HIGHLIGHTED STUDENT RESEARCH

Microbial community assembly in a multi-layer dendritic metacommunity

Nathan I. Wisnoski[1] · **Jay T. Lennon**[1]

Abstract

A major goal of metacommunity ecology is to infer the local- and regional-scale processes that underlie community assembly. In dendritic ecological networks, branching patterns and directional flow can alter the balance between local and regional factors during assembly. Vertical habitat structure may further affect community assembly in dendritic metacommunities. In this study, we analyzed the bacterial metacommunity of a fifth-order mountain stream network to assess differences in community assembly (1) between planktonic and benthic habitats, (2) across spatial scales, and (3) between headwater and downstream regions of the network. Using taxonomic and phylogenetic null modeling, we found habitat-specific spatial patterns of community assembly across the dendritic network. Compositional differences between planktonic and benthic communities were maintained by variable selection, but we also found evidence of local dispersal limitation between the two habitats. Planktonic community assembly was scale dependent, transitioning from homogeneous selection at local scales to variable selection at regional scales, while benthic community assembly was less scale dependent. Variable selection structured headwaters in both habitat types, but downstream communities were primarily structured by homogeneous selection, especially in sediments. Taken together, our results show that vertical habitat structure contributes to the scale-dependent processes of community assembly across the dendritic metacommunity.

Keywords Metacommunity · Community assembly · Stream · Microbial ecology · Dendritic network

Introduction

Metacommunity ecology examines the assembly, structure, and diversity of communities with an emphasis on the

---

Communicated by Jamie M. Kneitel.

In dendritic ecological networks, such as streams, branching patterns and directional flow can alter the balance between local and regional processes underlying community assembly. Streams also contain vertical habitat structure, encompassing planktonic and benthic habitats that impose contrasting sets of environmental filters and differ in spatial connectivity within the metacommunity. In this study, we demonstrated that the relative importance of community assembly processes in a stream bacterial metacommunity was not only spatially variable across the network, but also dependent on spatial scale. Furthermore, the strength and pattern of scale-dependence differed within and between vertical habitats in the watershed, suggesting the metacommunity resembles a multi-layer dendritic network.

✉ Nathan I. Wisnoski
    wisnoski@indiana.edu

[1] Department of Biology, Indiana University, Bloomington, IN, USA

interplay between local- and regional-scale processes (Leibold and Chase 2018). At the local scale, environmental filtering and species interactions influence assembly through the deterministic process of selection (Vellend 2016; Leibold and Chase 2018). Selection can lead to the assembly of similar communities in similar habitats (i.e., homogeneous selection) or dissimilar communities in dissimilar habitats (i.e., variable selection) (Stegen et al. 2015). The metacommunity framework also incorporates the effects of dispersal and stochastic processes on community assembly (Mouquet and Loreau 2003; Zhou and Ning 2017). For example, dispersal limitation can account for compositional dissimilarity between communities in similar habitats, while rampant dispersal can homogenize communities across dissimilar habitats due to mass effects. Therefore, selection should play a prevailing role in structuring communities when dispersal is low but non-limiting.

While the direction of dispersal in an idealized metacommunity is often assumed to be random, some ecosystems have physical features that impose directionality. For example, stream and river ecosystems represent dendritic networks with hierarchical, branching connectivity that

constrains and directionally orients dispersal (Fig. 1a) (Grant et al. 2007; Brown et al. 2011; Carrara et al. 2012; Altermatt 2013). As a result, some sites in dendritic networks are more isolated and less connected than others. For example, headwater streams are separated by elongated dispersal routes along the stream network that may exceed the dispersal capabilities of some organisms. At the same time, dispersal is counteracted by prevailing downstream flows that further reduce headwater connectivity within the metacommunity (Brown et al. 2011; Altermatt 2013; Tonkin et al. 2018). Many headwater communities (e.g., benthic macroinvertebrates) are assembled by selection, while downstream communities show greater environmental mismatch due to high rates of dispersal from upstream (i.e., mass effects) (Brown

Fig. 1 The dendritic metacommunity structure of stream ecosystems with vertical habitat structure. **a** Map of sampling locations within H. J. Andrews Experimental Forest ($n = 49$). Sampling was conducted extensively across the broader Lookout Creek watershed and intensively within small Watershed 1 (lower left). Imagery sourced from Google Earth Pro, with stream network sourced from H.J. Andrews Experimental Forest data portal. **b** A lateral cross-section showing the vertical habitat structure in the stream channel. Bacterioplankton occur in the water column, while sediment-attached biofilms line the benthic habitat. **c** A longitudinal cross-section of the differences in spatial connectivity between planktonic and benthic habitats, where plankton are hypothesized to have higher dispersal than sediment-attached bacteria
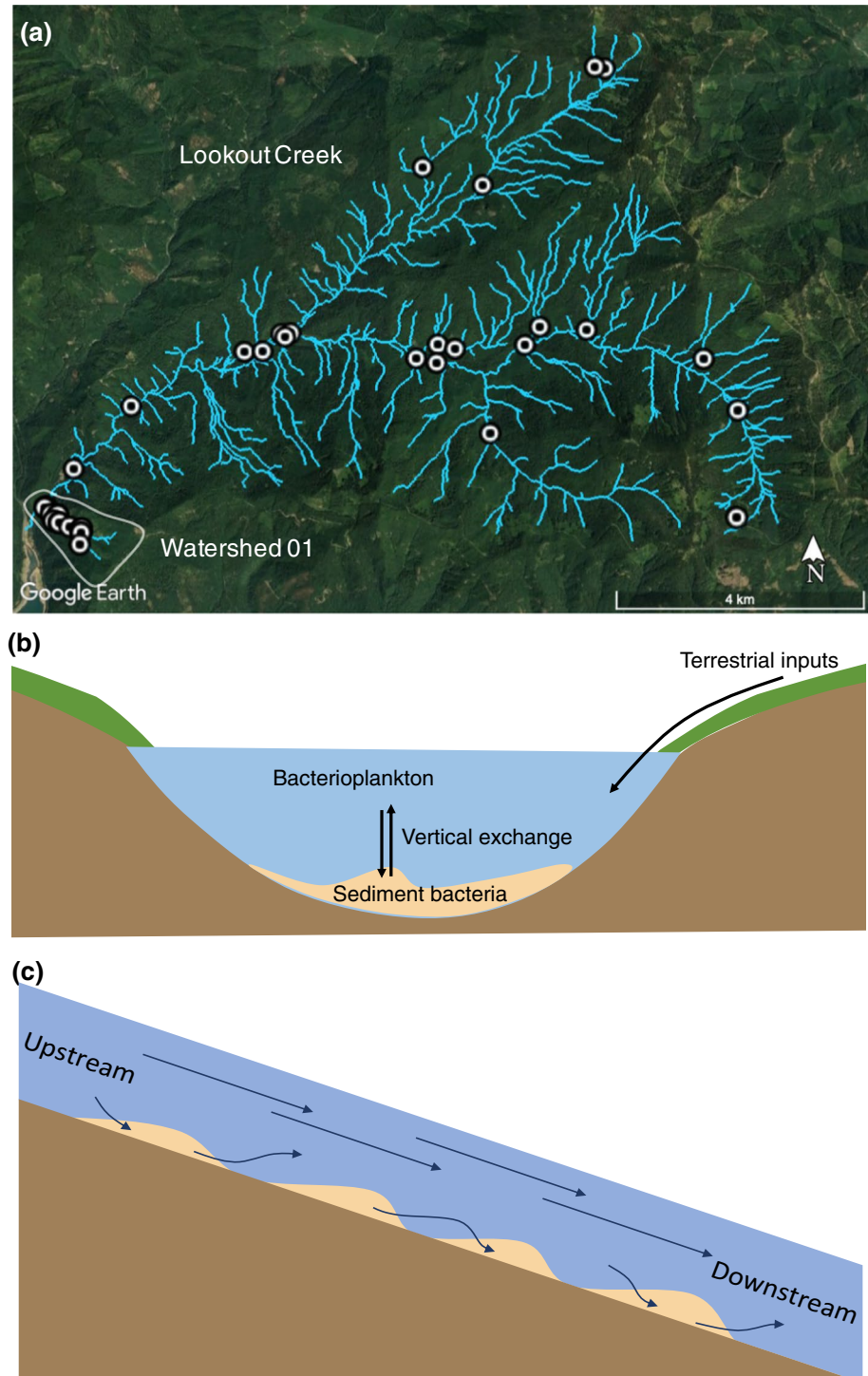
and Swan 2010; Tornwall et al. 2017). However, different patterns have been documented for other taxonomic groups with limited upstream-dispersal vectors, such as passively dispersing microorganisms. For these communities, headwater assemblages experience high rates of immigration from surrounding terrestrial ecosystems that can disrupt selection (Ruiz-González et al. 2015; Battin et al. 2016). Terrestrial-derived bacteria are gradually filtered out as they disperse downstream, where homogeneous selection becomes the dominant process as stable planktonic communities establish in reaches with longer residence times (Read et al. 2015; Savio et al. 2015; Ruiz-González et al. 2015; Hassell et al. 2018).

