofilm composition and diversity. Considering that substrate particles would correspond to a given habitat type (i.e. set of environmental characteristics), substrate heterogeneity may be described by change in particle composition, but also by change in particle configuration (i.e. spatial arrangement of particles). Therefore, based on the landscape ecology framework, it is expected that biofilm diversity would increase with substrate heterogeneity in terms of composition and configuration.

Aquatic larvae of caddisflies (Insecta, Trichoptera) form cases that provide protection and camouflage (Frandsen et al., 2023), using mineral materials (sand, small gravel), plant materials (fragments of dead leaves and wood) or a mix of them, collected in the environment. These cases constitute specific microenvironments that offer specific conditions favourable to the development of microbial communities: physical stability in terms of hydraulic constraints and exposure to light, as well as local nutrient enrichment from excretion by the larvae within the case (Bergey and Resh, 1994). The preferred materials and the architecture of the case vary according to the species. Several dozens of trichopteran species are potentially present in a stream, resulting in a large variability of cases shape, size, structure and composition.

Trichoptera cases are thus naturally replicated "landscape" systems that differ from surrounding substrates in terms of composition and configuration of the physical environment.

The objective of this study is to test whether landscape ecology predictions apply to biofilms at a microscale. The regional species pool is constituted of the microbial community growing on all the different kinds of substrates present in the stream, including materials used by larvae to build their cases. Our overall hypothesis is that species assembly of microbial communities on Trichoptera cases is deterministic, *i.e.* there is a species selection from the regional pool due to the composition and configuration of trichopteran cases, and that heterogeneity of substrate configuration at the stream and at the case level promote microbial diversity. After characterizing the composition and structure of cases (thereafter referred to as structured substrates) from various trichopteran species sampled in a stream, we compared the biofilm communities developed on these structured substrates to those found on other substrates present in the same sampling sites that were not structured by Trichoptera larvae (leaves, sand, gravels, wood pieces). We expected differences in the composition of microbial communities between structured and unstructured substrates used by the different trichopteran species to produce cases. An effect of the type of substrate (i.e. organic or mineral) was also expected. If our hypotheses are verified, we would observe higher biodiversity on cases than on unstructured substrate particles. For similar reasons, we expected that differences in heterogeneity at the case scale would affect microbial communities, *i.e.* increased species richness with increased case structural heterogeneity.

Material and method

Sampling

Trichoptera larvae, leaf and twig litter, sand and pebbles were collected in a sandy bottom circumneutral stream (le Petit Hermitage stream 48°26'6''N 1°34'7''O) located in the forest of Villecartier (Brittany, N.-W France) in Spring 2022. Six different species of Trichoptera were sampled. Two of these build their cases with exclusively mineral material: *Sericostoma personatum* (n = 5) and *Athripsodes aterrimus* (n = 5), thereafter referred to as min1 and min2, three with exclusively organic material derived from plant litter: *Halesus radiatus* (large woody cases, n = 6), *Chaetopteryx villosa* (small wood and leaf litter-based cases, n = 6), and *Lepidostoma basale* (with cases made of leaf litter cut in small squares, n = 6), thereafter referred to as org1, org2 and org3, and one creates mixed organic-mineral cases with a mostly mineral central part to which are generally attached two wooden twigs longer than the mineral central part (*Anabolia nervosa*, thereafter referred to as mixed cases, n = 5). Larvae were extracted from the cases, and larvae and cases were fixed in 96% ethanol rapidly after collection. Additionally, other mineral (sand, gravels) and organic substrates (wood, leaves) were sampled at the same time (5 to 6 replicates each). They represent the raw materials used by Trichoptera larvae to build their cases, but without the structuration done by larvae. These substrates will thereafter be referred to as unstructured substrates. They also represent the most abundant substrates in the study area.

Substrate characterisation

All the samples (cases and unstructured substrates) were photographed using a Leica M205 C stereomicroscope (Leica microsystems, Wetzlar, Germany) before the DNA extraction step. Pictures were then used to measure various structure parameters with ImageJ (Schneider et al., 2012). We measured classical landscape metrics transposed to this case study. Composition heterogeneity was assessed through substrate roughness. Case width was measured every millimetre along the largest dimension. These measures were used to calculate roughness, based

on the root-mean-square method (Huber et al., 2007; Jacobs et al., 2017). Roughness values close to 0 are indicative of smooth cases, while they increase with increasing three-dimensional heterogeneity. Heterogeneity of case configuration was assessed through an aggregation index, which analyses the degree of intermixing of different classes of particles. We measured each individual particle found along a line from the anterior to the posterior end of the case. Then, particles were pooled into seven size classes according to their visual distribution in the whole dataset and we calculated a juxtaposition index (JI) (Heinen and Cross, 1983) corresponding the number of successive changes of particle class from anterior to posterior end of each case divided by the total number of particles measured. The value of JI varies from 0 when all particles are of similar size class to 1 when all particles differ from the adjacent one. The coefficient of variation of particle size within case was also calculated from these measures. The total surface of the cases was also measured.

Microbial community characterisation

The microbial community associated with each sample (structured and unstructured substrates) was characterised using a metabarcoding approach. For DNA extraction on cases, only one half of each sample (cut in half following the median plane) was used, except for min2 (*A. aterrimus*) cases, which were too small. For the mixed cases, the mineral part and the organic part were separated and treated as different samples (thereafter referred as mixed-min and mixed-org). DNA was extracted using the NucleoSpin® Tissue purification kit (Macherey-Nagel, Düren, Germany), with an additional mechanical lysis step using a steel ball and TissueLyzer for 5 minutes at 30 Hz (Ferreira et al., 2020) before adding the first extraction buffer.

Three separate PCRs were performed on each extract. The first PCR targeted the rbcL plastid gene, which is specific of photosynthetic organisms, using a primer pair optimized for diatoms

(Tapolczai et al., 2019) based on previous observations that diatoms largely dominated phototroph community in this stream, as in most headwater streams (Allen et al., 2024). The second PCR targeted the ITS, to amplify the DNA of non-photosynthetic eukaryotic organisms, particularly fungi (Gardes and Bruns, 1993). The third PCR targeted the V4 and V5 regions of the 16S gene for prokaryotic organisms. All primer sequences are available in Table S1.

PCRs were performed in 25 µL of reaction mixture containing 12.5 µL of 2x Multiplex PCRMaster Mix (Qiagen®,Venlo, Netherlands), 9.5 µL of pure water, 0.5 µL of forward and reverse primers at 0.2 µM, and 2 µL of DNA extract. PCR conditions included an initial heat activation at 95 °C for 15 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 57 °C for 90 s and extension at 72 °C for 90 s, and a final extension step at 72 °C for 10 min. The quality of the amplifications was checked by gel electrophoresis using a 1.5% agarose gel made from a 1:1 mixture of standard and low-melting agarose. Amplification failed for some DNA extracts (corresponding to abnormally brown DNA extracts). For those samples (one leaf sample, two org1 and one org2), a 1:10 dilution of the DNA extract was used to obtain a correct amplification. A negative control was prepared by carrying out all the extraction and PCR procedures without the biological sample.

Amplicon sequencing was performed on the 180 samples (60 samples * 3 PCR) on an Illumina MiSeq in 2x250 bp, in paired-ended reads using V3 chemistry on the EcogenO platform (https://osur.univ-rennes.fr/EcogenOENG OSUR, Rennes, France).

DADA2 (Callahan et al., 2016) was used for the bioinformatic treatment of the demultiplexed MiSeq reads. Primer sequence removal was done using Cutadapt 4.3 (Martin, 2011). DNA reads were filtered for length and quality with the following parameters: Q score ≥ 2, a minimal length equal to 50. Sequences were truncated to 220 bp for rbcL. The maximum number of expected errors was set to 2 for forward and reverse reads. Given the large size of the dataset,

a process of pseudo-pooling was applied to samples in order to ensure rare variant resolution while keeping computation time reasonable (see DADA2 documentation for details). For the merging of forward and reverse reads, the minimum overlap was set to 12 bp and the maximum number of mismatches to 1. Chimeras and singletons were also removed from the dataset. The taxonomic assignment of Amplicon Sequence Variants (ASV) was performed automatically. The R package "Diat.barcode" (v.11.1 published on 25-05-2022) was used for rbcL (Rimet et al., 2019), Silva v138 (McLaren, 2020) for 16S, and Unite database (Abarenkov et al., 2022) for ITS. Analyses of microbial diversity were performed using the ASV contingency table (ASV frequency across samples). The correlation between the ASV richness and the species richness based on the taxonomic assignation of the ASV was checked for all three markers, considering the strong correlation between measurements made on an ASV or taxonomic basis, only results based on ASV are presented. For all statistical analyses, ASVs that totalise 5 reads or less across all samples were discarded to avoid false-positives. A rarefied ASV table was computed to account for differences in the number of reads per sample using the rarefy_even_depth function of the phyloseq R package (McMurdie and Holmes, 2013). For each marker, samples with fewer reads than the extraction blank were excluded as they might represent failure in the extraction or amplification step rather than true absence (3 samples were discarded for rbcL and ITS, 4 for 16S). The rarefaction depth was set as the minimum number of reads for the remaining samples (612, 1404 and 756 for rbcL, ITS and 16S respectively).

