Fluvial network organization imprints on microbial co-occurrence networks

Stefanie Widder[a], Katharina Besemer[b,c], Gabriel A. Singer[b,d], Serena Ceola[e], Enrico Bertuzzo[f], Christopher Quince[g], William T. Sloan[g], Andrea Rinaldo[f,h,1], and Tom J. Battin[b,c,1]

[a]Division of Computational Systems Biology, Department of Microbiology and Ecosystem Science, University of Vienna, A-1090 Vienna, Austria; [b]Division of Limnology, Department of Limnology and Oceanography, University of Vienna, A-1090 Vienna, Austria; [c]WasserCluster Lunz GmbH, A-3293 Lunz am See, Austria; [d]Leibniz-Institute of Freshwater Ecology and Inland Fisheries, D-12587 Berlin, Germany; [e]Dipartimento di Ingegneria Civile, Chimica, Ambientale e dei Materiali, Università di Bologna, I-40136 Bologna, Italy; [f]Laboratory of Ecohydrology, School of Architecture, Ecole Polytechnique Fédérale de Lausanne, CH-1015 Lausanne, Switzerland; [g]School of Engineering, University of Glasgow, Glasgow G12 8QQ, United Kingdom; and [h]Dipartimento di Ingegneria Civile, Edile e Ambientale, Università di Padova, I-35131 Padova, Italy

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.

stream networks | hydrological regime

Streams and rivers sculpt the continental surface, forming fluvial networks (1), within which the biodiversity ranks among the highest on Earth (2). The dendritic nature of fluvial networks has been shown to affect the spatial and temporal patterns of microbial, invertebrate, and fish biodiversity (3–8). Ecological theory and observations posit that the local environment governs the dynamics and diversity of ecological communities in headwaters, the smallest and most abundant streams in fluvial networks. In contrast, dispersal ensures that communities further downstream are shaped not only by their immediate environment but also by upstream processes (3–9). Thus, the dynamics of the metacommunity, which comprises all interconnected communities in a landscape (10), are inextricably linked to the organization and hydrology of the fluvial network (5–8). This perception is essential to understand, predict, and manage streams and rivers and their resistance and resilience to human alterations across scales (that is, from patches to the catchment) (11).

Ecological interactions are often usefully represented as networks (12). For example, analyses of food webs and mutualistic (e.g., pollination) networks have demonstrated that network organization can be linked to network persistence, to disturbance (12–16), or to species coexistence and richness (17). Microbial communities are so diverse and poorly studied that mapping out the interactions on the basis of biological knowledge is currently impossible for all but the simplest of habitats. Therefore, co-occurrence networks are increasingly used to infer microbial interactions (18, 19) in soils (20), oceans (21), lakes (22), and even in global genomic surveys (23).

A key question is whether the organization of microbial co-occurrence networks and their response to disturbance reflect physical characteristics inherent in fluvial networks such as geomorphology (1), resilience of the hydrological regime (24), and metacommunity dynamics (3–8). This question is important because microorganisms, often encapsulated in biofilms attached to the streambed, fulfill critical ecosystem functions in streams and rivers (25), which have an ongoing history of being perturbed by human activity (11, 26). We addressed this question by using co-occurrence networks based on 454 pyrosequencing data (8) of the 16S rRNA gene from benthic biofilms from 114 streams of the prealpine Ybbs catchment (Fig. 1) (8, 27). Our dataset comprised 955,691 sequences constituting 1,005 operational taxonomic units (OTUs) affiliated with 126 bacterial families, from which we constructed co-occurrence networks at OTU level and computed their fragmentation.

We computed the fragmentation as the relative fraction of disconnected compartments within a co-occurrence network (*Materials and Methods*) as a basic descriptor of network topology. Fragmentation patterns were explored as a function of fluvial network hydrology, which we suitably described using a probabilistic model based on daily rainfall and streamflow (24, 28) and leaning on the concept of metacommunity ecology (10).