Another feature of dendritic systems that is not considered by classical metacommunity theory is that they commonly exhibit vertical habitat structure (Fig. 1b). In streams, planktonic organisms inhabiting the water column experience vastly different physical environments than benthic organisms living in the sediment matrix of the streambed (Hart and Finelli 1999). As a result, different sets of environmental filters may influence the composition of planktonic and benthic bacterial communities (Besemer et al. 2012; Wilhelm et al. 2013). For example, planktonic microorganisms must contend with changes in resource availability, pH, predation, and hydrology (Fierer et al. 2007; Read et al. 2015; Niño-García et al. 2016), while benthic communities experience additional constraints, such as shear stress, space limitation in biofilms, and fluctuating redox conditions resulting from surface water-groundwater mixing (Battin et al. 2016). The different flow environments of benthic and planktonic habitats could also affect bacterial dispersal rates and community assembly (Battin et al. 2016). For example, bacterioplankton presumably have high dispersal rates that increase the potential for mass effects, while bacteria in sediment biofilms disperse downstream intermittently (Leff et al. 1992), increasing the potential for selection (Fig. 1c). However, the two habitats are not completely separate, as planktonic-benthic mixing introduces a vertical axis of dispersal allowing plankton to colonize sediments and sediment-associated bacteria to be suspended in the water column (Leff et al. 1992; Freimann et al. 2015), which may influence community structure at relevant scales of hydrological exchange. These habitat-specific differences in environmental filters and dispersal could alter the relative importance of community assembly processes underlying local and regional diversity by influencing their spatial distributions in the dendritic network.

In this study, we analyzed bacterial diversity in a dendritic metacommunity while considering not only directional flow, but also the vertical habitat structure separating stream sediments from the overlying water column in a fifth-order mountain stream network. Using taxonomic and phylogenetic approaches, we tested whether the relative importance of community assembly processes varied (1) between planktonic and benthic habitats, (2) across spatial scales, and (3) along the longitudinal (i.e., headwater versus downstream) stream dimension in the dendritic metacommunity.

Methods

Study site

H.J. Andrews Experimental Forest (44.2° N, 122.2° W) is a 6400-hectare conifer forest in the Western Cascade Range, Oregon, USA. Andrews Forest is a Long-Term Ecological Research (LTER) site that contains the Lookout Creek watershed, a fifth-order, mountainous (410–1630 m elevation) catchment of high gradient streams that drains to the McKenzie River (Fig. 1). The underlying geology is volcanic and dates back to the Oligocene, with Miocene-age andesite lava flows at higher elevations (Swanson and James 1975). Catchment topography is steep with confined valleys, and precipitation filters through loamy, organic soils to the stream (Harr 1977). Streams are boulder-dominated, with step–pool, riffle–pool, and cascade reaches. At lower elevations, vegetation is primarily Douglas fir (*Pseudotsuga menziesii*), western hemlock (*Tsuga heterophylla*), and western red cedar (*Thuja plicata*). Pacific silver fir (*Abies amabilis*) and noble fir (*Abies procera*) are present at higher elevations. The climate is Mediterranean, with peak precipitation between October and April. Mean annual precipitation is 230 cm at low elevations and 355 cm at high elevations (McKee and Bierlmaier 1987), and a sizeable snowpack accumulates above 900 m (Daly et al. 2010).

Sampling

In June 2015, we sampled streams in the Lookout Creek watershed of H.J. Andrews Experimental Forest (Fig. 1). Our sampling design was hierarchical, such that lower-order stream sites were nested within branches of higher-order stream sites. Our samples ($n = 49$) spanned all five stream orders of Lookout Creek, where headwaters are 1st-order streams (1st-order, $n = 9$; 2nd-order, $n = 24$; 3rd-order, $n = 11$; 4th-order, $n = 3$, and 5th-order, $n = 2$). We sampled more lower- and middle-order streams than higher-order streams, because lower-order streams comprise a larger portion of the stream network and are more spatially heterogeneous. We sampled major confluences across the catchment. Each sampling location was geo-referenced using handheld GPS. At each site, we measured

temperature, pH, and conductivity in the stream using a YSI 6920 V2-2 water quality sonde (YSI Incorporated, Yellow Springs, OH). We preserved water samples with HCl to pH 2 for chemical analyses in the laboratory. With the preserved water samples, we measured total nitrogen (TN) after persulfate digestion using the second derivative method (Bachmann and Canfield 1996) and total phosphorus (TP) using the ammonium molybdate method (Prepas and Rigler 1982). Dissolved organic carbon (DOC) was measured in 0.7-μm glass fiber filtered samples by oxidation and nondispersive infrared detection on a Shimadzu TOC-V (Kyoto, Japan). These environmental variables were used to capture longitudinal patterns in environmental conditions in the stream network.