Statistical analysis

Statistical analyses were conducted with R version 4.2.3 (R Core Team, 2023). To test the first hypothesis (*i.e.*, that the substrate heterogeneity of structured substrates favours microbial biodiversity compared with unstructured substrates), we analysed the effect of substrate structure (structured versus unstructured), considering also substrate type (mineral or organic),

on microbial community composition and richness. The effect of structure (structured or unstructured) and substrate type (mineral or organic) on ASV richness was determined independently for the data obtained with each barcode using two-way ANOVAs after controlling for residuals normality and homoscedasticity. ASV richness was compared between structured and unstructured substrates, based on ASV rarefaction curves drawn with the iNEXT R package (Hsieh et al., 2022).

To identify changes in community composition between different substrate types, we used non-metric multidimensional scaling (NMDS) on presence/absence datasets using Jaccard's dissimilarity index as the distance metric. To identify the significance of these differences, a PERMANOVA was performed using the vegan package (Oksanen et al., 2022).

To evaluate the contribution of neutral processes to community composition, community data were fitted on a Sloan neutral community model (Burns et al., 2016; Sloan et al., 2006). This model determines the relationship between the total number of reads of an ASV and its frequency across all samples and estimate the rate of migration from source community to local community. The model was constructed on a pool of all samples (structured and unstructured substrates). Observed ASV frequencies were compared to those predicted based on the neutral model. ASV were considered neutral when their frequency in structured substrates samples was within the 95% confidence interval of the neutral model. Over-represented and under-represented ASVs were determined as those present at a frequency higher or lower than the 95% confidence interval of the frequency predicted by the model. The proportion of deterministic processes was assessed as the proportion of non-neutral ASV. Models were well fitted for bacteria and diatoms but not for fungi. Normalised stochasticity ratio were computed following Ning et al. (2019). A multiblock sparse partial least square discriminant analysis (sPLS-DA) was used to identify the microbial groups specifically associated with structured and unstructured substrates (Singh et al., 2019).

We used *SourceTracker* (Knights et al., 2011) to identify the contribution of the different possible sources (within the unstructured substrates) of microbial communities on the cases.

To test the second hypothesis (i.e. that increased case heterogeneity favours microbial diversity), we analysed the effect of case heterogeneity (roughness, variation of particle size and JI) on the composition and ASV richness of diatoms, fungi and bacteria. To determine the effect of heterogeneity on community composition, we used a distance-based redundancy analysis (dbRDA; Legendre and Anderson, 1999) using Jaccard distances for the three microbial communities and the measured descriptors of case heterogeneity (roughness, variation of particle size and JI), case surface area and case substrate type, to construct our dbRDA model. An ANOVA was used to test the significance of the different terms included in the model. To test the effect of the same factors on ASV richness, we used a multiple linear regression approach. Best models were selected based on the Akaike Information Criterion and the genetic algorithm method using the glmulti package (Calcagno and Mazancourt, 2010).

Results

Description of the microbial communities in structured and unstructured substrate

The sequencing of DNA samples amplified with the RbcL primers generated 2,041,407 reads after quality filtering and chimera/singleton removal, which were distributed into 546 distinct ASVs. Of these, 529 ASVs were found to belong to 39 different genera of diatoms (fig1). ITS-amplified DNA samples produced 3857 ASVs, as obtained from 2,389,650 filtered reads. Among these ASVs, the dominant phyla were Ascomycota (37%) and Basidiomycota (14%) respectively (fig1). The sequencing of DNA samples amplified with 16S primers generated 1,997,863 filtered reads assigned to 13,436 distinct ASVs, principally representative of the Proteobacteria and Bacteroidota phyla (fig 1). For simplicity, the different communities

investigated using RbcL, ITS and 16S primers will thereafter be referred to as diatom, fungi and bacterial communities.

Comparison of microbial communities on structured and unstructured substrates

Through NMDS ordination, we demonstrated that communities differed between structured and unstructured substrates, and substrate type for bacterial, fungal and diatoms communities. For bacterial and fungal communities, we detected an additional interactive effect between both factors (fig 2; table 1).

The proportion of deterministic (non-neutral) processes in community composition was estimated by the quantification of the ASV that did not follow a neutral community model (table 1, fig S1). This approach also allowed us to determine the migration rate (e.g. the probability that a place in the local community is taken by an individual immigrating from source community). We detected 11.3% of bacterial ASVs and 16.7% of diatom ASVs on structured substrates that were present more frequently or less frequently than predicted by the neutral model. The diatom genera *Nitzschia*, *Navicula*, *Planothidium* and *Fragilaria* had a high proportion of over-represented ASVs (supplementary table S2). For bacteria, high proportion of ASVs belonging to the Rhodobacterales and Acetobacterales orders were overrepresented on structured substrates (supplementary table S4 Migration rates for diatoms and bacteria were 0.41 and 0.51 respectively.). The multiblock sPLS-DA performed on the three microbial communities indicated Staurosira, Pinnularia and Caloneis as diatom genus associated with structured substrates and strongly co-occuring with bacteria from the Phormidiaceae, Bryobacteraceae and Spirochaetaceae family (fig 3).

For diatoms, most of the community originated from organic unstructured substrates for all types of cases (fig 4). Gravels were detected as the major and nearly unique source of the fungal communities for min1 and min2, while leaf and woody litter were the major sources for org1,

org2 and org3 (fig 4). For bacteria, 50% to 80% of the community originated from mineral unstructured substrate, mainly the gravels for the two exclusively mineral cases (min1 and min2). The bacterial community of mixed and organic structured substrates originated from both mineral and organic unstructured substrates (fig 4).

Trichoptera cases significantly promoted ASV richness for diatoms, fungi and bacteria compared with unstructured substrates (fig 5, fig 6). Mineral substrates promoted fungal richness, but no effect of substrate type was observed for diatoms or bacteria. Similar results were observed when considering species or genus richness based on sequence annotations (fig 5). The richness of fungal community was higher on mineral substrates compared to organic ones (fig 5). Rarefaction curves indicate that a higher number of different ASVs are found on structured substrates than on unstructured substrates for a similar number of samples for diatoms and fungi (fig 6).

In diatoms, the structured substrates sample rarefaction curve presents a steeper slope than that of unstructured substrates, indicating a higher diversity within and among samples collected on trichopteran cases (fig 6). The extrapolations of the rarefaction curves indicate that for a higher number of samples the richness of unstructured substrates and cases may have converged to around 200 ASVs. This indicates that there are no diatom species or ASV specific to Trichoptera cases but that these structured substrates support a high number of ASVs from the local pool (fig 6). For the fungal and bacterial diversity, the rarefaction curves did not reach a stable state, indicating that our sampling did not cover all fungal and bacterial ASV richness. With respect to fungal diversity, structured substrates harboured both a steeper curve and a higher number of ASVs when extrapolating the curves for a higher number of samples, indicating that a part of the fungal diversity was specific to the environment created by the cases. For the bacterial diversity, nearly no difference appears in the slope, but the extrapolation

of the rarefaction curves indicates a higher richness on structured substrates than on unstructured substrates (fig 6).

Influence of spatial heterogeneity within structured substrates

Structured substrates spatial heterogeneity influenced the composition and richness microbial communities associated with cases. More especially, the case heterogeneity of composition (i.e. particle roughness) influenced both fungal and bacterial communities but not diatoms, while the heterogeneity of configuration (JI and variation of particle size) influenced only bacterial communities. Structured substrates type affected community composition for all three groups of microorganisms while the size of the case had a significant effect only for bacterial communities (fig 7).

Bacterial ASV richness was affected positively by two of the variables used to characterise the heterogeneity of structured substrates: roughness and JI. Bacterial richness was also affected positively by case size (table 3). Fungal richness was found to decrease with increasing case size. Heterogeneity affected only marginally fungal richness by the interaction between case size and JI (table 3).

