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Fluvial network organization imprints on microbial co-occurrence networks

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Recent studies highlight linkages among the architecture of ecological networks, their persistence facing environmental disturbance, and the related patterns of biodiversity. A hitherto unresolved question is whether the structure of the landscape inhabited by organisms leaves an imprint on their ecological networks. We analyzed, based on pyrosequencing profiling of the biofilm communities in 114 streams, how features inherent to fluvial networks affect the co-occurrence networks that the microorganisms form in these biofilms. Our findings suggest that hydrology and metacommunity dynamics, both changing predictably across fluvial networks, affect the fragmentation of the microbial co-occurrence networks throughout the fluvial network. The loss of taxa from co-occurrence networks demonstrates that the removal of gatekeepers disproportionately contributed to network fragmentation, which has potential implications for the functions biofilms fulfill in stream ecosystems. Our findings are critical because of increased anthropogenic pressures deteriorating stream ecosystem integrity and biodiversity.

Significance

Microbial communities orchestrate most biogeochemical processes on Earth. In streams and rivers, surface-attached and matrix-enclosed biofilms dominate microbial life. Despite the relevance of these biofilms for ecosystem processes (e.g., metabolism and nutrient cycling), it remains unclear how features inherent to stream and river networks affect the fundamental organization of biofilm communities in these ecosystems. We combined co-occurrence analyses of biofilms based on next-generation sequencing with a probabilistic hydrological model, and showed how fragmentation of microbial co-occurrence networks change across stream networks. Our analyses offer potential insights into the response of microbial community organization and persistence to human alterations that increasingly change the hydrological regime and biodiversity dynamics in fluvial networks.

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In fluvial networks, the scaling relationship is characterized by a power law, $\lambda = k^{-0.54}$ (Table S1), where $\lambda$ is the catchment size and $k$ is the number of nodes in the network. The Jaccard similarity coefficient for biofilm communities decreases from 0.94 in the smallest to 0.48 in the large streams (Table S1), indicating a decrease in similarity across catchment sizes.

The hydrological regime of the fluvial network of the Ybbs River. Maps showing the distribution of the hydrologic parameter defining the hydrologic regime ($\lambda/k$) (A) and the coefficient of variation of daily streamflow ($CV_0$) (B) throughout the River Ybbs fluvial network as derived from the probabilistic model (Materials and Methods). $\lambda/k$ values range from 0.72 in small streams to 0.82 in large streams (281 nodes and 362 edges) (Fig. 3). The model revealed that the hydrological regime changed from upstream to downstream. For instance, the hydrological responsiveness ranged from 0.75 d in upstream to 4.8 d in downstream catchments, which translates into $\lambda/k$ values increasing from 0.4 to nearly 2.5 in the respective catchments (Fig. 2A). Values of $CV_0$ ranged from 0.94 in the smallest to 0.48 in the largest streams (Fig. 2B). These numerical values suggest that pronounced variability in discharge and ephemeral regimes (i.e., zero-flow days exist) and fast hydrological response to rainfall events characterize small streams in the upstream catchments. In contrast, reduced fluctuations in discharge and slower hydrological response to rainfall events characterize larger streams with persistent streamflows.

Next, we assessed how the fragmentation of co-occurrence networks of biofilm communities may change with catchment size as a continuous and scalable parameter that varies with the hydrologic regime (Fig. 3C). To do so, we divided sampling sites into bins based on log-scaled catchment size to take into account the relative distribution of small versus larger streams in fluvial networks. An even larger number of bins would compromise the statistical rigor of the co-occurrence networks because they would be derived from fewer communities. Our co-occurrence networks were computed from 10 to 15 individual communities, all of them upstream of confluences (Materials and Methods). We found generally elevated fragmentation ($\lambda/k$ ranging from 0.78 to 0.82) of biofilm co-occurrence networks in the small (0.1–3.2 km$^2$) and large (8.3–20.4 km$^2$) catchments but significantly lower fragmentation ($\lambda/k = 0.73$) in midsized catchments (3.6–8.3 km$^2$) (Fig. 3D). We note that the lower fragmentation of co-occurrence networks in midsized streams is not related to spatial clustering of these streams in the Ybbs network (Fig. S1). We also note that the use of Strahler order instead of catchment size as a binning criterion yielded similar patterns of fragmentation.

