

Dendritic connectivity controls biodiversity patterns in experimental metacommunities

Francesco Carrara^{a,1}, Florian Altermatt^{b,1,2}, Ignacio Rodriguez-Iturbe^c, and Andrea Rinaldo^{a,d,2}

^aLaboratory of Ecohydrology (ECHO/IEE/ENAC), École Polytechnique Fédérale Lausanne, 1015 Lausanne, Switzerland; ^bDepartment of Aquatic Ecology, Eawag: Swiss Federal Institute of Aquatic Science and Technology, 8600 Dübendorf, Switzerland; ^cDepartment of Civil and Environmental Engineering, Princeton University, Princeton, NJ 08544; and ^dDipartimento di Ingegneria Civile, Edile ed Ambientale, Università di Padova, 35131 Padova, Italy

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Biological communities often occur in spatially structured habitats where connectivity directly affects dispersal and metacommunity processes. Recent theoretical work suggests that dispersal constrained by the connectivity of specific habitat structures, such as dendrites like river networks, can explain observed features of biodiversity, but direct evidence is still lacking. We experimentally show that connectivity per se shapes diversity patterns in microcosm metacommunities at different levels. Local dispersal in isotropic lattice landscapes homogenizes local species richness and leads to pronounced spatial persistence. On the contrary, dispersal along dendritic landscapes leads to higher variability in local diversity and among-community composition. Although headwaters exhibit relatively lower species richness, they are crucial for the maintenance of regional biodiversity. Our results establish that spatially constrained dendritic connectivity is a key factor for community composition and population persistence.

microbial microcosms | directional dispersal | community assembly | nonneutral dynamics

A major aim of community ecology is to identify processes that define large-scale biodiversity patterns (1–8). For simplified landscapes, often described geometrically by linear or lattice structures, a variety of local environmental factors have been brought forward as the elements creating and maintaining diversity among habitats (9–12). Many highly diverse landscapes, however, exhibit hierarchical spatial structures that are shaped by geomorphological processes and neither linear nor 2D environmental matrices may be appropriate to describe biodiversity of species living within dendritic ecosystems (13, 14). Furthermore, in many environments intrinsic disturbance events contribute to spatiotemporal heterogeneity (14, 15). Riverine ecosystems, among the most diverse habitats on earth (16), represent an outstanding example of such mechanisms (7, 17–19).

Here, we investigate the effects of directional dispersal imposed by the habitat-network structure on the biodiversity of metacommunities (MCs), by conducting a laboratory experiment using aquatic microcosms. Experiments were conducted in 36-well culture plates (Fig. 1), thus imposing by construction a metacommunity structure (20, 21): Each well hosted a local community (LC) within the whole landscape and dispersal occurred by periodic transfer of culture medium among connected LCs (22), following two different geometries (*Materials and Methods*, Fig. S1, and *SI Materials and Methods*). We compared spatially heterogeneous MCs following a river network (RN) geometry (Fig. 1D), with spatially homogeneous MCs, in which every LC has a 2D lattice of four nearest neighbors (2D) (Fig. 1E). The coarse-grained RN landscape is derived from a scheme (13) known to reproduce the scaling properties observed in real river systems (Fig. 1A).

To single out the effects of connectivity, we deliberately avoided reproducing other geomorphic features of real river networks, such as the bias in downstream dispersal, the growing habitat capacity with accumulated contributing area, or other environmental conditions connected to topographic elevation. Directional dispersal refers to the pathway constrained by the habitat connectivity

and does not imply downstream-biased dispersal kernels; that is, in all treatments dispersal kernels were identical and symmetric. Disturbance consisted of medium replacement and reflects the spatial environmental heterogeneity inherent to many natural systems (*Materials and Methods*).