To characterize bacterial communities, we sampled planktonic and sediment-associated microbial biomass for high-throughput community sequencing at each site. We sampled planktonic microorganisms by filtering 1 L of surface water onto 47 mm 0.2-μm Supor Filters (Pall, Port Washington, NY) in the field. We sampled sediment-associated communities (of sediment grain < 1 cm in diameter) using a sediment corer. All samples were frozen on dry ice in the field and preserved at − 20 °C until processing. To get an integrated sample of sediment-associated bacteria at each site (and to sample a larger mass of sediments than would fit in an extraction kit tube), we detached bacterial cells from sediment biofilms by gently sonicating 5 g of sediment in a 1% tetrasodium pyrophosphate solution for 10 min in pulses of 10 s on, 5 s off. We then used the cell suspension for downstream analysis of the sediment-associated community.

Sequence preparation and processing

We characterized bacterial community composition by sequencing the 16S rRNA gene (Caporaso et al. 2012). We extracted DNA from surface water samples using the PowerWater DNA isolation kit (MoBio, Carlsbad, CA) and from the sediment extractions using the PowerSoil DNA isolation kit (MoBio, Carlsbad, CA). While differences in extraction kit chemistry have the potential to introduce biases in downstream analyses, we selected kits optimized to specific substrates with the assumption that it would yield representative samples of each habitat type. Recent comparisons demonstrate the high quality of PowerWater and PowerSoil extractions from their respective substrates, with similar inferences of community structure from a known mock community (Hermans et al. 2018). We PCR-amplified the V4 region of the 16S rRNA gene using barcoded primers (515F and 806R) for the Illumina MiSeq platform. Per each 50 μl reaction, PCR conditions were the following: 5 μl of 10X Perfect Taq Plus PCR Buffer (5Prime), 10 μl 5P

solution (5Prime), 0.25 μl Perfect Taq Plus DNA Polymerase (5Prime), 1 μl dNTP mix (10 mM each), 1 μl 515F forward primer (10 μM), 1 μl 806R reverse primer (10 μM), and 10 ng of template. Thermal cycler conditions were 3 min at 94 °C, 30 cycles of (45 s at 94 °C, 30 s at 50 °C, and 90 s at 72 °C), then 10 min at 72 °C. Sequence libraries were cleaned using AMPure XP purification kit, quantified using Quant-iT PicoGreen dsDNA assay kit (Invitrogen), and pooled at equal concentrations of 10 ng per library. We sequenced the pooled libraries on the Illumina MiSeq platform at the Indiana University Center for Genomics and Bioinformatics using 300 × 300 bp paired end reads (600-cycle Reagent Kit v3). We processed the raw reads using *mothur* (v. 1.41.1) to remove non-bacterial sequences and low-quality reads (quality score < 25), and removed chimeras with VSEARCH (Schloss et al. 2009; Rognes et al. 2016). We classified OTUs with the OptiClust algorithm (Westcott and Schloss 2017) based on 97% similarity using the SILVA rRNA database version 132 (Quast et al. 2013). All further analyses were conducted in R version 3.5.3 (R Core Team 2018).

Diversity analyses

We analyzed taxonomic patterns of diversity within and between planktonic ($n = 29$) and benthic sediment ($n = 20$) habitats in the metacommunity. First, we rarefied each sample to a total number of 10,623 reads (the smallest sample with > 10,000 reads), and relativized reads for each OTU to the size of each sample using the R package *vegan* (v. 2.5-6) (Oksanen et al. 2019). As a measure of within-site (α) diversity, we used the exponential of Shannon's index, which corresponds to the number of equally abundant species needed to obtain the value of Shannon diversity obtained on the original data (Jost 2007). To measure differences in community structure among sites (β-diversity), we calculated pairwise dissimilarities between communities using the Bray–Curtis dissimilarity. To determine whether β-diversity was related to categorical features of the stream network, such as habitat type, stream order, and watershed, we used PERMANOVA (Anderson 2001). We used distance-based redundancy analysis (dbRDA) to quantify the importance of quantitative environmental variables (TP, TN, DOC, pH, elevation, conductivity) for explaining β-diversity (Legendre and Legendre 2012). We used multiple regression to quantify how community dissimilarity changed with increasing dendritic distance (i.e., along the stream network path) between sites within and between habitat types. We calculated dendritic distances in Google Earth using GIS layers of the H.J. Andrews stream network created from LIDAR imaging.

Community assembly processes

We used a null model approach to distinguish deterministic selection from stochastic assembly processes across the stream network (Chase et al. 2011; Chase and Myers 2011; Stegen et al. 2015). In this approach, we used taxonomic and phylogenetic information from the bacterial sequencing efforts. Phylogenies organize bacterial taxa by their evolutionary history and can inform mechanisms of community assembly if broad-scale, ecologically relevant traits map onto phylogenetic relatedness (Cadotte and Davies 2016). Thus, environments may select for phylogenetically similar subsets of taxa from the metacommunity that possess traits necessary to colonize the local habitat. Homogeneous selection was inferred when pairwise phylogenetic β-diversity was lower than expected under stochastic assembly. In contrast, heterogeneous selection (i.e., dissimilar environments favoring dissimilar taxa) was inferred when phylogenetic β-diversity was greater than stochastic expectations.

To calculate phylogenetic β-diversity, we first created a phylogeny of all the OTUs in the stream network

$$\beta_{RC,Bray-Curtis} = 2\left(\frac{1 * \sum(Bray_{null} > Bray_{observed}) + 0.5 * \sum(Bray_{null} = Bray_{observed})}{1000} - 0.5\right)$$

using a double-precision, approximately maximum-likelihood approach with the program FastTree v. 2.1.8 (Price et al. 2010). Using the *picante* R package (Kembel et al. 2010), we computed the β-Mean Nearest Taxon Distance (βMNTD), an abundance-weighted community-scale measure of the mean phylogenetic relatedness of each OTU within a community compared to its most closely related OTU in a second community. We generated null distributions ($n = 999$) of βMNTD by randomly shuffling the tips of the phylogenetic tree. Because the contribution of rare taxa to βMNTD is small yet computationally intensive, we performed this analysis using only the OTUs detected at least 10 times in the metacommunity ($n \approx 5,700$). For each pair of sites i and j, we then compared the observed βMNTD values to the null distribution for the site-pair to calculate the β-Mean Nearest Taxon Index (βNTI), which quantifies the degree of phylogenetic turnover relative to expected turnover under stochastic community assembly:

$$\beta NTI_{i,j} = \frac{\beta MNTD_{i,j} - \mu_{i,j}}{\sigma_{i,j}},$$

where $\beta MNTD_{i,j}$ is the observed mean nearest taxon distance and the null distribution is described by its mean ($\mu_{i,j}$) and variance ($\sigma_{i,j}$). Thus, βNTI is a z-score, and deviations are considered significant if $|\beta NTI| > 2$, where values greater

than 2 indicate variable selection and values less than –2 indicate homogeneous selection.