Discussion

Structured substrates harbour different microbial communities and higher diversity than unstructured substrates

Our first hypothesis was that structured substrates microbial communities were deterministic compared to the unstructured substrate. Indeed, by associating substrates in a stable conformation Trichoptera larvae create a stable microscale landscape that offers particular ecological niches for microbes. Our results confirmed this hypothesis as we found marked

significant compositional differences between structured and unstructured substrates for bacterial, fungal and diatoms communities (fig 2, table 1). The comparison with a neutral community model confirmed that the observed differences are not due only to neutral processes but results from deterministic processes. Diatom taxa occurring more frequently on structured than on unstructured substrates are diverse, including large motile diatoms (*Nitzschia* sp., *Navicula* sp.) and small adnate diatoms (*Planothidium* sp.) (Rimet and Bouchez, 2012). For bacteria, we noticed phototrophic species-containing groups such as *Rhodobacter* sp., *Rhodoferax* sp. and *Rhodomicrobium* sp. among the bacterial ASV more frequent on the cases. This result reinforces a previous study that highlights that net-spinning Trichoptera create different microenvironments hosting different microbial communities (Bertagnolli et al., 2023). More generally, several studies have shown that aquatic macroorganisms bear host-specific microbial communities (Chiarello et al., 2020; Falasco et al., 2018; Receveur et al., 2020). This host-specificity is generally attributed to direct interaction between the host and its microbiome through the secretion of a mucus containing diverse compounds that may affect microbial community (notably antimicrobial compounds) and immune cells (Esteban, 2012). In the present study, we focused on microbial community associated with Trichoptera cases. This approach limits the direct effect of the animal compared to study where skin or internal microbial community is used, while animals' behaviour may still affect the microbial community. Considering that a sand grain or a piece of leaf litter would have similar physical and chemical properties whether they are part of a case or not, the observed differences in microbial community are more likely a direct effect of microscale landscape through substrate spatial structuration.

Such host-specificity was shown here through the deterministic proportion of microbes that was associated with cases. Further analysis could investigate the relationships between Trichoptera species or larvae phenological development on microbial communities associated with cases

and analyse the underlying mechanisms of this specificity. We also expected higher microbial diversity on structured substrates than on the unstructured substrates, because cases represent areas with stable exposure to light and water current compared to sand and litter. This expectation was verified for the three groups of microorganisms studied (fig 5, 6). While these substrates are regularly remobilised by the current, Trichoptera larvae activity and case structure stabilize the case environmental conditions (Otto and Johansson, 1995; Statzner and Holm, 1989), and therefore offer a more stable habitat to microorganisms.

The nature of the substrate also affects microbial communities

Our first hypothesis assumed that the nature of the substrate may affect its colonisation by microorganisms. In our results we observed, as expected, an important effect of organic versus mineral substrates on microbial community composition (fig 2, table 1) for all taxonomic groups. Indeed, the surface properties, that may strongly differ between mineral and organic substrates, affect diatoms, fungi and bacteria adhesion and colonization abilities (Kearns and Bärlocher, 2008; Laviale et al., 2019; Rosenhahn et al., 2010) and organic substrate may also be used as a carbon and nutrient source by some organisms. Substrate type influenced also richness but only for fungi (fig 5). The observed lower richness of fungi on organic substrate may be explained by the abundance of decomposers on organic substrates that can exclude other fungal species (Ferreira et al., 2010). Migration from the source community contribute around 40-50% to the community at the surface of the cases and the assemblages are mostly but not uniquely driven by stochasticity. Microbial assemblages colonizing cases are originated from the dominant substrates in the streams, but we demonstrated that species of other substrate types colonized cases and contributed to the microbial assemblages: for instance, diatom communities associated with mineral structured substrates are recruited from organic

unstructured substrates, while bacterial communities associated with organic structured substrates are originated partly from mineral unstructured substrates (Fig 4).

Microscale landscape heterogeneity affects microbial diversity

To analyse the relationship between microscale substrate heterogeneity and microbial diversity, we tested among the different structured substrate types the extent to which the spatial heterogeneity of composition and configurations affected microbial community composition and richness. Heterogeneity contributed to explaining differences in community composition for fungi and bacteria (fig 7). However, the expected heterogeneity-diversity relationship was found only for bacterial community (table 3). More especially, fungi responded to only compositional heterogeneity (case roughness), while both compositional and configurational heterogeneity influenced bacterial communities (high turnover between particle size, high coefficient of variation in size). Diatoms were not influenced by small-scale heterogeneity, likely because diatoms are large and motile they are less affected by factors at the scale considered.

The size of the case was also found to affect ASV composition in bacteria, and ASV richness in bacteria and fungi, with opposite effects between fungi and bacteria (table 3). Biofilm microbial communities are hotspots of interactions that occurs notably between diatoms, fungi and bacteria (e.g. Allen et al., 2017; Zancarini et al., 2017). These interactions may have been interfering with the effect of the microscale landscape parameters (*e.g*. effect on fungi, possibly not due directly to microscale landscape but mediated by competition with bacteria). Considering these interactions may be key in the downscaling of landscape ecology at the microbial scale. However, cases represent a complex structure with a highly fractal structuration, and many parameters should be considered to completely characterise these

structures as it can be done to characterise the heterogeneity of macroscale landscapes (Riera et al., 2023).

Conclusion

Landscape heterogeneity is known to promote diversity at the macroscale. We expected to find the same relationship for stream microbial communities. In this study, this question was explored at two levels: at the stream level we found that Trichoptera larvae, creating heterogeneity by the simple structuration of substrates already present in the stream, change microbial community and promote diversity of diatoms, fungi and bacteria. At the case level, we found some insights that heterogeneity of structured substrates in terms of configuration affects bacterial and fungal communities and promotes bacterial diversity.

Overall, our results show that by creating diverse structurally complex heterogeneous microscale landscapes, Trichoptera larvae act as engineer species and increase microbial diversity in streams. This suggests a positive relationship between diversity of Trichoptera larvae and microbial diversity. The decline in the diversity of benthic macroinvertebrates in streams is therefore likely to lead to an invisible decline in the diversity of microorganisms.

Acknowledgements: Funding was provided by the LTSER "Zone atelier armorique" and the "Observatoire des Sciences de l'Environnement de Rennes" (OSERennes). We thank L. Favier for technical help for the sampling and L. Pellan for help with the identification of the larvae. We are thankful to the molecular ecology platform of Ecobio laboratory and particularly V. Daburon and C. Roose-Amsaleg for help with DNA extraction. We acknowledge R. Causse-Védrines and the EcoGeno platform that performed the sequencing.

Data availability statement: All data and R code to replicate the analyses and to reproduce the figures are deposited on a public database https://doi.org/10.48579/PRO/HDWUK8.

References

Abarenkov, K., Zirk, A., Piirmann, T., Pöhönen, R., Ivanov, F., Nilsson, R.H., Kõljalg, U., 2022. UNITE general FASTA release for eukaryotes. Version 16.10.2022. https://doi.org/10.15156/BIO/2483913

Allen, J., Danger, M., Wetzel, C.E., Felten, V., Laviale, M., 2024. Diatom Primary Production in Headwater Streams, in: Diatom Photosynthesis. John Wiley & Sons, Ltd, pp. 327–349. https://doi.org/10.1002/9781119842156.ch11

Allen, J.L., Leflaive, J., Bringuier, C., Ten-Hage, L., Chauvet, E., Cornut, J., Danger, M., 2017. Allelopathic inhibition of primary producer growth and photosynthesis by aquatic fungi. Fungal ecology 29, 133–138.

Battin, T.J., Besemer, K., Bengtsson, M.M., Romani, A.M., Packmann, A.I., 2016. The ecology and biogeochemistry of stream biofilms. Nature Reviews Microbiology 14, 251–263.

Battin, T.J., Sloan, W.T., Kjelleberg, S., Daims, H., Head, I.M., Curtis, T.P., Eberl, L., 2007. Microbial landscapes: new paths to biofilm research. Nat Rev Microbiol 5, 76–81. https://doi.org/10.1038/nrmicro1556

Bergey, E.A., Resh, V.H., 1994. Interactions between a stream caddisfly and the algae on its case: factors affecting algal quantity. Freshwater Biology 31, 153–163. https://doi.org/10.1111/j.1365-2427.1994.tb00849.x

Bergmann, G.E., Leveau, J.H.J., 2022. A metacommunity ecology approach to understanding microbial community assembly in developing plant seeds. Front. Microbiol. 13. https://doi.org/10.3389/fmicb.2022.877519

Bertagnolli, A.D., Maritan, A.J., Tumolo, B.B., Fritz, S.F., Oakland, H.C., Mohr, E.J., Poole, G.C., Albertson, L.K., Stewart, F.J., 2023. Net-spinning caddisflies create denitrifier-enriched niches in the stream microbiome. ISME COMMUN. 3, 1–6. https://doi.org/10.1038/s43705-023-00315-8

Besemer, K., 2016. Microbial biodiversity in natural biofilms. Aquatic biofilms: ecology, water quality and wastewater treatment 63–88.

Besemer, K., 2015. Biodiversity, community structure and function of biofilms in stream ecosystems. Research in microbiology 166, 774–781.