The microcosm communities were composed of nine protozoan and one rotifer species, which are naturally co-occurring in freshwater habitats, with bacteria as a common food resource (21). These species cover a wide range of body sizes (Fig. 1B), intrinsic growth rates, and other important biological traits (23) (Table S1). Thus, the microcosm communities cover substantial biological complexity in terms of more structured trophic levels and species interactions that cannot be entirely captured by any model (24) (*Materials and Methods* and *SI Materials and Methods*). Previous microbial experiments found that spatiotemporal heterogeneity among local communities induced by disturbance (25) and dispersal (26–28) events has a strong influence on species coexistence and biodiversity. In previous works (20, 22, 26, 28) the focus was mostly on dispersal distance, dispersal rates, and dispersal kernels and how they affect diversity patterns in relatively simple landscapes. These factors, directly affecting the history of community assembly (29, 30), introduce variability in community composition in terms of abundances and local species richness. We specifically studied basic mechanisms of dispersal and landscape structure on diversity patterns in metacommunities mimicking realistic network structures. Thus, our replicated and controlled experimental design sheds light on the role of connectivity in more structured metacommunities, disentangling complex natural systems' behavior (31).

Results and Discussion

We compared the RN and the 2D landscapes, focusing on three measures of biodiversity: the number of species present in a local community (α -diversity), among-community diversity (β -diversity), and the number of LCs in which a given species is present (species occupancy) (7). We found a significantly broader α -diversity distribution (Figs. 2 and 3A and B) in the RN compared with the 2D landscapes [measured as the coefficient of variation (CV), $\overline{CV}_{RN} = 0.265$, $\overline{CV}_{2D} = 0.122$, paired t test, $t_5 = 7.05$, $P = 0.0009$]. Furthermore β -diversity, here described by the spatial decay of Jaccard's similarity index (*Materials and Methods* and *SI Materials and Methods*), was higher in the RN compared with the 2D landscapes (Fig. 3C). Mean local species richness in RN was significantly lower compared with that in 2D landscapes

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¹F.C. and F.A. contributed equally to this work.

²To whom correspondence may be addressed. E-mail: florian.altermatt@eawag.ch or andrea.rinaldo@epfl.ch.

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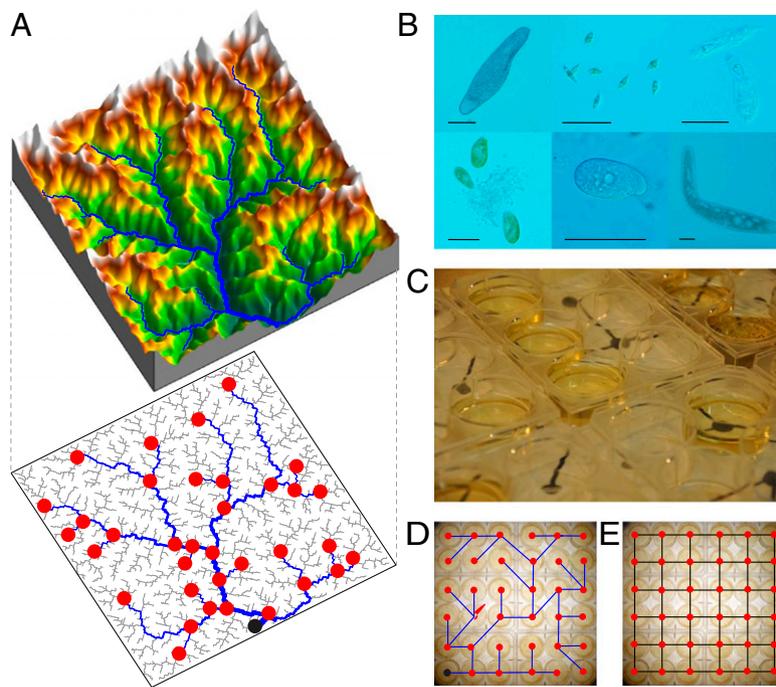


Fig. 1. Design of the connectivity experiment. (A) The river network (RN) landscape (*Lower*: red points label the position of LCs, and the black point is the outlet) derives from a coarse-grained optimal channel network (OCN) that reflects the 3D structure of a river basin (*Upper*). (B–E) The microcosm experiment involves protozoan and rotifer species. (B) Subset of the species (for names see *SI Materials and Methods*). (Scale bars, 100 μm .) (C) Communities were kept in 36-well plates. (D and E) Dispersal to neighboring communities follows the respective network structure: blue lines are for RN (D), same network as in A, and black lines are for 2D lattice with four nearest neighbors (E).