To further explore the fragmentation patterns, we evaluated the contribution of individual biofilm communities to the fragmentation of mean co-occurrence networks randomized over the entire Ybbs network (Materials and Methods). We found that the contributions of biofilms from the various streams to the mean co-occurrence networks varied broadly from upstream to downstream (Fig. S2). However, midsized streams (catchment size: 3.6–8.3 km$^2$) were the only streams that significantly ($P < 0.05$, two-tailed t test) decreased the fragmentation of mean co-occurrence networks (Table S2). This analysis suggests that biofilm communities in the midsized streams drive at least in part the presence of zero-flow days, and $\lambda/k > 1$ indicates perennial streams.
1). It is well known that changes in

Microbial co-occurrence network

We analyzed the responsiveness of co-occurrence networks from upstream communities to disturbance. Here we specifically assess the impact of removal of nodes (that is, OTUs), that are contained in a large fraction of shortest edge paths between distinct nodes and which thus have a high betweenness centrality (31). Such nodes are also termed gatekeepers (32), which interact simultaneously with different compartments of the network through transfer of energy and matter, for instance; they are thought to be crucial for ecological network structure and persistence because they literally hold the network together (14, 32, 33). We found that betweenness centrality among the nodes was heterogeneous across the network, which indeed indicates the presence of gatekeepers (Fig. 4D). We iteratively computed the fragmentation upon random removal of single OTUs from the largest connected component of the network (the so-called giant component) of eight co-occurrence networks with more than 75 nodes in the giant component (Table S1). The positive relationship between fragmentation and betweenness centrality suggests that the removal of OTUs with higher betweenness centrality from the co-occurrence networks contributes disproportionately to their fragmentation (Fig. 4B).

Discussion

The present study links co-occurrence patterns in microbial communities to potential real-world agents of disturbance in a fluvial network. It expands our current understanding on the relationship between organization and fragility of theoretical and mutualistic networks (14–17) to microbial co-occurrence networks. We suggest hydrological disturbance and metacommunity dynamics as potential controls on the co-occurrence patterns of benthic biofilm communities in fluvial networks. Not unexpectedly, the probabilistic hydrological model shows how the hydrological regime, as a major control on benthic microbial life, changes from upstream to downstream as catchment size increases. What may run counter to the logical perception is the nonlinear response of co-occurrence network fragmentation along this hydrological gradient. In fact, we anticipated fragmentation to parallel decreasing flow-induced disturbance downstream. For instance, the hydrological regime in small streams is characterized by notable temporal fluctuations and even by zero flow days (i/k < 1). It is well known that changes in flow rates can physically disturb (e.g., by abrasion and erosion) benthic biofilms (30) and affect their functioning (34) and community succession (35). Farther downstream, temporal flow variability becomes reduced because of the larger contributing area, which may alleviate physical stress on benthic biofilms. This scenario would be in line with observations on food chain length that scales with catchment size and flow variability in streams (29).

We postulate mechanisms linked to hydrology and metacommunity dynamics, as supported by theoretical and empirical evidence. The lower fragmentation of the large co-occurrence network derived from all upstream communities (Fig. 3A) is not driven by the number of nodes (that is, taxa richness) (Fig. S3).

Table 1. Summary statistics for co-occurrence network fragmentation (f)

<table>
<thead>
<tr>
<th>Catchment type</th>
<th>Position in catchment</th>
<th>Fragmentation f</th>
<th>95% CI</th>
<th>P from randomness</th>
<th>Jaccard similarity index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catchment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small (&lt;5 km²)</td>
<td>Position in catchment</td>
<td>0.54 (0.50; 0.60)</td>
<td>0.5 x 10⁻⁶</td>
<td>0.72</td>
<td></td>
</tr>
<tr>
<td>Large (&gt;5 km²)</td>
<td>Position in catchment</td>
<td>0.68 (0.66; 0.73)</td>
<td>0.2 x 10⁻⁶</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small catchment</td>
<td>Upstream from confluence</td>
<td>0.48 (0.43; 0.54)</td>
<td>0.1 x 10⁻⁶</td>
<td>0.76</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Downstream from confluence</td>
<td>0.79 (0.74; 0.81)</td>
<td>0.1 x 10⁻⁷</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large catchment</td>
<td>Upstream from confluence</td>
<td>0.64 (0.64: 0.72)</td>
<td>0.2 x 10⁻⁸</td>
<td>0.79</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Downstream from confluence</td>
<td>0.78 (0.75; 0.81)</td>
<td>0.3 x 10⁻⁷</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Upstream (<5 km²) and downstream (>5 km²) networks refer to the networks in Fig. 3 A and B. For pairwise comparison, non-overlapping 95% CI indicate significantly different fragmentation. See Materials and Methods for bootstrapping and deviation from randomness. The Jaccard similarity refers to the degree of shared edges between the respective co-occurrence networks.
evidence (3–10), to drive the observed patterns of co-occurrence network fragmentation. Although hydrological variation is indeed elevated in small headwater streams, the constrained contributing area of these catchments limits the size of the metacommunity, from which local biofilm communities assemble in these streams. Along with the relative isolation of such headwater streams in fluvial networks (5, 8), we assume that this leads to dispersal limitation in small compared with large streams. We propose that the combined effects of hydrological regime and dispersal limitation lead to biofilm communities of relatively distinct and idiosyncratic composition in small streams. Besemer et al. (8) showed that these effects may increase beta diversity in headwaters, which in turn may reduce the strength and detectability of co-occurrence patterns across these streams.