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 pressures that increasingly change the hydrological regime and biodiversity dynamics in fluvial networks.
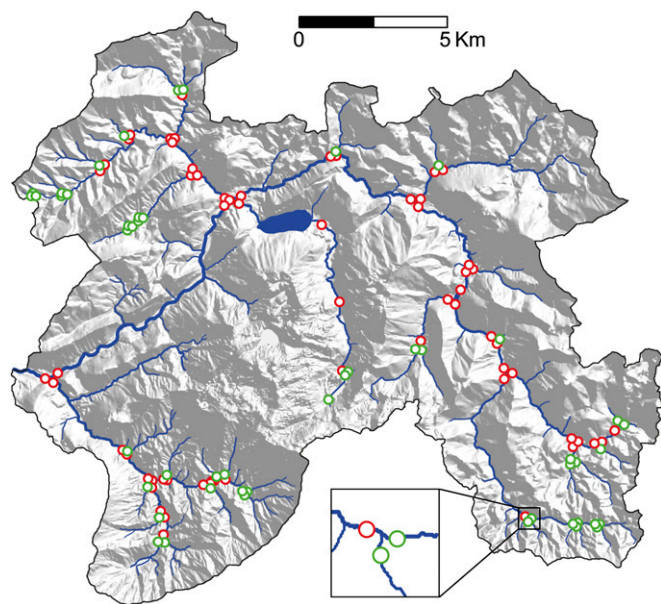
ENVIRONMENTAL SCIENCES

Fig. 1. The Ybbs network. The River Ybbs catchment (254 km^2, Austria) and its fluvial network suitably extracted from a digital elevation model. We sampled benthic biofilms from 50 and 64 streams upstream (green circles) and downstream (red circles), respectively.

Results

Fluvial Network Hydrology. In fluvial networks, the scaling relationship between the hydrological regime and catchment size mechanistically links fluvial network topology to biological inputs from terrestrial ecosystems into streams and to both environmental and ecological stability therein (1, 29). To explore how the hydrological regime as a potential source of disturbance to benthic and microbial life in streams (29, 30) changes across the Ybbs network, we adopted a probabilistic model (24, 28) (*Materials and Methods*).

Notably, we quantified the hydrologic regime at stream reach level via a dimensionless parameter (λ/k) relating the frequency of runoff-forming rainfall events, λ (d^{-1}), to the mean duration of the flow pulse released from the catchment after an effective rainfall event, $1/k$ (days). λ/k essentially depends on catchment-scale morphological, hydrological, and climatological attributes. For each stream, we also evaluated the coefficient of variation of daily flow (CV$_Q$). 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 λ/k values increasing from 0.4 to nearly 2.5 in the respective catchments (Fig. 2*A*). Values of CV$_Q$ ranged from 0.94 in the smallest to 0.48 in the largest streams (Fig. 2*B*). 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.

Fragmentation of Microbial Co-occurrence Networks Across a Fluvial Network. In a next step, we explored co-occurrence patterns of biofilm communities (*Materials and Methods*) at three different spatial scales in the Ybbs network. First, we constructed comprehensive co-occurrence networks from 50 upstream sites (that is, catchments smaller than the largest first-order catchment with an approximate size of 5 km^2; referred to as small streams hereafter) and from 64 downstream sites (catchments larger than

5 km^2; referred to as large streams hereafter) in the Ybbs catchment (Fig. 3 *A* and *B*). This apportionment agrees with previous work showing that the mean local diversity (that is, alpha diversity) of benthic biofilms and its dispersion change from upstream to downstream (8). We found that the fragmentation of the co-occurrence networks was significantly lower in small ($f = 0.54$) than in large ($f = 0.68$) streams (Table 1). The Jaccard similarity (0.72; *Materials and Methods* and Table S1) between both co-occurrence networks suggests high similarity and supports the appropriateness to compare both networks. The higher number of nodes (that is, OTUs; $n = 595$) and edges ($n = 1,299$) in the co-occurrence network from the small streams compared with the large streams (281 nodes and 362 edges) (Fig. 3 *A* and *B* and Table S1) agrees with the previously reported patterns of higher alpha diversity upstream in the Ybbs network (8).