(Fig. 2 A–D, $\overline{\langle \alpha \rangle}_{\text{RN}} = 5.72$, $\overline{\langle \alpha \rangle}_{\text{2D}} = 6.72$, paired t test, $t_5 = 9.23$, $P = 0.0003$). These results confirm theoretical predictions on the role of directional dispersal from both individual- and metacommunity-based models (7, 32, 33). Specifically, we experimentally observe that the anisotropy induced by directional dispersal has a strong impact on the spatial configuration of the species occupancy, reflected in α - and β -diversity (Figs. 2 A–D and 3E). Anisotropy results in radically different distributions of closeness centrality, i.e., the mean geometric geodesic distance (34) and the mean distance l between all LC pairs (Fig. S2) in RN vs. 2D landscapes ($l_{\text{RN}} = 5.33$, $l_{\text{2D}} = 3$) (*SI Materials and Methods*).

In parallel to the experiment we developed a stochastic model, generalizing across spatial and temporal scales (*Materials and Methods*). The model embeds spatiotemporal environmental heterogeneity and is based on a Lotka–Volterra competition model. We simulated the dynamics of species competing for space and food resources on the same trophic level, subjected to periodic perturbation events consisting of partial habitat destruction. The model is an approximation to our experimental system, but does not contain trophic dynamics that may occur in the protozoa communities. Dispersal to neighboring patches can generate recolonization.

We measured species-specific intrinsic growth rates and carrying capacities in pure cultures (Fig. S3 and Table S1), and we used these specific values in the stochastic model, without fitting parameters (*Materials and Methods*). Even if estimates on growth rates and carrying capacities were already available for some species (21), we repeated these experiments to get direct values for our specific experimental conditions, i.e., illumination, nutrient levels, chamber temperature, and particular environment provided by well plates (volume and ratio of area to volume). The model confirmed the experimental observations: a higher variability for α -diversity (Fig. 2 E and F) and a higher β -diversity (Fig. 3C) in dendrites compared with lattice landscapes. These patterns were robust over a long time interval relative to species-intrinsic growth rates (Fig. 3D, Fig. S4, and *SI Materials and*

Methods). Furthermore, the patterns are consistent also at different spatial scales (Fig. 3D).

The bimodal shape of the α -diversity distribution observed in both model and experiment for the river network geometry (Fig. 3A) called for an analysis based on the degree of connectivity, d , which gives the number of connected neighboring nodes to a LC. In the “headwater” (H) class, LCs have $d_{\text{H}} = 1$ and are connected uniquely to their “downstream” node whereas in the “confluence” (C) class, LCs are characterized by $d_{\text{C}} = 3$ and are connected to two “upstream” and one downstream nodes. In our scenario, the terms downstream and upstream refer only to the position of the connected LC with respect to the outlet. They do not refer to a mass flow as dispersal is not directionally biased (7) (*Materials and Methods*). The outlet (O) of the network, connected only to its upstream node ($d_{\text{O}} = 1$), falls into the H class.

We found that the α -diversity distribution for Hs peaks at a significantly lower value compared with the peak of the Cs’ distribution ($\overline{\langle \alpha \rangle}_{\text{H}} = 5.29$, $\overline{\langle \alpha \rangle}_{\text{C}} = 6.10$, paired t test, $t_5 = 7.24$, $P = 0.0008$; Fig. S5) and exhibits higher variability (Fig. 4A). Fig. 2 A and E shows this pattern, in which the backbone of the river network exhibits on average a higher species richness with respect to peripheral communities.

To explain the variability of the local species richness in the RN, we included two other factors in our analysis: the “ecological diameter” l_i of the LC i (strictly related to its closeness centrality) and the temporal distribution of disturbance events. The ecological diameter is simply defined as the average distance $l_i = \langle d_{ij} \rangle_j$ of i from all of the other LCs j in the RN, where d_{ij} represents the shortest (geodesic) distance between i and j (34). We found that connectivity significantly affected α -diversity in the RN landscape (ANOVA, $F_{1,5} = 12.09$, $P = 0.0006$), whereas neither time to the last disturbance nor network centrality significantly affected local species richness (ANOVA, $F_{6,5} = 1.66$, $P = 0.13$; and $F_{4,5} = 0.71$, $P = 0.59$) (Fig. S6 and *SI Materials and Methods*).