In midsized streams, the size of the upstream metacommunity increases and local community assembly may become relieved from dispersal limitation. These conditions seem favorable to stronger co-occurrence patterns (that is, lower fragmentation) of biofilm communities, implying elevated biotic interactions (18) or species sorting mediated by the local environment (36) in these streams. The notion of elevated interactions is indirectly supported by theory and experimental observations showing elevated biodiversity in more connected communities that occupy a central position in fluvial networks (6, 7); biodiversity may in turn promote interactions in microbial communities (37). Previous work reporting maximal values of alpha diversity in midsized streams of the Ybbs network (8) further corroborates this notion.

Farther downstream, we assume that metacommunity dynamics rather than hydrology become a more important control on the co-occurrence patterns of biofilms. As contributing area increases, the metacommunity from where microorganisms can immigrate increases as well (5–8). At the same time, the hydrological stochasticity in the abundant and rapidly responding streams in the upstream catchments (i.e., high values of $\lambda/k$) and hence the microbial diversity they transport downstream may shape the composition and dynamics of the downstream metacommunity. Thus, asynchronous contributions of microbial diversity from these upstream catchments may affect dispersal and assembly dynamics of local communities downstream, likely via neutral processes (that is, demographic stochasticity) (10), and the co-occurrence networks they form.

The co-occurrence patterns that we have revealed as potentially related to metacommunity dynamics and hydrology at the scale of the entire catchment apparently hold true at the scale of stream confluences. The larger contributing area downstream of a confluence constitutes a step jump in metacommunity size (potentially scaling with the contributing area of a catchment) and concomitantly in dispersal of microorganisms mixing at the confluence from each of the upstream catchments. Mixing may carry the signatures of hydrological and microbial processes occurring in each of the contributing catchments and affecting community assembly and co-occurrence downstream of the confluence. Biotic interactions, including competition, are commonly thought to increase co-occurrence in microbial networks as they refer to common resources and environmental conditions (12, 18, 19). The fact that we found elevated fragmentation downstream of confluences points to stronger influence of stochastic processes (e.g., neutrality) (10), rather than competition, organizing the biofilm communities. The higher fragmentation downstream of confluences may be reinforced by elevated physical disturbance related to flow patterns and sedimentary dynamics (38), for instance, which continuously rework and redistribute microbial niches downstream of confluences.

Taken together, our findings insinuate that the inverse gradients of hydrology and metacommunity paired with a discrete and step-wise influence of confluences generate the observed pattern of biofilm co-occurrence network fragmentation.

Ecological network theory predicts that communities of tightly connected species should be more fragile (12, 14, 39). Network organization counteracts this trend leading to robust species-rich communities, yet at the expense of pronounced fragility due to selected removal of nodes from the network (12, 14, 39). However, empirical studies that explore effects of real-world disturbance on co-occurrence patterns of microbial communities are few (19, 40). In this study, we randomly removed single nodes from the co-occurrence networks and assessed the impact on the network fragmentation. Given that the interactions between the hundreds of microbial taxa (as OTUs) are not understood, no mechanistic assumptions were made for this simulation. Our findings suggest that the loss of gatekeepers contributes disproportionately to co-occurrence network fragmentation, which essentially agrees with reports on food web and mutualistic networks (where mechanistic assumptions are made) showing high fragility of these networks upon selective removal of species (13, 15, 38). Sequencing data allowed us to identify gatekeepers as being affiliated with *Saprospiraceae* and *Sphingomonadaceae* (Fig. 4C). These OTUs had high betweenness centrality values (up to 0.5 and 0.15, respectively) and were consistently present in the giant component of the co-occurrence networks. This is notable as these are typical freshwater families, often dwelling in headwater streams where dissolved organic carbon is often aromatic and humic and where flow-induced erosion can be high. The loss of these gatekeepers may have adverse consequences for the integrity and function of biofilm communities.
The need to perceive streams and rivers as networks or macro-systems consisting of connected and interacting systems is increasingly recognized (4, 11, 41). Our findings are relevant in that context because they suggest the type of linkage that governs the organization of microbial communities in relation to flow dynamics across fluvial networks. Distinct anthropogenic pressures, such as interbasin water transfer, damming, and mountain top mining and valley filling (26), but also climate change effects on the hydrological regime and its resilience (24) alter fundamental attributes of fluvial networks. It is imperative to understand the consequences of these for microbial interactions and for the persistence of biofilm communities, which are critical for ecosystem processes.