Burns, A.R., Stephens, W.Z., Stagaman, K., Wong, S., Rawls, J.F., Guillemin, K., Bohannan, B.J., 2016. Contribution of neutral processes to the assembly of gut microbial communities in the zebrafish over host development. ISME J 10, 655–664. https://doi.org/10.1038/ismej.2015.142

Calcagno, V., Mazancourt, C. de, 2010. glmulti: An R Package for Easy Automated Model Selection with (Generalized) Linear Models. Journal of Statistical Software 34, 1–29. https://doi.org/10.18637/jss.v034.i12

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2 : High-resolution sample inference from Illumina amplicon data. Nature Methods 13, 581–583. https://doi.org/10.1038/nmeth.3869

Chiarello, M., Auguet, J.-C., Graham, N.A.J., Claverie, T., Sucré, E., Bouvier, C., Rieuvilleneuve, F., Restrepo-Ortiz, C.X., Bettarel, Y., Villéger, S., Bouvier, T., 2020. Exceptional but vulnerable microbial diversity in coral reef animal surface microbiomes. Proceedings of the Royal Society B: Biological Sciences 287, 20200642. https://doi.org/10.1098/rspb.2020.0642

Dang, H., Lovell, C.R., 2015. Microbial Surface Colonization and Biofilm Development in Marine Environments. Microbiology and Molecular Biology Reviews 80, 91–138. https://doi.org/10.1128/mmbr.00037-15

Esteban, M.Á., 2012. An Overview of the Immunological Defenses in Fish Skin. International Scholarly Research Notices 2012, 853470. https://doi.org/10.5402/2012/853470

Fahrig, L., Baudry, J., Brotons, L., Burel, F.G., Crist, T.O., Fuller, R.J., Sirami, C., Siriwardena, G.M., Martin, J.-L., 2011. Functional landscape heterogeneity and animal biodiversity in agricultural landscapes. Ecology Letters 14, 101–112. https://doi.org/10.1111/j.1461-0248.2010.01559.x

Falasco, E., Bo, T., Ghia, D., Gruppuso, L., Bona, F., Fenoglio, S., 2018. Diatoms prefer strangers: non-indigenous crayfish host completely different epizoic algal diatom communities from sympatric native species. Biol Invasions 20, 2767–2776. https://doi.org/10.1007/s10530-018-1728-x

Ferreira, S., Ashby, R., Jeunen, G.-J., Rutherford, K., Collins, C., Todd, E.V., Gemmell, N.J., 2020. DNA from mollusc shell: a valuable and underutilised substrate for genetic analyses. PeerJ 8, e9420. https://doi.org/10.7717/peerj.9420

Ferreira, V., Gonçalves, A.L., Pratas, J., Canhoto, C., 2010. Contamination by uranium mine drainages affects fungal growth and interactions between fungal species and strains. Mycologia 102, 1004–1011. https://doi.org/10.3852/09-248

Fierer, N., 2008. Microbial Biogeography: Patterns in Microbial Diversity across Space and Time, in: Accessing Uncultivated Microorganisms. John Wiley & Sons, Ltd, pp. 95–115. https://doi.org/10.1128/9781555815509.ch6

Frandsen, P.B., Holzenthal, R.W., Espeland, M., Breinholt, J., Thomas, J.A., Simon, S., Kawahara, A.Y., Plotkin, D., Hotaling, S., Li, Y., Nelson, C.R., Niehuis, O., Mayer, C., Podsiadlowski, L., Donath, A., Misof, B., Lemmon, E.M., Lemmon, A., Morse, J.C., Pauls, S., Zhou, X., 2023. Phylogenomics recovers multiple origins of portable case-making in caddisflies (Insecta: Trichoptera), the world's most common underwater architects. https://doi.org/10.1101/2023.12.21.572910

Gardes, M., Bruns, T.D., 1993. ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. Molecular Ecology 2, 113–118. https://doi.org/10.1111/j.1365-294X.1993.tb00005.x

Heinen, J., Cross, G.H., 1983. An Approach to Measure Interspersion, Juxtaposition, and Spatial Diversity from Cover-Type Maps. Wildlife Society Bulletin (1973-2006) 11, 232–237.

Hsieh, T.C., Ma, K.H., Chao, A., 2022. iNEXT: iNterpolation and EXTrapolation for species diversity [WWW Document]. URL http://chao.stat.nthu.edu.tw/wordpress/software-download/

Hubbell, S.P., 2001. The Unified Neutral Theory of Biodiversity and Biogeography. Princeton University Press.

Huber, G., Gorb, S., Hosoda, N., Spolenak, R., Arzt, E., 2007. Influence of surface roughness on gecko adhesion. Acta Biomaterialia 3, 607–610. https://doi.org/10.1016/j.actbio.2007.01.007

Hutchinson, G.E., 1957. Concluding Remarks. Cold Spring Harb Symp Quant Biol 22, 415–427. https://doi.org/10.1101/SQB.1957.022.01.039

Jacobs, T.D.B., Junge, T., Pastewka, L., 2017. Quantitative characterization of surface topography using spectral analysis. Surface Topography: Metrology and Properties 5, 013001. https://doi.org/10.1088/2051-672X/aa51f8

Kearns, S.G., Bärlocher, F., 2008. Leaf surface roughness influences colonization success of aquatic hyphomycete conidia. Fungal Ecology 1, 13–18. https://doi.org/10.1016/j.funeco.2007.07.001

Knights, D., Kuczynski, J., Charlson, E.S., Zaneveld, J., Mozer, M.C., Collman, R.G., Bushman, F.D., Knight, R., Kelley, S.T., 2011. Bayesian community-wide culture-independent microbial source tracking. Nat Methods 8, 761–763. https://doi.org/10.1038/nmeth.1650

Laviale, M., Beaussart, A., Allen, J., Quilès, F., El-Kirat-Chatel, S., 2019. Probing the Adhesion of the Common Freshwater Diatom Nitzschia palea at Nanoscale. ACS Appl. Mater. Interfaces 11, 48574–48582. https://doi.org/10.1021/acsami.9b17821

Legendre, P., Anderson, M.J., 1999. Distance-Based Redundancy Analysis: Testing Multispecies Responses in Multifactorial Ecological Experiments. Ecological Monographs 69, 1–24. https://doi.org/10.1890/0012-9615(1999)069[0001:DBRATM]2.0.CO;2

Li, Y., Ma, K., Song, W., Zhou, J., Liu, X., Wang, M., Tu, Q., 2023. Environmental heterogeneity and dispersal limitation simultaneously determine the spatial scaling of different microbial

functional groups. Science of The Total Environment 885, 163854. https://doi.org/10.1016/j.scitotenv.2023.163854
Lortie, C.J., Brooker, R.W., Choler, P., Kikvidze, Z., Michalet, R., Pugnaire, F.I., Callaway, R.M., 2004. Rethinking plant community theory. Oikos 107, 433–438. https://doi.org/10.1111/j.0030-1299.2004.13250.x
Macarthur, R.H., Wilson, E.O., 1967. The Theory of Island Biogeography, REV-Revised. ed. Princeton University Press.
Marsland, R., Cui, W., Mehta, P., 2020. A minimal model for microbial biodiversity can reproduce experimentally observed ecological patterns. Sci Rep 10, 3308. https://doi.org/10.1038/s41598-020-60130-2
Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.journal 17, 10–12. https://doi.org/10.14806/ej.17.1.200
McLaren, M., 2020. Silva SSU taxonomic training data formatted for DADA2 (Silva version 138) (Version 2). https://doi.org/10.5281/zenodo.3986799
McMurdie, P.J., Holmes, S., 2013. phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. PLOS ONE 8, e61217. https://doi.org/10.1371/journal.pone.0061217
Mittelbach, G.G., Schemske, D.W., 2015. Ecological and evolutionary perspectives on community assembly. Trends in Ecology & Evolution 30, 241–247. https://doi.org/10.1016/j.tree.2015.02.008
Mony, C., Vandenkoornhuyse, P., Bohannan, B.J.M., Peay, K., Leibold, M.A., 2020. A Landscape of Opportunities for Microbial Ecology Research. Frontiers in Microbiology 11.
Ning, D., Deng, Y., Tiedje, J.M., Zhou, J., 2019. A general framework for quantitatively assessing ecological stochasticity. Proceedings of the National Academy of Sciences 116, 16892–16898. https://doi.org/10.1073/pnas.1904623116
Oksanen, J., Simpson, G.L., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., Caceres, M.D., Durand, S., Evangelista, H.B.A., FitzJohn, R., Michael Friendly, Brendan Furneaux, Hannigan, G., Hill, M.O., Lahti, L., McGlinn, D., Ouellette, M.-H., Cunha, E.R., Tyler Smith, Stier, A., Braak, C.J.F.T., Weedon, J., 2022. vegan: Community Ecology Package [WWW Document]. URL https://CRAN.R-project.org/package=vegan
Otto, C., Johansson, A., 1995. Why do some caddis larvae in running waters construct heavy, bulky cases? Animal Behaviour 49, 473–478. https://doi.org/10.1006/anbe.1995.0061
R Core Team, 2023. R: A Language and Environment for Statistical Computing.
Receveur, J.P., Fenoglio, S., Benbow, M.E., 2020. Insect-associated bacterial communities in an alpine stream. Hydrobiologia 847, 331–344. https://doi.org/10.1007/s10750-019-04097-w
Riera, E., Mauroy, B., Francour, P., Hubas, C., 2023. Unleashing the Potential of Artificial Reefs Design: A Purpose-Driven Evaluation of Structural Complexity.
Rimet, F., Bouchez, A., 2012. Life-forms, cell-sizes and ecological guilds of diatoms in European rivers. Knowl. Managt. Aquatic Ecosyst. 01. https://doi.org/10.1051/kmae/2012018
Rimet, F., Gusev, E., Kahlert, M., Kelly, M.G., Kulikovskiy, M., Maltsev, Y., Mann, D.G., Pfannkuchen, M., Trobajo, R., Vasselon, V., Zimmermann, J., Bouchez, A., 2019. Diat.barcode, an open-access curated barcode library for diatoms. Sci Rep 9, 15116. https://doi.org/10.1038/s41598-019-51500-6
Rosenhahn, A., Schilp, S., Jürgen Kreuzer, H., Grunze, M., 2010. The role of "inert" surface chemistry in marine biofouling prevention. Physical Chemistry Chemical Physics 12, 4275–4286. https://doi.org/10.1039/C001968M
Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. Nat Methods 9, 671–675. https://doi.org/10.1038/nmeth.2089