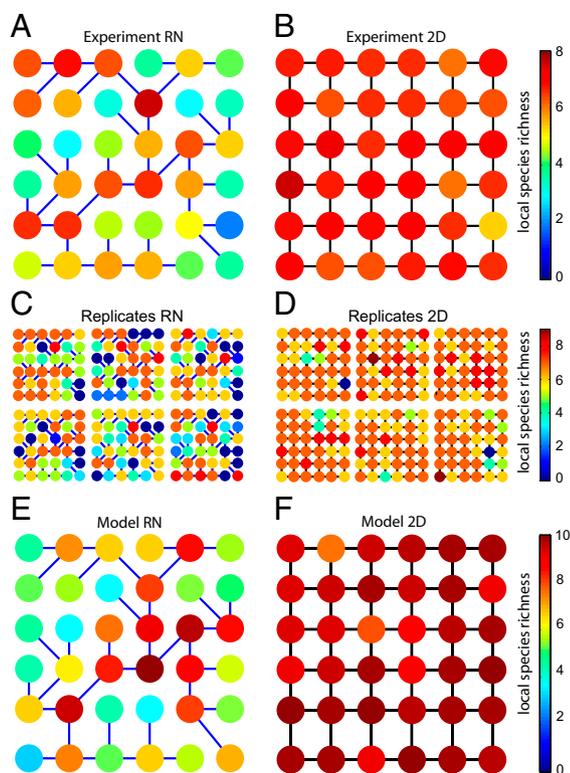


Fig. 2. Experimental and theoretical local species richness in river network (RN) and lattice (2D) landscapes. (A and B) Mean local species richness (α -diversity, color coded; every dot represents a LC) for the microcosm experiment averaged over the six replicates. (C and D) Species richness for each of these replicates individually. (E and F) The stochastic model predicts similar mean α -diversity patterns (note different scales).

We obtained β -diversity separately for headwaters and confluences, to test the difference in species composition within the river network structure. Headwaters exhibit not only a higher variability in α -diversity, but also a higher β -diversity compared with confluences (Fig. 4B), confirming patterns found in natural river basins (16, 18). Therefore, the difference in the loss of spatial correlation relative to lattice landscapes appeared even higher when only headwaters were considered in the comparison. These results reveal the crucial importance of headwaters as a source of biodiversity for the whole landscape. In natural systems other local environmental factors may play a role in structuring ecosystems (35). Nevertheless, our causal approach sheds light on the sole effect of directional dispersal on biodiversity. Note that the patterns we found in river network geometry are predicted to be even stronger in the presence of a downstream dispersal, which is typical for many passively transported riparian and aquatic species in river basins (19, 33).

We observed a lower mean α -diversity in the experiment compared with the theoretical predictions ($\Delta(\bar{\alpha})_{RN} = 37\%$, $\Delta(\bar{\alpha})_{2D} = 42\%$), but a rescaling to the experimental mean produced a consistent local species richness distribution (Fig. 3A and B). Species occupancies are presented in Fig. 3E as a rank-occupancy curve: Both the model and the experiment revealed that well-connected 2D landscapes presented higher spatial persistence compared with river network environments, but the sharp decrease in experimental rank-occupancy curves observed in both landscapes suggests that some species are disadvantaged. It is likely that species competition in the experiment had stronger effects on the persistence of weaker species than that generated in the model by pure competition for space (SI Materials and Methods).

At this point of the discussion the following question arises: How does the system react over these spatiotemporal scales, without any disturbance–dispersal events? We tested species' ability to coexist in an “isolation” treatment, under the same environmental conditions (Materials and Methods). We hypothesized that under stress (space saturation and reduced availability of bacteria) larger protozoans, such as *Blepharisma* and *Spirostomum* sp., could predate on smaller protozoans, such as *Chilomonas*, *Tetrahymena*, and *Colpidium* sp. (Table S1 for species' traits). The latter appeared to be strongly inferior competitors (Fig. S6). Note that predation could happen even at low protist densities and high bacterial densities.