Materials and Methods

Study Sites and Sampling. We sampled benthic biofilms from 114 streams upstream and downstream from confluences in the river Ybbs (Austria) (8, 27) at base flow in winter. For details on sampling and field measurements, see SI Materials and Methods.

Probabilistic Hydrological Model of the Ybbs Fluvial Network. To characterize the hydrological regime of the Ybbs River network, we adopted a probabilistic characterization of temporal discharge dynamics by coupling a stochastic analysis of daily rainfall events to catchment transport dynamics (24, 28). The model expresses the probabilistic structure of discharge in terms of three parameters, namely, α, j, and k, which describe the magnitude and frequency of discharge-producing rainfall events and the characteristic response time of the catchment (that is, the time needed by effective rainfall inputs to reach the outlet), respectively. The ratio j/k identifies the shape of the probability distribution and the hydrological regime (Supporting Information). For the Ybbs River network, we assumed spatially uniform rainfall, where j = 0.53 d−1 (that is, on average there is a rainfall event effectively producing discharge almost every 2 d) and α = 9.61 mm. The response time k is assumed to increase as a power law relation with total contribution area (k) as a direct result of the fact that drainage area, and thus, the channel paths determining hydrograph recession rates predictably increase downstream (Supporting Information).

Sequencing. DNA was extracted from microbial biomass (8), and the V4 and V5 regions of the 16S rRNA gene were amplified using the primers 515F 5′-GTGNCAGCMGCC GCGGTAA-3′ and 926R 5′-CCGYCAATTYMTTTRAGTTT-3′ (Invitrogen) (42). Equal amounts of the barcode PCR products were sequenced at the Centre for Genomic Research (Liverpool, United Kingdom) on a 454 GS20 FLX Titanium platform. See ref. 8 and SI Methods for details on the bioinformatics. The final dataset consisted of 955,691 sequences which constituted 1,005 OTUs and were affiliated to 126 bacterial families.

Co-occurrence Networks and Fragmentation. OTUs affiliated with families were used to construct the co-occurrence networks. We filtered out OTUs present in fewer than three sampling sites involved in the particular network inference. Then the dataset was Hellinger-transformed. All possible Spearman rank correlations between OTUs across sampling sites and corresponding P values were calculated. We corrected the false discovery rate according to Benjamini-Hochberg (43). We considered a valid co-occurrence event to be robust if the Spearman correlation coefficient ρ > 0.6 and statistically significant at P < 0.01 (20).

Co-occurrence network fragmentation (f) was calculated as the ratio of the number of disconnected subgraphs (CL) to the overall number of nodes (N) in each network as log10(CL)/log(N). Values of f range between 0 and 1 independent of network size and display an elevated resolution for less fragmented networks. Relative fragmentation patterns remained robust when various levels of filtering stringency were applied (Table S3). Co-occurrence networks were bootstrapped, and the computed fragmentation was validated against randomness as described in SI Materials and Methods. The degree of shared edges between the between the analyzed co-occurrence networks was assessed using the Jaccard similarity index (Supporting Information) as the ratio of the intersection of samples (similar edges) against their union (all present edges). We also assessed the relative contribution (%) of distance biofilm communities to the fragmentation of mean co-occurrence networks randomized over the Ybbs catchment based on 39 sites randomly chosen from 77 sites upstream of the confluences (Supporting Information).

To assess the relative contribution of OTUs to overall CB of a respective network, we examined the frequency distribution of CB using co-occurrence networks according to a minimal size criterion (>75 nodes) for the giant component. We conducted in silico experiment and tested how random removal of nodes affects community fragmentation, f, of the giant component of the co-occurrence networks. OTUs with a potential gatekeeper function were identified by combining graph-topological and pyrosequencing data. Further details are provided in SI Materials and Methods.

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