To classify assembly in sites with non-significant βNTI values (i.e., weak selection), we compared observed taxonomic β-diversity to expectations generated by a stochastically assembled null model. For a pair of sites, high dispersal should decrease β-diversity from stochastic expectations, but dispersal limitation should increase β-diversity (Chase et al. 2011; Chase and Myers 2011). To quantify the contributions of these two processes, we used the abundance-based Raup–Crick approach to generate distributions of expected dissimilarity values for each site-pair using Bray–Curtis dissimilarity ($n = 999$ permutations) (Stegen et al. 2013). The stochastic assembly null model was performed in the following way: OTUs were randomly selected in proportion to their regional site incidence, individuals were then sequentially and randomly added to local communities in proportion to their regional relative abundances, and total abundances of assembled communities were constrained to match observed total abundances. For each pair of sites, observed Bray–Curtis dissimilarity was compared to the site-specific null distribution to compute $\beta_{RC, Bray–Curtis}$:

where $\sum(Bray_{null} > Bray_{observed})$ is the number of null Bray–Curtis dissimilarities greater than observed values and $\sum(Bray_{null} = Bray_{observed})$ is the number of ties. After this calculation, $\beta_{RC, Bray–Curtis}$ ranges from −1 to 1. Deviations from null expectation were inferred when $|\beta_{RC, Bray–Curtis}| > 0.95$, with $\beta_{RC, Bray–Curtis} > 0.95$ indicating possible dispersal limitation and $\beta_{RC, Bray–Curtis} < -0.95$ indicating potential mass effects. Remaining site pairs were classified as undominated by any particular assembly mechanism (Stegen et al. 2015).

Scale-dependent and longitudinal patterns of assembly

Finally, we investigated whether the relative importance of community assembly processes varied across spatial scales and along the longitudinal axis of the stream network. When assessing the scale-dependence of community assembly processes in the dendritic metacommunity, we only compared sites that were hydrologically connected by flow (i.e., hierarchical upstream–downstream linkages but not among hydrologically disconnected headwaters). We calculated the dendritic distance separating each pair of sites, rounding distances to the nearest $\log_{10}(m)$ to generate discrete distance classes spanning five orders of magnitude (pairwise comparisons per distance class: 1–10 m, $n = 24$;

10–100 m, $n = 24$; 100–1000 m, $n = 216$; 1–10 km, $n = 284$; > 10 km, $n = 148$). We calculated the proportion of each assembly mechanism inferred within each distance class and quantified the frequencies of community assembly mechanisms at increasing spatial scales within and between planktonic and benthic habitats. In addition, we leveraged the nested structure of our sampling design, evaluating patterns of diversity within the overall Lookout Creek watershed and within the nested sub-watershed, Watershed 01 (Fig. 1a).

Given the dendritic structure of the stream network, we also examined the longitudinal variation (i.e., headwaters versus downstream) in community assembly processes. Specifically, we quantified how βNTI and $\beta_{RC, \, Bray–Curtis}$ varied with habitat type (i.e., within benthic sediments, within planktonic samples, and between habitats) and network position (headwater streams versus downstream). For this analysis, we analyzed all pairs of sites (flow-connected and unconnected), and grouped them into the following categories: headwater plankton ($n = 10$), headwater sediments ($n = 6$), downstream plankton ($n = 276$), downstream sediments ($n = 120$), headwater sediment-plankton comparisons ($n = 20$), and downstream sediment-plankton comparisons ($n = 384$). We inferred assembly processes as described above and then computed the proportion of each community assembly process observed in each category.

Results

Patterns of α- and β-diversity

Planktonic and benthic bacterial communities differed in α-diversity. On average, we observed 20% higher α-diversity in the bacterioplankton than in sediment-associated communities (species equivalents: 1789 ± 101 in sediments, 2210 ± 131 in plankton, $p = 0.002$, $F_{1,47} = 10.28$). Bacterioplankton also contained > threefold more habitat-specific taxa (i.e., taxa never found in sediment samples) than sediment-associated communities ($20.5 \pm 0.9\%$ unique in planktonic taxa vs. $6.2 \pm 0.7\%$ unique sediment taxa, $p < 0.001$, $F_{1,47} = 219.3$).

Patterns of β-diversity suggest key differences in community structure within and between habitat types, across stream orders, and across spatial scales. Across the network, variation in bacterial community structure was explained primarily by the habitat from which the samples were taken (PERMANOVA, $R^2 = 0.15$, $p = 0.001$), and moderately by the stream order of the sampling site ($R^2 = 0.036$, $p = 0.002$) and the spatial extent of the drainage basin (i.e., spanning the entire Lookout Creek watershed or the smaller, nested Watershed 01), where the samples were collected ($R^2 = 0.04$, $p = 0.006$). Distance-based redundancy analysis (dbRDA)
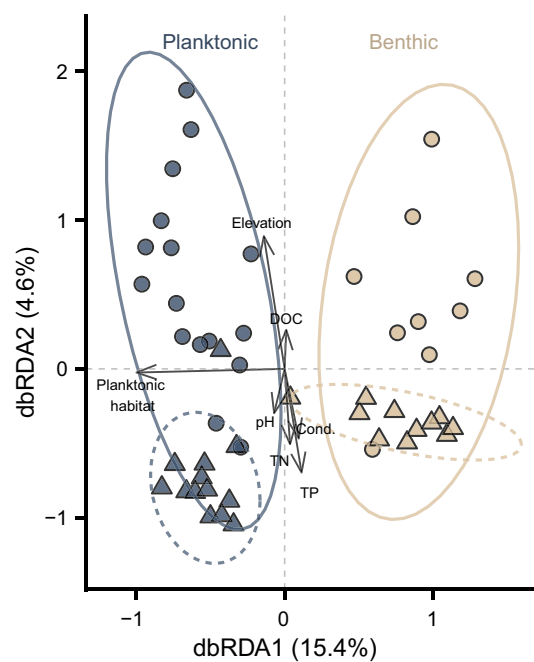

Fig. 2 Taxonomic β-diversity revealed compositional differences between and within benthic and planktonic habitats. Distance-based redundancy analysis (dbRDA) found the primary axis of variation in community composition could be explained by habitat type (i.e., planktonic or benthic). Within habitats, a secondary axis of variation explained a gradient from high elevation, low conductivity sites in the headwaters, to low elevation sites with high conductivity in the higher order streams. The dbRDA2 axis also captured differences in spatial scale of sampling, with sites from Watershed 01 clustering together (triangles, dashed ellipses, $n = 24$), nested within sites distributed across the broader Lookout Creek catchment (circles, solid ellipses, $n = 25$). Beige symbols indicate benthic samples ($n = 20$) and blue samples indicate planktonic samples ($n = 28$). Ellipses are 95% confidence intervals for the group locations in the dbRDA subspace

detected a separation between bacterioplankton and sediment samples along dbRDA1, which explained 15.4% of the variation (Fig. 2). Along dbRDA2, samples separated along a gradient that captured elevation and resource availability. Specifically, we identified communities that clustered in high elevation sites with relatively high dissolved organic carbon (DOC) concentrations and communities that clustered in low elevation sites with higher total phosphorus (TP), total nitrogen (TP), conductivity, and pH. Sites in Watershed 01 also clustered together along dbRDA2 more tightly than sites dispersed across the broader Lookout Creek watershed.