Singh, A., Shannon, C.P., Gautier, B., Rohart, F., Vacher, M., Tebbutt, S.J., Lê Cao, K.-A., 2019. DIABLO: an integrative approach for identifying key molecular drivers from multi-omics assays. Bioinformatics 35, 3055–3062. https://doi.org/10.1093/bioinformatics/bty1054

Sloan, W.T., Lunn, M., Woodcock, S., Head, I.M., Nee, S., Curtis, T.P., 2006. Quantifying the roles of immigration and chance in shaping prokaryote community structure. Environmental Microbiology 8, 732–740. https://doi.org/10.1111/j.1462-2920.2005.00956.x

Statzner, B., Holm, T.F., 1989. Morphological adaptation of shape to flow: Microcurrents around lotic macroinvertebrates with known Reynolds numbers at quasi-natural flow conditions. Oecologia 78, 145–157. https://doi.org/10.1007/BF00377150

Stoodley, P., Sauer, K., Davies, D.G., Costerton, J.W., 2002. Biofilms as complex differentiated communities. Annu Rev Microbiol 56, 187–209. https://doi.org/10.1146/annurev.micro.56.012302.160705

Tapolczai, K., Keck, F., Bouchez, A., Rimet, F., Kahlert, M., Vasselon, V., 2019. Diatom DNA Metabarcoding for Biomonitoring: Strategies to Avoid Major Taxonomical and Bioinformatical Biases Limiting Molecular Indices Capacities. Frontiers in Ecology and Evolution 7.

Telford, R.J., Vandvik, V., Birks, H.J.B., 2006. Dispersal Limitations Matter for Microbial Morphospecies. Science 312, 1015–1015. https://doi.org/10.1126/science.1125669

Vellend, M., 2010. Conceptual Synthesis in Community Ecology. The Quarterly Review of Biology 85, 183–206. https://doi.org/10.1086/652373

Wiens, J.A., 1995. Habitat fragmentation: island v landscape perspectives on bird conservation. Ibis 137, S97–S104. https://doi.org/10.1111/j.1474-919X.1995.tb08464.x

Zancarini, A., Echenique-Subiabre, I., Debroas, D., Taïb, N., Quiblier, C., Humbert, J.-F., 2017. Deciphering biodiversity and interactions between bacteria and microeukaryotes within epilithic biofilms from the Loue River, France. Sci Rep 7, 4344. https://doi.org/10.1038/s41598-017-04016-w

Zheng, S., Bawazir, M., Dhall, A., Kim, H.-E., He, L., Heo, J., Hwang, G., 2021. Implication of Surface Properties, Bacterial Motility, and Hydrodynamic Conditions on Bacterial Surface Sensing and Their Initial Adhesion. Frontiers in Bioengineering and Biotechnology 9.

Tables

Table 1: Results of the PERMANOVA on the microbial communities

	F	p-value	
Diatoms			
Substrat (organic-mineral)	2.5351	0.007	**
Structure (case-unstructured)	2.9545	0.002	**
interaction	1.0690	0.316	

Fungi			
Substrat (organic-mineral)	2.7259	0.001	***
Structure (case-unstructured)	1.9774	0.001	***
interaction	1.2961	0.036	*
Bacteria			
Substrat (organic-mineral)	3.6117	0.001	***
Structure (case-unstructured)	3.6564	0.001	***
interaction	1.8877	0.002	**

630

631

632 **Table 2: Results of the neutral community model and normalised stochasticity ratio.**

	Migration rate [confidence interval]	Above	Below	Neutral	non-neutral proportion (%)	R^2	Normalised Stochasticity ratio
Diatoms	0.41 [0.33;0.49]	35	11	229	16.7	0.86	0.59
Fungi	0.0084 [0.0076;0.0094]	185	28	1595	11.8	0.07	0.88
Bacteria	0.51 [0.48; 0.55]	388	73	3636	11.3	0.46	0.84

633

634

635 **Table 3. Results of the multiple regression for the best models for fungal and bacterial ASV richness**
636 **on cases.**

	Fungi		Bacteria	
	t	p-value	t	p-value
JI			2.761	0.012
Roughness			3.312	0.003
Variation of particle size			-1.461	0.159
Case size	-2.408	0.023	3.807	0.001
JI: Roughness			-2.680	0.014
Case size:JI	-2.107	0.044		
Roughness: Variation of particle size			1.973	0.062
Roughness: Case surface			-5.405	<0.001
Case size:Substrate(org.)			4.699	<0.002
Adjusted R^2	0.43		0.64	

Figures

Fig. 1 Treemaps presenting the representation of ASV among genera (for RbcL) or phylum (for ITS and 16S).

Fig. 2. Non-metric multidimensional scaling ordination (NMDS) of microbial communities.

Fig. 3. Results of the *SourceTracker* analysis indicating the contribution of different sources to the microbial communities found on Trichoptera cases.

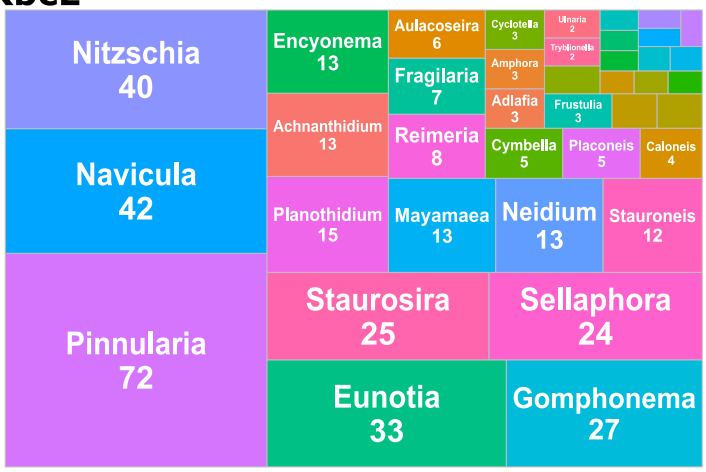
Fig. 4. Co-occurrence diagram presenting the correlations greater than 0.6 between different diatoms (grouped by genus) bacteria (grouped by family) and fungi (grouped by genus). Open symbols denote groups that are over-represented on unstructured substrates while closed symbols denotes groups over-represented on structured substrate. Presented groups are those selected by the multiblock sPLS-DA algorithm to better discriminate better structured and unstructured substrates.

Fig. 5. Richness of microbial communities on the different types of substrates, expressed as ASV richness (a, c, e) and taxonomic richness (b, d, f) for diatoms (a-b) fungi (c-d) and bacteria (e-f). Significant effect of the structure (structured vs. unstructured) and the substrate type (organic vs. mineral) on ASV and taxonomic richness based on a ANOVA is indicated by asterisks (*: $p<0.05$, **: $p<0.01$, ***: $p<0.001$, ns: not significant).

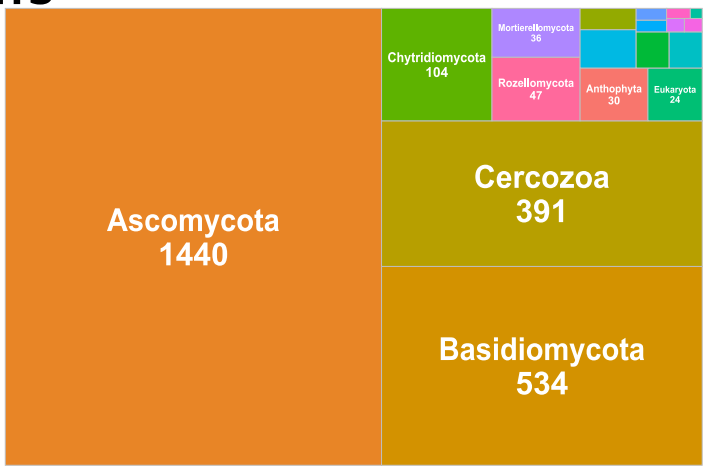
Fig. 6. Rarefaction curves for substrates grouped as structured and unstructured substrates. Shaded area around rarefaction curves indicates 95% confidence intervals.