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. 3*C*). To do so, we divided sampling sites into six 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 (f 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 ($f = 0.73$) in midsized catchments (3.6–8.3 km^2) (Fig. 3*D*). 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
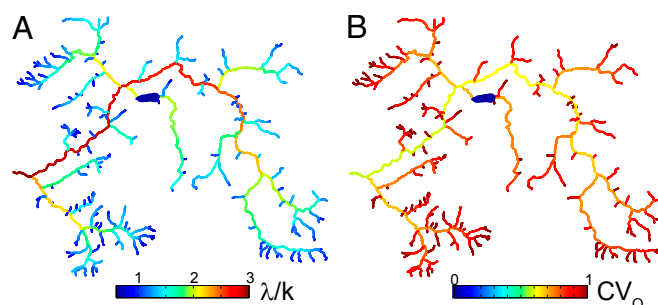
Fig. 2. Hydrological regime of the fluvial network of the Ybbs River. Maps showing the distribution of the hydrologic parameter defining the hydrologic regime (λ/k) (*A*) and of the coefficient of variation of daily streamflow (CV$_Q$) (*B*) throughout the River Ybbs fluvial network as derived from the probabilistic model (*Materials and Methods*). $\lambda/k < 1$ indicates ephemeral streams with the presence of zero-flow days, and $\lambda/k > 1$ indicates perennial streams.
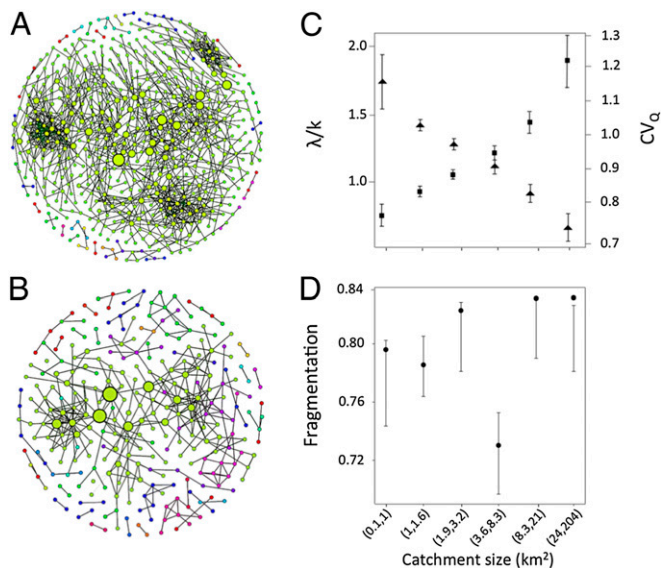
Fig. 3. Microbial co-occurrence networks based on 454 pyrosequencing of benthic biofilm communities. Co-occurrence networks from communities in 50 upstream (<5 km²; first to third stream order) (*A*) and 64 downstream (>5 km², second- to fifth-order streams) sites (*B*). Fragmentation of the co-occurrence networks from upstream communities is significantly lower than that from downstream communities (Table 1). The size of the nodes is scaled to their betweenness centrality. Giant components are indicated in green for both co-occurrence networks; other colors denote smaller components. (*C*) Fragmentation patterns differ from patterns of CV_Q (triangles) and λ/k (squares) across the bins of catchment size (as in *D*). (*D*) Fragmentation (*f*; 95% confidence interval) is highest in small and large streams but significantly lower in midsized streams. Co-occurrence networks were computed from 10, 10, 14, 15, 14, and 14 communities for the six bins of catchment size (log scale). We used only sites upstream of confluences to avoid dependencies and effects of confluences (see text).

the lower fragmentation of the large co-occurrence network derived from all upstream communities (Fig. 3*A*). We note that the difference in fragmentation between small and large streams (Fig. 3 *A* and *B*) is not driven by the number of nodes (that is, taxa richness) (Fig. S3).