As expected, spatially isolated sites in the dendritic network were more compositionally dissimilar than nearby sites (Fig. 3). However, dissimilarity increased at the same rate in both planktonic and sediment communities (Table 1). The dissimilarity between communities in different habitat types was consistently higher than within-habitat differences from local (y-intercept) to regional scales (~ 10 km).
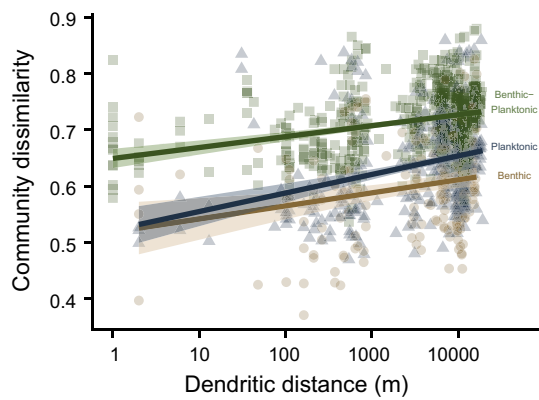
Fig. 3 Community dissimilarity (Bray–Curtis) increased with dendritic distance in the network. Comparisons between sites in different habitat types (green squares, $n = 404$) had the highest dissimilarity at all spatial scales. Comparisons within planktonic samples (blue triangles, $n = 286$) and within benthic samples (beige circles, $n = 126$) were not statistically different from one another. The rates of increase in community dissimilarity with dendritic distance were not statistically different for any of the habitat comparisons. Linear model output for these relationships can be found in Table 1

Scale-dependent community assembly

Bacterial community assembly in the larger Lookout Creek stream network was habitat and scale dependent. Overall, hydrologically connected communities predominantly showed evidence of selection (homogeneous: $246/696 = 35.3\%$ of comparisons; variable: $374/696 = 53.7\%$ of comparisons), with some evidence for dispersal limitation ($60/696 = 8.6\%$), as well as assembly that was undominated by any particular mechanism ($16/696 = 2.3\%$) (Fig. 4). We did not detect a signature of mass effects in the catchment. Within communities of the same habitat type, homogeneous selection was the dominant process (sediments:

$88/134 = 65.7\%$; plankton: $142/214 = 66.4\%$). Planktonic communities showed strong evidence for homogeneous selection from local to regional scales, but we detected increasing evidence for variable selection ($32/46 = 70\%$ of comparisons > 1 km apart) at broader scales. Benthic communities showed strong signatures of homogeneous selection across all spatial scales in the catchment (1 m–10 km), with variable selection also playing a role at the reach scale (< 10 m) and at larger scales (> 100 m). In benthic communities, evidence for assembly undominated by any one mechanism occurred at the local (1–10 m) scale and at increasingly broad (> 1 km) scales. Within both habitats, dispersal limitation became increasingly evident at broader spatial scales (> 1 km).

Between communities in different habitats, variable selection was the dominant assembly mechanism ($286/348 = 82.2\%$ of comparisons), increasing in importance at broader spatial scales. In contrast to within-habitat comparisons, dispersal limitation between planktonic and benthic habitats occurred at local scales and decreased in importance with increased scale.

Longitudinal trends in community assembly

We also found evidence of habitat-specific differences in assembly between headwater and downstream bacterial communities (Fig. 5). In particular, variable selection maintained differences among headwaters ($7/10 = 70\%$ of planktonic comparisons, $5/6 = 83.3\%$ of benthic comparisons, and $17/20 = 85\%$ of planktonic-benthic comparisons). Downstream, homogeneous selection was the dominant process in planktonic ($137/276 = 49.6\%$ of comparisons) and benthic ($79/120 = 65.8\%$ of comparisons) communities, but variable selection remained the dominant process operating between habitats ($346/384 = 90.1\%$ of comparisons). We found an instance of dispersal limitation between headwater plankton ($1/10 = 10\%$ of comparisons) and between

Table 1 Linear model output for the relationship depicted in Fig. 3

Model term	Estimate	Std. error	t value	P value
Intercept	0.52	0.02	24.84	2×10^{-16}
Log_{10} (dendritic distance + 1)	0.024	5.9×10^{-3}	4.06	5.3×10^{-5}
Habitat (planktonic)	4.5×10^{-3}	0.027	0.17	0.87
Habitat (planktonic-benthic)	0.13	0.024	5.53	4×10^{-8}
Dendritic distance × habitat (planktonic)	9.4×10^{-3}	7.4×10^{-3}	1.27	0.21
Dendritic distance × habitat (planktonic-benthic)	-3.6×10^{-3}	6.6×10^{-3}	-0.54	0.59