Fig. 7. dbRDA biplot of the diatom, fungal and bacterial community on Trichoptera cases. Arrows indicates environmental factors. Asterisks indicate factors that significantly affect microbial community (* : <0.05, **: <0.01, ***: <0.001).

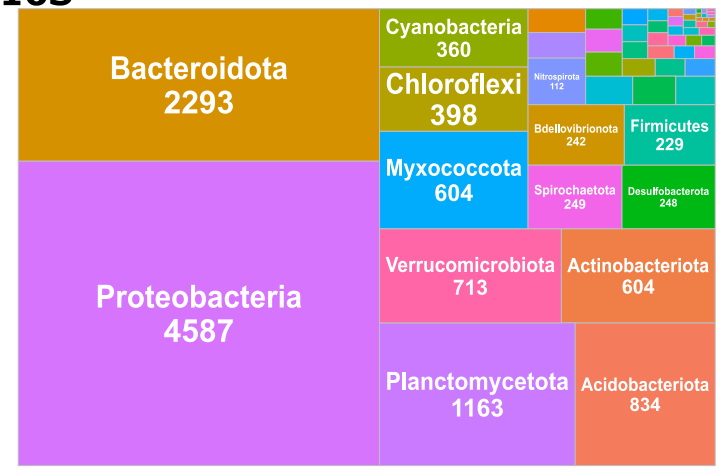
RbcL


Nitzschia 40
Navicula 42
Pinnularia 72
Encyonema 13
Aulacoseira 6
Fragilaria 7
Achnanthidium 13
Reimeria 8
Cyclotella 3
Ulnaria 2
Amphora 3
Adlafia 3
Frustulia 3
Cymbella 5
Placoneis 5
Caloneis 4
Planothidium 15
Mayamaea 13
Neidium 13
Stauroneis 12
Staurosira 25
Sellaphora 24
Eunotia 33
Gomphonema 27

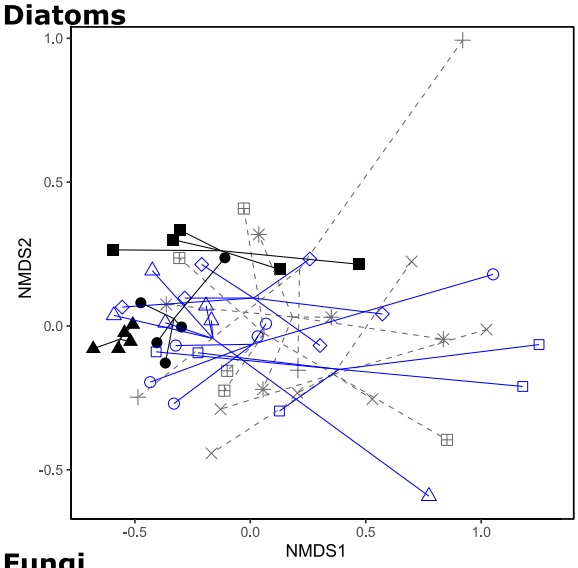
ITS


Ascomycota 1440
Chytridiomycota 104
Mortierellomycota 36
Rozellomycota 47
Anthophyta 30
Eukaryota 24
Cercozoa 391
Basidiomycota 534

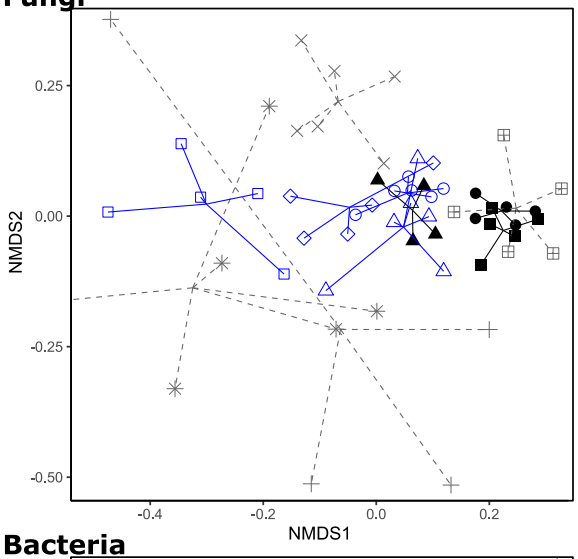
16S


Bacteroidota 2293
Proteobacteria 4587
Cyanobacteria 360
Chloroflexi 398
Myxococcota 604
Nitrospirota 112
Bdellovibrionota 242
Firmicutes 229
Spirochaetota 249
Desulfobacterota 248
Verrucomicrobiota 713
Actinobacteriota 604
Planctomycetota 1163
Acidobacteriota 834