Finally, at a smaller scale we studied the possible effect of confluences on co-occurrence patterns of biofilm communities. Confluences are the nodes of fluvial networks, where water, sediments, and biodiversity from different catchments mix and marked increments in total contributing area occur. We constructed co-occurrence networks for biofilms sampled upstream and downstream of confluences in the small (<5 km²) and large (>5 km²) catchments, respectively. We found that fragmentation of co-occurrence networks was significantly higher in biofilm communities downstream than upstream of confluences; this pattern was consistent for both small and large streams (Table 1).

Betweenness Centrality and Fragmentation of Co-occurrence Networks. We analyzed the responsiveness of co-occurrence network fragmentation to random removal of nodes as this may provide insights into the susceptibility of co-occurrence networks of biofilms to disturbance. Here we specifically assess consequences 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 heterogeneously distributed in the co-occurrence networks, which indeed indicates the presence of gatekeepers (Fig. 4*A*). 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. 4*B*).

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 ($\lambda/k < 1$). 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

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

Catchment type	Position in catchment	Fragmentation *f*, 95% CI	*P* from randomness	Jaccard similarity index
Catchment	Small (<5 km²)	0.54 (0.50; 0.60)	0.5×10^{-6}	0.72
	Large (>5 km²)	0.68 (0.66; 0.73)	0.2×10^{-6}	
Small catchment	Upstream from confluence	0.48 (0.43; 0.54)	0.1×10^{-4}	0.76
	Downstream from confluence	0.79 (0.74; 0.81)	0.1×10^{-7}	
Large catchment	Upstream from confluence	0.64 (0.64; 0.72)	0.2×10^{-8}	0.79
	Downstream from confluence	0.78 (0.75; 0.81)	0.3×10^{-7}	

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.
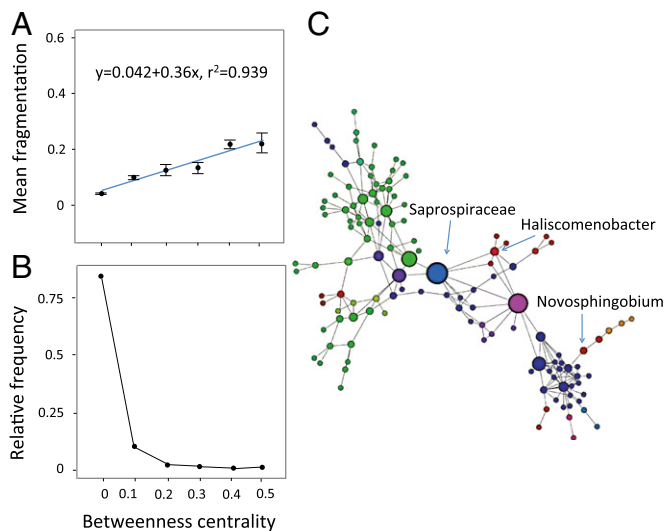
Fig. 4. Fragmentation, persistence and betweenness centrality of biofilm co-occurrence networks. (*A*) Random removal of nodes from the co-occurrence network generates a positive relationship between betweenness centrality (CB) and fragmentation (*f*). This suggests that the removal of gatekeepers (that is, higher CB) contributes disproportionately to fragmentation. The analysis is based on 1,818 nodes in nine co-occurrence networks (giant component); error bars depict SE. (*B*) Frequency distribution showing the heterogeneous contribution of the various nodes to betweenness centrality (CB) and the disproportionally low contribution of gatekeepers to co-occurrence networks. Statistics were done on the respective giant components. (*C*) Depiction of the giant component of the co-occurrence network of the downstream communities (Fig. 3*D*). The size of the nodes is scaled to CB to indicate their gatekeeping properties; coloring is according to compartment structure. Gatekeeping nodes that consistently occurred in all networks are indicated.