The model was constructed using simple multiple regression of the Bray–Curtis dissimilarity between sites on the $\log_{10}$ (dendritic distance + 1 m) between the sites, the differences in habitat type between the site comparisons, and the interaction between dendritic distance and habitat type. In the model, the intercept, dendritic distance, and between-habitat terms were significant. The significant distance term indicates that increasing distance between sites was associated with increased Bray–Curtis dissimilarity. The habitat terms indicate that, compared to the arbitrary baseline of the benthic habitat, pairwise comparisons in the bacterioplankton had similar average dissimilarities, but between-habitat comparisons had higher average dissimilarities. The interaction terms were not significant, indicating no differences among habitats in the rates at which community dissimilarity increased with dendritic distance
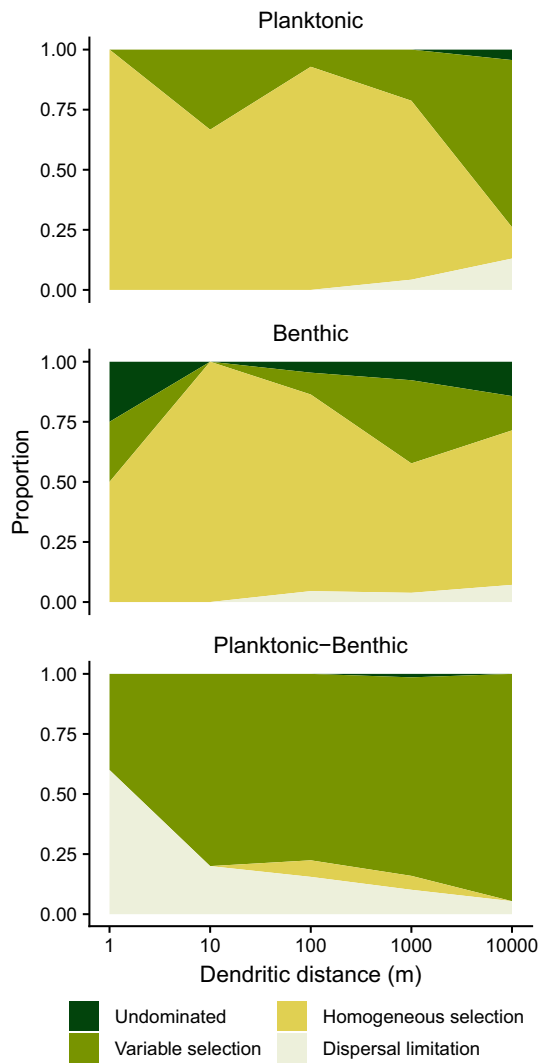
Fig. 4 Habitat and scale dependent community assembly mechanisms in the dendritic metacommunity. Within planktonic habitats, there was a transition from homogeneous to variable selection with increasing spatial scale. In benthic communities, homogeneous selection was the dominant assembly mechanism across all spatial scales. Variable selection and undominated assembly occurred at local and regional scales. Dispersal limitation within habitats increased in frequency with spatial scale. Between planktonic and benthic habitats, variable selection was the dominant mechanism inferred across most spatial scales, but dispersal limitation between habitats was common at local scales. The number of comparisons within each distance class was: 1–10 m ($n = 24$), 10–100 m ($n = 24$), 100–1000 m ($n = 216$), 1–10 km ($n = 284$), and > 10 km ($n = 148$)

Fig. 5 Habitat dependence in longitudinal patterns of community assembly. Across headwater communities, variable selection was the dominant assembly mechanism. Downstream, benthic and planktonic communities shifted towards primarily homogeneous selection (with variable selection, dispersal limitation, and undominated assembly also detected), while variable selection remained the dominant process underlying differences between planktonic and benthic communities. The number of site comparisons for each habitat and network position were: headwater plankton ($n = 10$), headwater sediments ($n = 6$), downstream plankton ($n = 276$), downstream sediments ($n = 120$), headwater sediment-plankton comparisons ($n = 20$), and downstream sediment-plankton comparisons ($n = 384$)

one headwater planktonic-benthic comparison, but most evidence for dispersal limitation was detected downstream (49/276 = 17.8% of planktonic comparisons, 9/120 = 7.5% of benthic comparisons, and 29/384 = 7.6% of planktonic-benthic comparisons). Last, we found that some downstream communities were undominated by any individual assembly process (12/276 = 4.3% of planktonic comparisons, 15/120 = 12.5% of benthic comparisons, and 1/384 = 0.26% of planktonic-benthic
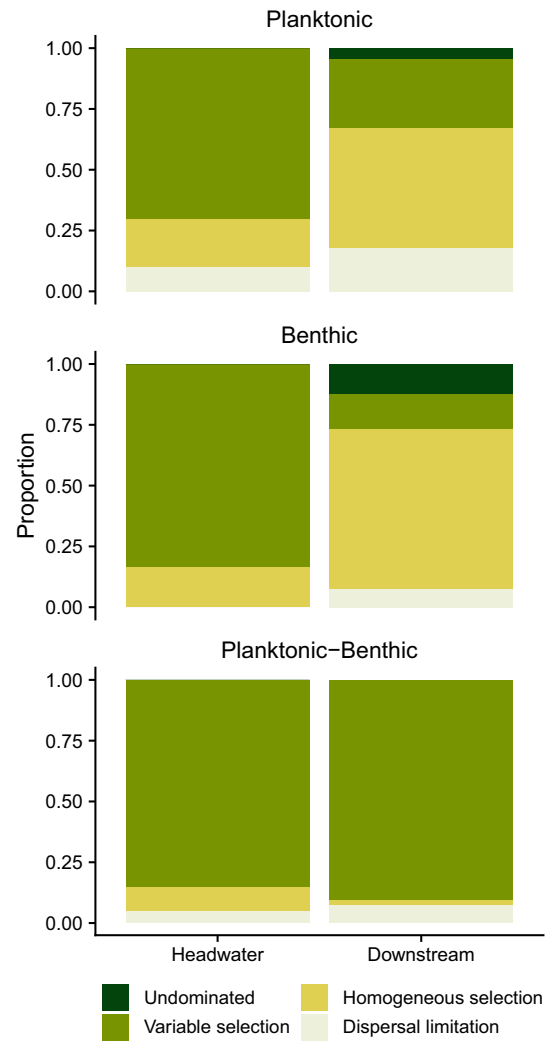
comparisons). Thus, variable selection among headwaters transitions to habitat-dependent shifts in community assembly processes downstream.

Discussion

We have shown that bacterial community assembly in a dendritic metacommunity depends on vertical habitat structure, spatial scale, and network position. Overall, deterministic

selection was the predominant assembly mechanism across the stream network. Homogeneous selection was consistently strong across spatial scales within benthic communities, while planktonic communities transitioned from homogeneous to variable selection with increasing spatial scale. Dispersal limitation within each habitat emerged at broader spatial scales. Variable selection maintained compositional differences between habitats at all scales, but local-scale dispersal limitation between planktonic and benthic habitats suggested that sufficient vertical mixing occurs at larger spatial scales. In the longitudinal dimension of the stream network, variable selection was common among all headwater communities, while homogeneous selection was the dominant assembly mechanism within downstream planktonic and benthic communities. Thus, community assembly in dendritic metacommunities was strongly habitat- and scale-dependent, which may help reconcile taxonomic differences in dendritic metacommunity organization through tighter integration of spatial scale and vertical habitat structure.

Compositionally distinct planktonic and benthic communities

Several lines of evidence support the view that deterministic processes were responsible for differences in planktonic and benthic microbial communities. First, based on the higher α-diversity and greater proportion of habitat-specific taxa detected in the plankton, our data suggest that many planktonic taxa do not successfully colonize the streambed. This pattern may also reflect the fact that sources other than benthic sediments (e.g., nearby soils) also contribute to planktonic diversity (Battin et al. 2016). Across the watershed, community structure was consistently distinct between planktonic and benthic habitats due to variable selection, similar to what has been reported for stream bacterial communities in alpine (Besemer et al. 2012; Wilhelm et al. 2013) and arid (Kaestli et al. 2019) ecosystems. Such differences may be due to the reduced turnover of the sediment habitat matrix relative to the water column, as well as the physiochemical environmental differences between the two habitats (Hermans et al. 2020). In light of these results, our inferred community assembly processes support a prevailing role for variable selection between planktonic and benthic communities.