We found that a consistent subset of four species survived at the end of the isolation experiment (Fig. S7), whereas all other species went mostly extinct, resulting in lower values of both α - and β -diversity ($\overline{CV}_{\text{Isolation}} = 0.086$, $\langle \bar{\alpha} \rangle_{\text{Isolation}} = 4.17$). The results confirmed the importance of dispersal and connectivity for maintaining higher levels of biodiversity observed in fragmented landscapes (Fig. 4A) (36, 37), at temporal scales over which competitive exclusion dynamics have emerged in isolated communities. Clearly, competition, although stronger than just for space and resources, has not altered the connectivity-induced patterns highlighted by both the theoretical and the experimental approaches.

Because the types of dispersal and disturbances used in our system are not specific to riverine environments, the above results apply to a variety of heterogeneous and fragmented environments. We suggest that species constrained to disperse within dendritic corridors face reduced spatial persistence and higher extinction risks. On the other hand, heterogeneous habitats sustain higher levels of among-community biodiversity that can be altered by modifying the connectivity of the system, with implications for community ecology and conservation biology.

Materials and Methods

Aquatic Communities. Each LC within a MC was initialized with nine protozoan species, one rotifer species, and a set of common freshwater bacteria as a food resource. The nine protozoan species were *Blepharisma* sp., *Chilomonas* sp., *Colpidium* sp., *Euglena gracilis*, *Euplotes aediculatus*, *Paramecium aurelia*, *Paramecium bursaria*, *Spirostomum* sp. and *Tetrahymena* sp., and the rotifer was *Cephalodella* sp.). *Blepharisma* sp., *Chilomonas* sp., and *Tetrahymena* sp. were supplied by Carolina Biological Supply, whereas all other species were originally isolated from a natural pond (38) and have also been used for other studies (21, 22). We use the same nomenclature as in such studies, except for *Cephalodella* sp., which has been previously identified as *Rotaria* sp. All species are bacterivores whereas *E. gracilis*, *E. aediculatus*, and *P. bursaria* can also photosynthesize. Furthermore, *Blepharisma* sp., *Euplotes aediculatus*, and *Spirostomum* sp. may not only feed on bacteria but also can predate on smaller flagellates. Twenty-four hours before inoculation with protozoans and rotifer, three species of bacteria (*Bacillus cereus*, *Bacillus subtilis*, and *Serratia marcescens*) were added to each community. LCs were located in 10-mL multiwell culture plates containing a solution of sterilized local spring water, 1.6 g·L⁻¹ of soil, and 0.45 g·L⁻¹ of Protozoan Pellets (Carolina Biological Supply). Protozoan Pellets and soil provide nutrients for bacteria, which are consumed by protozoans. We conducted the experiment in a climatized room at 21 °C under constant fluorescent light. On day 0, 100 individuals of each species were added, except for *E. gracilis* (500 individuals) and *Spirostomum* (40 individuals), which naturally occur, respectively, at higher and lower densities. We determined species' intrinsic growth rate r and carrying capacity K in pure cultures, at identical conditions (Species' Traits: Population Growth below).

Landscapes. Each MC consisted of 36 LCs, connected according to two different schemes: a lattice network in which each LC has four nearest neighbors with periodic boundaries (2D landscape) and a coarse-grained RN structure, obtained from a 200 × 200 space filling optimal channel network (OCN) (13, 39, 40), with an appropriate threshold on the drainage area (SI Materials and Methods). In the RN landscape a LC has either three nearest neighbors (C) or one nearest neighbor (H). Landscapes of these two dispersal treatments were replicated six times. Furthermore, we had MCs of the isolation treatment, replicated three times.

macroscopic solutions may not be negligible. Thus, we performed numerical simulations using the Gillespie algorithm (45), which allows us to produce time series that exactly recover the solution of the multivariate master equation in Eq. 11 with transition probabilities in Eqs. 9 and 10. Edge effects in the lattice landscape are removed by imposing periodic boundary conditions. The dynamics of the system are stochastically perturbed to include diffusive dispersal of individuals across patches and spatially uncorrelated environmental disturbances, reflecting the experimental conditions. A simulation ends when the system has reached monodominance. Actually, in the experimental disturbance regime (and without any speciation process taken

into account), only the species with the highest growth rate survives in the simulations.

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