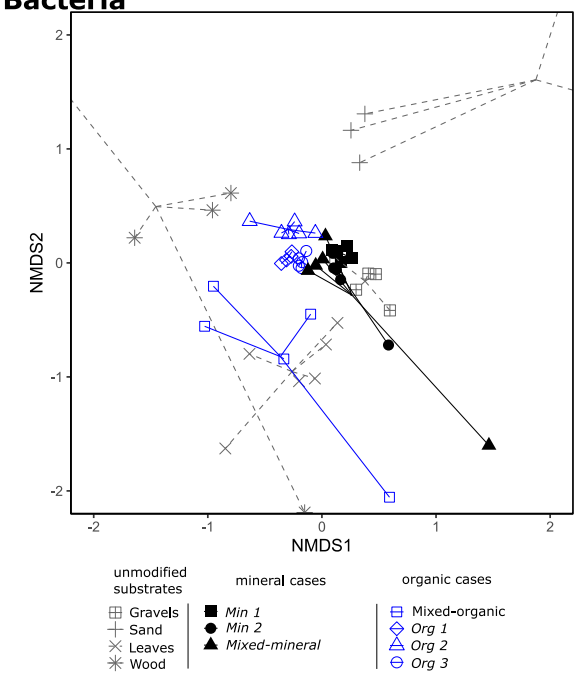
Diatoms

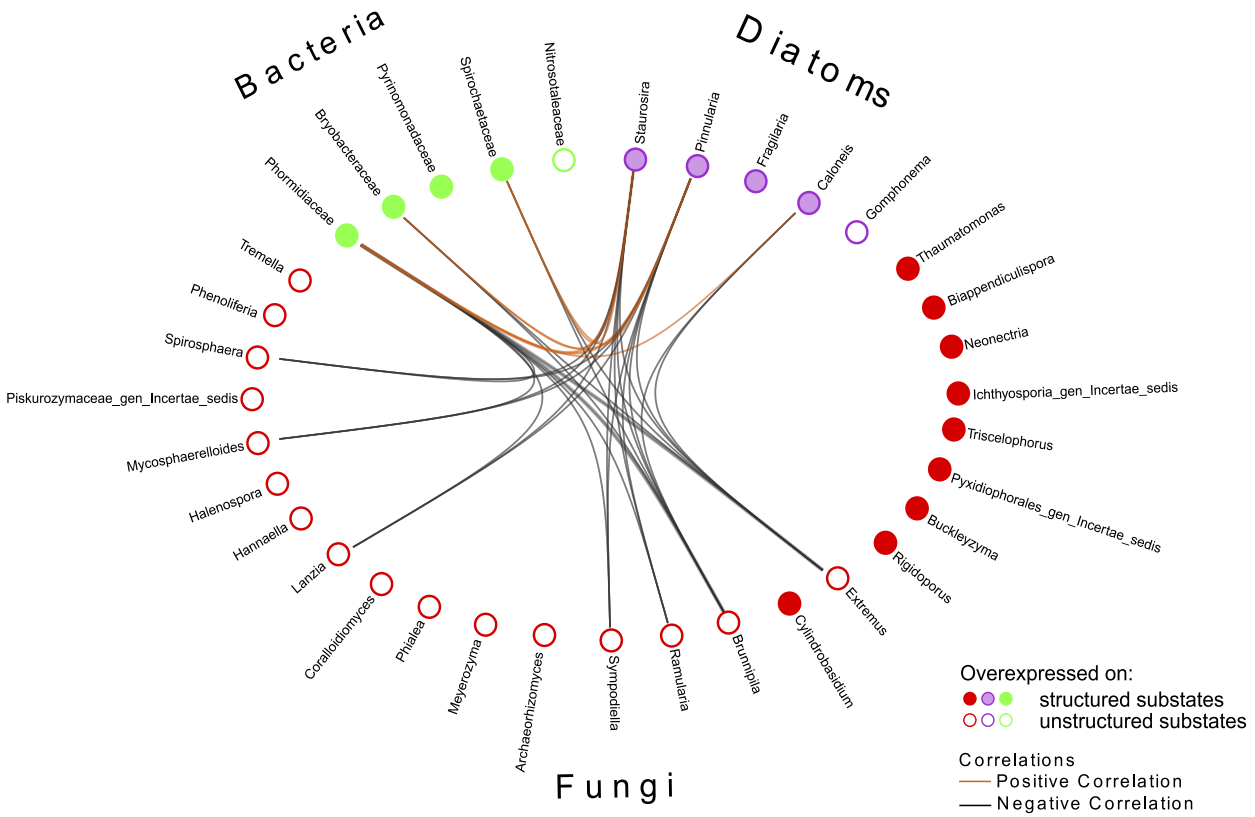


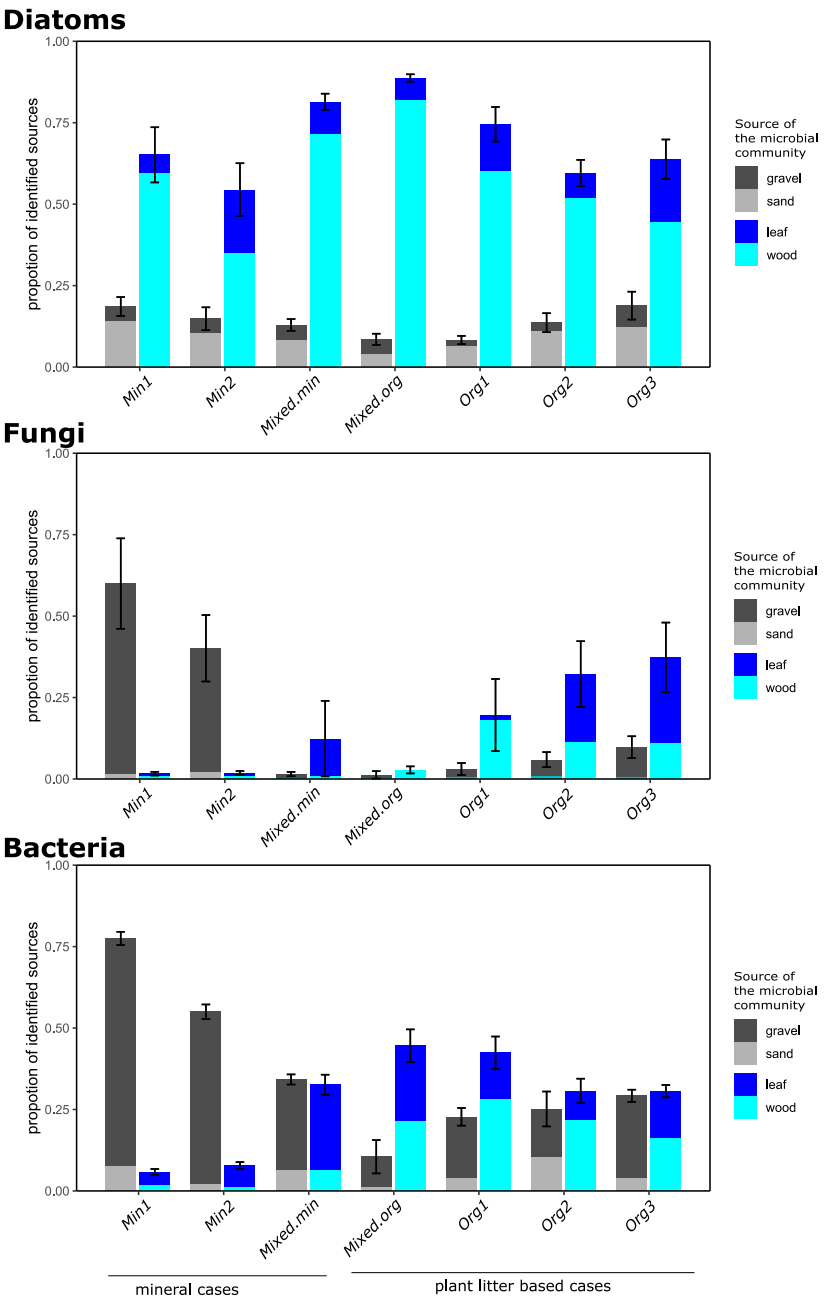
Fungi

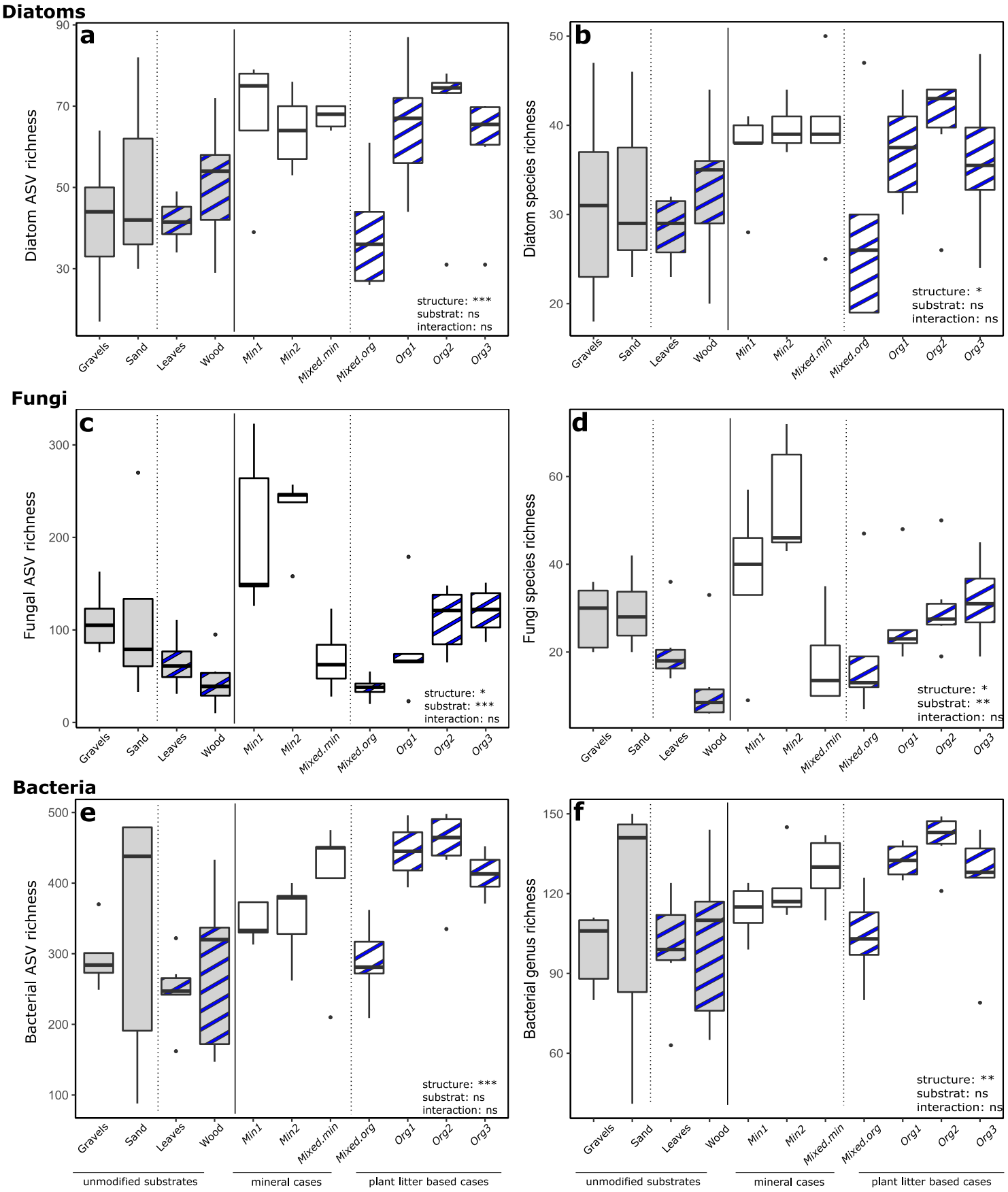


Bacteria

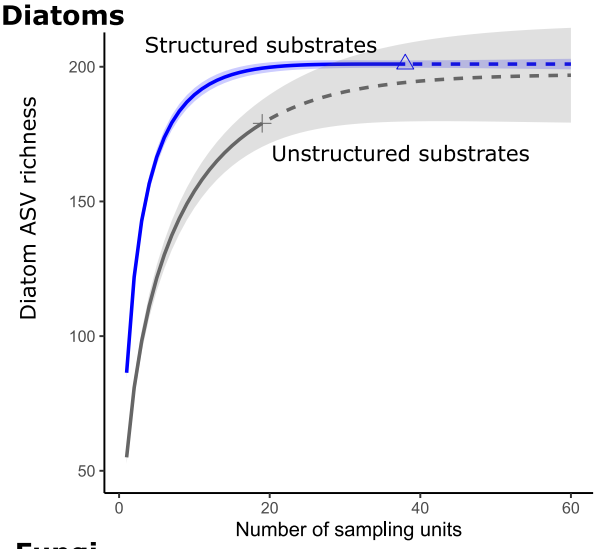



Bacteria
Diatoms
Fungi
Pyrinomonadaceae
Spirochaetaceae
Nitrosotaleaceae
Staurosira
Pinnularia
Fragilaria
Caloneis
Gomphonema
Bryobacteraceae
Phormidiaceae
Tremella
Phenoliferia
Spirosphaera
Piskurozymaceae_gen_Incertae_sedis
Mycosphaerelloides
Halenospora
Hannaella
Lanzia
Thaumatomonas
Biappendiculispora
Neonectria
Ichthyosporia_gen_Incertae_sedis
Triscelophorus
Pyxidiophorales_gen_Incertae_sedis
Buckleyzyma
Rigidoporus
Extremus
Cylindrobasidium
Brunnipila
Ramularia
Symmetrospermella
Archaeorhizomyces
Meyerozyma
Phialea
Coralloidiomyces
Overexpressed on:
structured substates
unstructured substates
Correlations
Positive Correlation
Negative Correlation