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 λ/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. 4*C*). 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 sedimentary environments and associated with surfaces. They comprise members known to degrade aromatic compounds (i.e., *Novosphingobium* sp.) and to confer physical structure to the communities (i.e., *Haliscomenobacter* sp.), properties which appear advantageous to microorganisms forming biofilms in 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 macrosystems 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 therefore 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, λ, 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 λ/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 $\lambda = 0.53$ d^{-1} (that is, on average there is a rainfall event effectively producing discharge almost every 2 d) and $\alpha = 9.61$ mm. The response time k is assumed to increase as a power law relation with total contributing area $1/k \propto A^{1/3}$ 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 50-GTGNCAGCMGCC GCGGTAA-30 and 926R 50-CCGYCAATTYMTTTRAGTTT-30 (Invitrogen) (42). Equal amounts of the barcoded 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 Materials and 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 $\rho > |0.6|$ 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 log(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 cites 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 an 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*.

1. Rodriguez-Iturbe I, Rinaldo A (1997) *Fractal River Basins: Chance and Self-Organisation* (Cambridge Univ Press, Cambridge, UK).
2. de Aguiar MAM, Baranger M, Baptestini EM, Kaufman L, Bar-Yam Y (2009) Global patterns of speciation and diversity. *Nature* 460(7253):384–387.
3. Fagan WF (2002) Connectivity, fragmentation and extinction risk in dendritic metapopulations. *Ecology* 83(12):3243–3249.
4. Muneepeerakul R, et al. (2008) Neutral metacommunity models predict fish diversity patterns in Mississippi-Missouri basin. *Nature* 453(7192):220–222.
5. Carrara F, Altermatt F, Rodriguez-Iturbe I, Rinaldo A (2012) Dendritic connectivity controls biodiversity patterns in experimental metacommunities. *Proc Natl Acad Sci USA* 109(15):5761–5766.
6. Carrara F, Rinaldo A, Giometto A, Altermatt F (2014) Complex interaction of dendritic connectivity and hierarchical patch size on biodiversity in river-like landscapes. *Am Nat* 183(1):13–25.
7. Altermatt F (2013) Diversity in riverine metacommunities: A network perspective. *Aquat Ecol* 47(3):365–377.
8. Besemer K, et al. (2013) Headwaters are critical reservoirs of microbial diversity for fluvial networks. *Proc Biol Sci* 280(1771):20131760.
9. Brown BL, Swan CM (2010) Dendritic network structure constrains metacommunity properties in riverine ecosystems. *J Anim Ecol* 79(3):571–580.
10. Holyoak M, Leibold MA, Holt RD (2005) *Metacommunities: Spatial Dynamics and Ecological Communities* (Univ of Chicago Press, Chicago).
11. McCluney KE, et al. (2014) Riverine macrosystems ecology: Sensitivity, resistance, and resilience of whole river basins with human alterations. *Front Ecol Environ* 12(1):45–58.
12. Montoya JM, Pimm SL, Solé RV (2006) Ecological networks and their fragility. *Nature* 442(7100):259–264.
13. Stouffer DB, Bascompte J (2011) Compartmentalization increases food-web persistence. *Proc Natl Acad Sci USA* 108(9):3648–3652.
14. Saavedra S, Stouffer DB, Uzzi B, Bascompte J (2011) Strong contributors to network persistence are the most vulnerable to extinction. *Nature* 478(7368):233–235.
15. Pocock MJO, Evans DM, Memmott J (2012) The robustness and restoration of a network of ecological networks. *Science* 335(6071):973–977.
16. Thébault E, Fontaine C (2010) Stability of ecological communities and the architecture of mutualistic and trophic networks. *Science* 329(5993):853–856.
17. Suweis S, Simini F, Banavar JR, Maritan A (2013) Emergence of structural and dynamical properties of ecological mutualistic networks. *Nature* 500(7463): 449–452.
18. Fuhrman JA (2009) Microbial community structure and its functional implications. *Nature* 459(7244):193–199.
19. Faust K, Raes J (2012) Microbial interactions: From networks to models. *Nat Rev Microbiol* 10(8):538–550.
20. Barberán A, Bates ST, Casamayor EO, Fierer N (2012) Using network analysis to explore co-occurrence patterns in soil microbial communities. *ISME J* 6(2):343–351.
21. Steele JA, et al. (2011) Marine bacterial, archaeal and protistan association networks reveal ecological linkages. *ISME J* 5(9):1414–1425.
22. Eiler A, Heinrich F, Bertilsson S (2012) Coherent dynamics and association networks among lake bacterioplankton taxa. *ISME J* 6(2):330–342.
23. Freilich S, et al. (2010) The large-scale organization of the bacterial network of ecological co-occurrence interactions. *Nucleic Acids Res* 38(12):3857–3868.
24. Botter G, Basso S, Rodriguez-Iturbe I, Rinaldo A (2013) Resilience of river flow regimes. *Proc Natl Acad Sci USA* 110(32):12925–12930.
25. Battin TJ, Kaplan LA, Denis Newbold J, Hansen CM (2003) Contributions of microbial biofilms to ecosystem processes in stream mesocosms. *Nature* 426(6965):439–442.
26. Palmer MA, et al. (2010) Science and regulation. Mountaintop mining consequences. *Science* 327(5962):148–149.
27. Ceola S, et al. (2014) Hydrologic controls on basin-scale distribution of benthic invertebrates. *Water Resour Res* 50(4):2903–2920.
28. Botter G, Porporato A, Daly E, Rodriguez-Iturbe I, Rinaldo A (2007) Probabilistic characterization of base flows in river basins: Roles of soil, vegetation and geomorphology. *Water Resour Res* 43(6):W06404.