The strength of variable selection between communities in different habitats was scale dependent. At local scales, habitat differences in community structure were affected by dispersal limitation, but variable selection played an increasingly important role as spatial scale increased. This scale dependence may arise from insufficient vertical hydrological exchange at the scale of a local stream reach. That is, planktonic and benthic bacteria may be dispersal limited with respect to colonizing the opposite habitat at short distances (e.g., due to insufficient vertical hydrological mixing). At larger spatial scales, enough vertical exchange may have occurred between sites such that dispersal is not limiting, thereby allowing variable selection to structure differences between communities at broader scales. In our study system, it has been shown that vertical hydrological exchange plays a more important role in headwaters than in downstream reaches (Ward et al. 2019), as channels widen and the relative importance of vertical exchange to discharge diminishes. The reduction in hydrological exchange may explain why vertical dispersal limitation was slightly more important downstream than in headwaters.

Longitudinal and scale-dependent transitions in planktonic community assembly

We found mixed support for the expectation that bacterioplankton community assembly is driven primarily by dispersal. In our study, planktonic communities generally transitioned from homogeneous selection at the reach scale (< 1 km) to variable selection at the watershed scale (1–10 km). At the largest spatial scales (~ 10 km), we also detected an increasing frequency of undominated assembly and dispersal limitation. Contrary to our expectations, we did not detect a signal of mass effects in the null modeling analysis of bacterioplankton communities. While there could be methodological limitations to detecting mass effects from field surveys, previous simulation results have shown that the null modeling approach we employed can distinguish mass effects from homogeneous selection (Stegen et al. 2015). The dominance of selection over mass effects is consistent with the low-to-intermediate discharge in the Lookout Creek watershed at the time of sampling (i.e., between spring snowmelt and baseflow conditions). Therefore, our results suggest that dispersal was sufficiently non-limiting for abiotic/biotic conditions to structure bacterioplankton communities at the local scale, but low enough to prevent mass effects.

Plankton transitioned from variable selection among headwaters to a combination of assembly processes downstream. Variable selection among headwater plankton suggests that environmental differences underlie the assembly of headwater bacterioplankton communities. As previously suggested, immigration from terrestrial ecosystems can also influence headwater bacterial diversity (Read et al. 2015; Savio et al. 2015; Ruiz-González et al. 2015; Hassell et al. 2018), but local-scale dispersal connectivity between terrestrial soils and bacterioplankton may be weak or transient (Hermans et al. 2020; Wisnoski et al. 2020). Mass effects of terrestrial-derived bacteria could contribute to the inference of variable selection among headwaters if different headwater streams receive phylogenetically distinct immigrants. Downstream plankton

were primarily assembled by homogeneous selection, suggesting that similar environmental filters selected for similar bacterial communities across higher-order streams. Constrained ordination showed that longitudinal patterns in community structure reflected environmental gradients from high to low elevation sites that may be related to the environmental filters that underlie selection on drifting bacterioplankton. Therefore, the water column may serve as a dispersal corridor for terrestrial-derived bacteria, which progressively undergo homogeneous selection as they drift downstream.

We did, however, detect dispersal limitation at the largest spatial scales (e.g., from 1–10 km), likely due to the large spatial distances between high- and low-elevation sites. For example, low-elevation headwaters of the smaller Watershed 01 were tightly clustered within the range of communities spanning the broader Lookout Creek (Fig. 2), although watershed had a significant, yet modest, explanatory power in the PERMANOVA. The lower dispersion of Watershed 01 samples nested within the widely dispersed Lookout Creek samples may indicate that some high-elevation taxa were dispersal-limited with respect to colonizing Watershed 01 and vice versa. Thus, our results suggest that terrestrial-derived bacteria, environmental gradients, and dispersal limitation may explain changes in planktonic diversity across spatial scales and from headwaters to downstream reaches of the network.

Benthic community assembly showed weaker scale dependence than, but similar longitudinal trends to, planktonic assembly

In the benthic communities, our results suggest homogeneous selection was the dominant community assembly process across a range of spatial scales. This inference is consistent with the colonization of a phylogenetically similar subset of taxa from the overlying water column across the stream network (i.e., taxa that can live in sediment habitats). While homogeneous selection was the dominant process, we also found evidence for variable selection at the reach scale (e.g., due to stream versus riffle microhabitats) and at broader scales (e.g., due to differences in underlying geology or terrestrial inputs). As spatial scale increased, dispersal limitation and undominated assembly were more frequently detected. The increasing frequency of undominated assembly at the largest spatial scales (1–10 km) could reflect the idiosyncratic effects of disturbance history (e.g., large floods, debris slides, logging) that are common across the Lookout Creek watershed (Swanson and Jones 2002). In contrast to planktonic communities, where there was a scale transition from locally homogeneous to regionally variable selection, homogeneous selection remained important at all spatial scales.

In the longitudinal dimension of the network, we observed variable selection among headwater benthic communities. This suggests that, despite homogeneous selection within reaches (i.e., similar communities assemble in nearby sites regardless of network position), different headwaters favor the assembly of phylogenetically distinct benthic communities. Variable selection among headwaters may reflect dissimilar resource inputs among headwaters draining different terrestrial areas, or spatial variation in terrestrial sources that contribute to stream sediment assembly. The transition to homogeneous selection downstream may reflect longitudinal gradients in the homogenization of microhabitat structure (e.g., sediment size) and resource complexity (e.g., allochthonous vs. autochthonous organic matter) from lower- to higher-order streams (Vannote et al. 1980). However, we also found evidence downstream for variable selection (e.g., between third- and fifth-order streams), dispersal limitation, and undominated selection, indicating that not all downstream benthic communities are highly similar to one another. Thus, variable selection among headwaters was common in both benthic and planktonic communities among headwaters, likely reflecting divergent environmental filters, but downstream transitions to homogeneous selection were more habitat dependent.

Multi-layer dendritic metacommunities

Our work provides an empirical demonstration that the community assembly processes structuring metacommunities in dendritic networks vary not only with network position, but also across spatial scales and along the vertical dimension of streams, which encompasses planktonic and benthic habitats. The joint consideration of spatial scales and vertical habitat structure may be crucial to resolving taxonomic differences in diversity patterns in dendritic metacommunities (Schmera et al. 2018). For example, aquatic taxonomic groups (e.g., riparian plants, benthic invertebrates, and microorganisms) in dendritic networks span wide ranges of body sizes and generation times, disperse via different dispersal corridors throughout the stream network, and occupy benthic and planktonic habitats in vastly different ways. These key differences suggest the potential for a broader synthesis of metacommunity dynamics in stream networks built on a revised perspective embracing multi-layer dendritic networks with varying rates of dispersal and habitat use in the vertical and longitudinal dimensions.