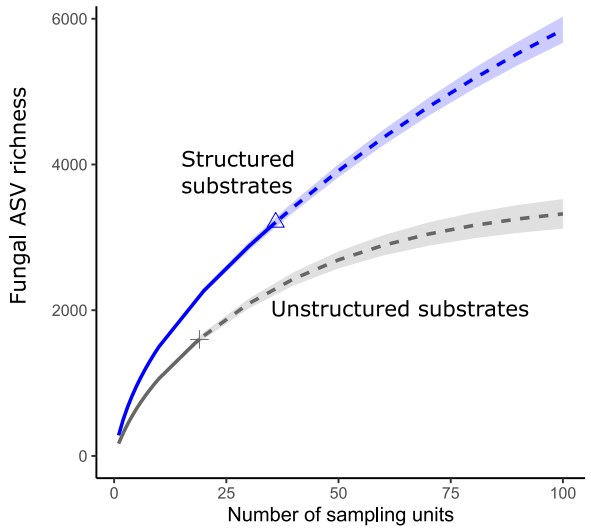

Diatoms
propotion of identified sources
1.00
0.75
0.50
0.25
0.00
Min1
Min2
Mixed.min
Mixed.org
Org1
Org2
Org3
Source of the microbial community
gravel
sand
leaf
wood
Fungi
propotion of identified sources
1.00
0.75
0.50
0.25
0.00
Min1
Min2
Mixed.min
Mixed.org
Org1
Org2
Org3
Source of the microbial community
gravel
sand
leaf
wood
Bacteria
propotion of identified sources
1.00
0.75
0.50
0.25
0.00
Min1
Min2
Mixed.min
Mixed.org
Org1
Org2
Org3
Source of the microbial community
gravel
sand
leaf
wood
mineral cases
plant litter based cases


Diatoms
a
Diatom ASV richness
structure: ***
substrat: ns
interaction: ns
b
Diatom species richness
structure: *
substrat: ns
interaction: ns
Fungi
c
Fungal ASV richness
structure: *
substrat: ***
interaction: ns
d
Fungi species richness
structure: *
substrat: **
interaction: ns
Bacteria
e
Bacterial ASV richness
structure: ***
substrat: ns
interaction: ns
f
Bacterial genus richness
structure: **
substrat: ns
interaction: ns
Gravels
Sand
Leaves
Wood
Min1
Min2
Mixed.min
Mixed.org
Org1
Org2
Org3
unmodified substrates
mineral cases
plant litter based cases

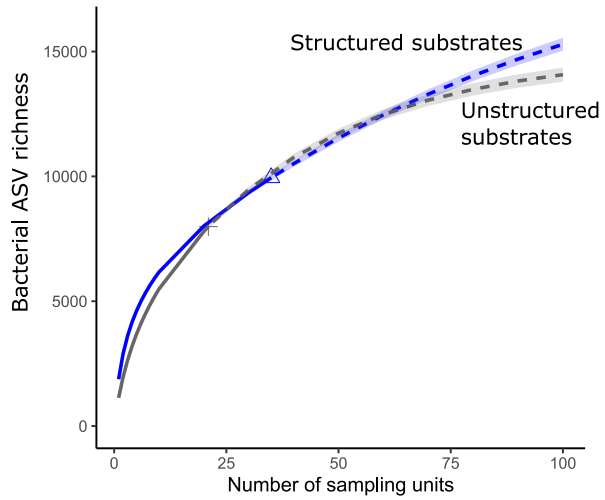
Diatoms

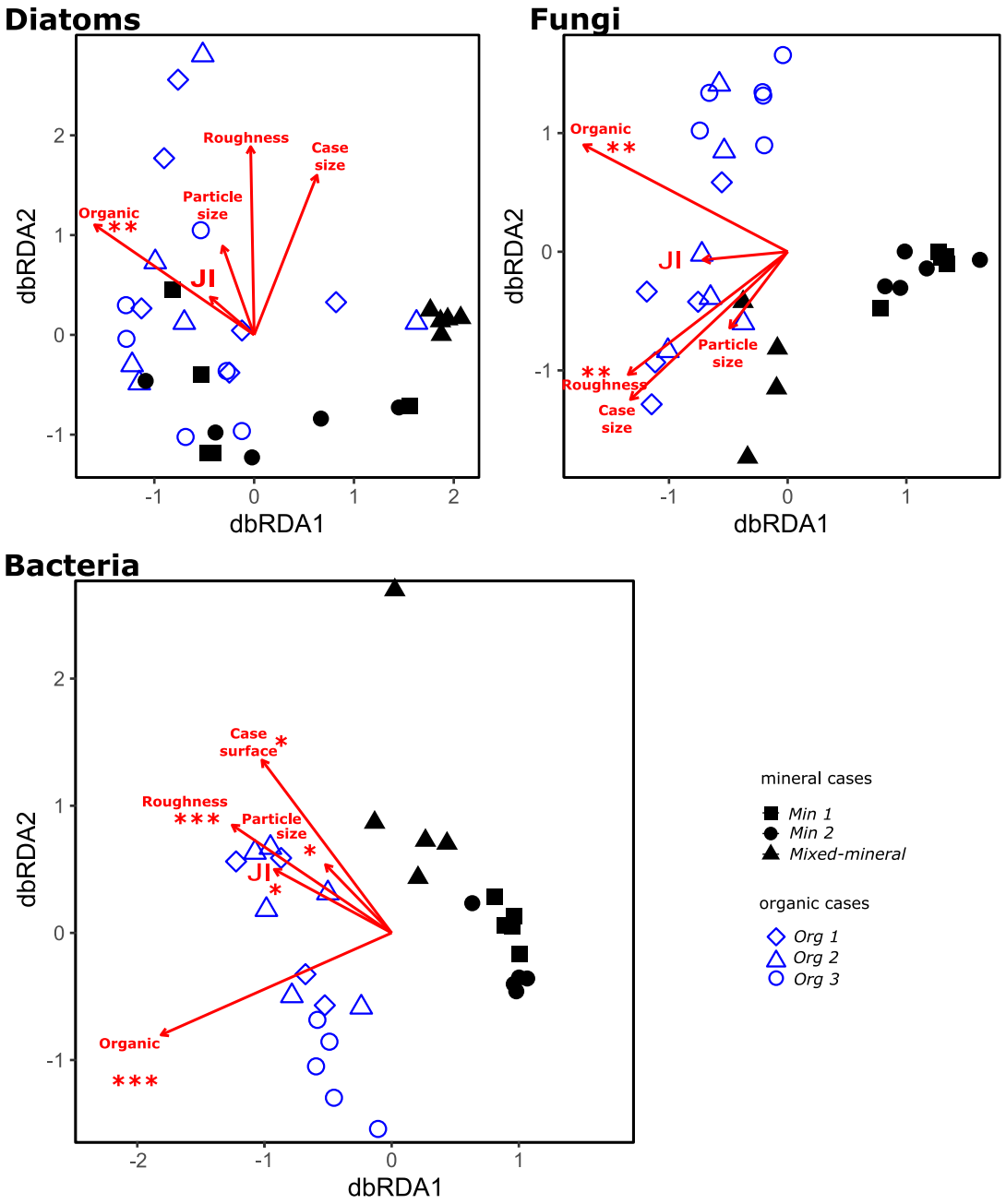


Fungi



Bacteria




Diatoms
Roughness
Case size
Particle size
Organic
**
JI
dbRDA2
dbRDA1
Fungi
Organic
**
JI
Particle size
**
Roughness
Case size
dbRDA2
dbRDA1
Bacteria
Case surface
*
Roughness
***
Particle size
*
JI
*
Organic
***
dbRDA2
dbRDA1
mineral cases
Min 1
Min 2
Mixed-mineral
organic cases
Org 1
Org 2
Org 3