ENVIRONMENTAL SCIENCES

29. Sabo JL, Finlay JC, Kennedy T, Post DM (2010) The role of discharge variation in scaling of drainage area and food chain length in rivers. *Science* 330(6006): 965–967.
30. Blenkinsopp SA, Lock MA (2004) The effect of storm-flow on river biofilms. *J Phycol* 30(5):807–818.
31. Freeman LC (1977) A set of measures of centrality based on betweenness. *Sociometry* 40(1):35–41.
32. Freeman LC (1980) The gatekeeper, pair-dependency and structural centrality. *Qual Quant* 14(4):585–592.
33. Fann SL, Borrett SR (2012) Environ centrality reveals the tendency of indirect effects to homogenize the functional importance of species in ecosystems. *J Theor Biol* 294:74–86.
34. Timoner X, Acuna V, von Schiller D, Sabater S (2012) Functional responses of stream biofilms to flow cessation, desiccation and rewetting. *Freshw Biol* 57(8):1565–1578.
35. Besemer K, et al. (2007) Biophysical controls on community succession in stream biofilms. *Appl Environ Microbiol* 73(15):4966–4974.
36. King AJ, Farrer EC, Suding KN, Schmidt SK (2012) Co-occurrence patterns of plants and soil bacteria in the high-alpine subnival zone track environmental harshness. *Front Microbiol* 3(47):347.
37. Czárán TL, Hoekstra RF, Pagie L (2002) Chemical warfare between microbes promotes biodiversity. *Proc Natl Acad Sci USA* 99(2):786–790.
38. Benda L, et al. (2004) The network dynamics hypothesis: How channel networks structure riverine habitats. *Bioscience* 54(5):413–427.
39. Solé RV, Montoya JM (2001) Complexity and fragility in ecological networks. *Proc Biol Sci* 268(1480):2039–2045.
40. Zhou J, et al. (2010) Functional molecular ecological networks. *MBio* 1(4):e00169-10.
41. Battin TJ, et al. (2008) Biophysical controls on organic carbon fluxes in fluvial networks. *Nat Geosci* 1(2):95–100.
42. Quince C, Lanzen A, Davenport R, Turnbaugh P (2011) Removing noise from pyrosequenced amplicons. *BMC Bioinf* 12:38.
43. Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: A practical and powerful approach to multiple testing. *J R Stat Soc B* 57(1):289–300.