References

Altermatt F (2013) Diversity in riverine metacommunities: a network perspective. Aquat Ecol 47:365–377. https://doi.org/10.1007/s10452-013-9450-3

Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. Austral Ecol 26:32–46. https://doi.org/10.1111/j.1442-9993.2001.01070.pp.x

Bachmann RW, Canfield DE (1996) Use of an alternative method for monitoring total nitrogen concentrations in Florida lakes. Hydrobiologia 323:1–8. https://doi.org/10.1007/BF00020542

Battin TJ, Besemer K, Bengtsson MM et al (2016) The ecology and biogeochemistry of stream biofilms. Nat Rev Microbiol 14:251–263. https://doi.org/10.1038/nrmicro.2016.15

Besemer K, Peter H, Logue JB et al (2012) Unraveling assembly of stream biofilm communities. ISME J 6:1459–1468. https://doi.org/10.1038/ismej.2011.205

Brown BL, Swan CM (2010) Dendritic network structure constrains metacommunity properties in riverine ecosystems. J Anim Ecol 79:571–580. https://doi.org/10.1111/j.1365-2656.2010.01668.x

Brown BL, Swan CM, Auerbach DA et al (2011) Metacommunity theory as a multispecies, multiscale framework for studying the influence of river network structure on riverine communities and ecosystems. J North Am Benthol Soc 30:310–327. https://doi.org/10.1899/10-129.1

Cadotte MW, Davies TJ (2016) Phylogenies in ecology: a guide to concepts and methods. Princeton University Press, Princeton

Caporaso JG, Lauber CL, Walters WA et al (2012) Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J 6:1621–1624. https://doi.org/10.1038/ismej.2012.8

Carrara F, Altermatt F, Rodriguez-Iturbe I, Rinaldo A (2012) Dendritic connectivity controls biodiversity patterns in experimental metacommunities. Proc Natl Acad Sci 109:5761–5766. https://doi.org/10.1073/pnas.1119651109

Chase JM, Myers JA (2011) Disentangling the importance of ecological niches from stochastic processes across scales. Philos Trans R Soc B Biol Sci 366:2351–2363. https://doi.org/10.1098/rstb.2011.0063

Chase JM, Kraft NJB, Smith KG et al (2011) Using null models to disentangle variation in community dissimilarity from variation in α-diversity. Ecosphere 2:art24. https://doi.org/10.1890/ES10-00117.1

Daly C, Conklin DR, Unsworth MH (2010) Local atmospheric decoupling in complex topography alters climate change impacts. Int J Climatol 30:1857–1864. https://doi.org/10.1002/joc.2007

Fierer N, Morse JL, Berthrong ST et al (2007) Environmental controls on the landscape-scale biogeography of stream bacterial communities. Ecology 88:2162–2173. https://doi.org/10.1890/06-1746.1

Freimann R, Bürgmann H, Findlay SEG, Robinson CT (2015) Hydrologic linkages drive spatial structuring of bacterial assemblages and functioning in alpine floodplains. Front Microbiol 6:1–15. https://doi.org/10.3389/fmicb.2015.01221

Grant EHC, Lowe WH, Fagan WF (2007) Living in the branches: population dynamics and ecological processes in dendritic networks. Ecol Lett 10:165–175. https://doi.org/10.1111/j.1461-0248.2006.01007.x

Harr RD (1977) Water flux in soil and subsoil on a steep forested slope. J Hydrol 33:37–58. https://doi.org/10.1016/0022-1694(77)90097-X

Hart DD, Finelli CM (1999) Physical-biological coupling in streams: the pervasive effects of flow on benthic organisms. Annu Rev Ecol Syst 30:363–395. https://doi.org/10.1146/annurev.ecolsys.30.1.363

Hassell N, Tinker KA, Moore T, Ottesen EA (2018) Temporal and spatial dynamics in microbial community composition within a temperate stream network. Environ Microbiol 20:3560–3572. https://doi.org/10.1111/1462-2920.14311

Hermans SM, Buckley HL, Lear G (2018) Optimal extraction methods for the simultaneous analysis of DNA from diverse organisms and sample types. Mol Ecol Resour 18:557–569. https://doi.org/10.1111/1755-0998.12762

Hermans SM, Buckley HL, Case BS, Lear G (2020) Connecting through space and time: catchment-scale distributions of bacteria in soil, stream water and sediment. Environ Microbiol 22:1000–1010. https://doi.org/10.1111/1462-2920.14792

Jost L (2007) Partitioning diversity into independent alpha and beta components. Ecology 88:2427–2439. https://doi.org/10.1890/06-1736.1

Kaestli M, Munksgaard N, Gibb K, Davis J (2019) Microbial diversity and distribution differ between water column and biofilm assemblages in arid-land waterbodies. Freshw Sci 38:869–882. https://doi.org/10.1086/706106

Kembel SW, Cowan PD, Helmus MR et al (2010) Picante: R tools for integrating phylogenies and ecology. Bioinformatics 26:1463–1464. https://doi.org/10.1093/bioinformatics/btq166

Leff LG, McArthur JV, Shimkets LG (1992) Information spiraling: movement of bacteria and their genes in streams. Microb Ecol 24:11–24. https://doi.org/10.1007/BF00171967

Legendre P, Legendre L (2012) Numerical ecology, 3rd edn. Elsevier, Amsterdam

Leibold MA, Chase JM (2018) Metacommunity ecology. Princeton University Press, Princeton

McKee A, Bierlmaier F (1987) H. J. Andrews Experimental Forest, Oregon. In: Greenland D (ed) The climates of the long-term ecological research sites. Institute of Arctic and Alpine Research. University of Colorado, Boulder, pp 11–17

Mouquet N, Loreau M (2003) Community patterns in source-sink metacommunities. Am Nat 162:544–557. https://doi.org/10.1086/378857

Niño-García JP, Ruiz-González C, del Giorgio PA (2016) Interactions between hydrology and water chemistry shape bacterioplankton biogeography across boreal freshwater networks. ISME J 10:1755–1766. https://doi.org/10.1038/ismej.2015.226

Oksanen J, Blanchet FG, Friendly M, et al (2019) vegan: community ecology package. R package version 2.5–4. Version 2.5–4

Prepas EE, Rigler FH (1982) Improvements in quantifying the phosphorus concentration in lake water. Can J Fish Aquat Sci 39:822–829. https://doi.org/10.1139/f82-112

Price MN, Dehal PS, Arkin AP (2010) FastTree 2—approximately maximum-likelihood trees for large alignments. PLoS ONE 5:e9490–e9490. https://doi.org/10.1371/journal.pone.0009490

Quast C, Pruesse E, Yilmaz P et al (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 41:590–596. https://doi.org/10.1093/nar/gks1219

R Core Team (2018) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Read DS, Gweon HS, Bowes MJ et al (2015) Catchment-scale biogeography of riverine bacterioplankton. ISME J 9:516–526. https://doi.org/10.1038/ismej.2014.166

Rognes T, Flouri T, Nichols B et al (2016) VSEARCH: a versatile open source tool for metagenomics. PeerJ 4:e2584–e2584. https://doi.org/10.7717/peerj.2584

Ruiz-González C, Niño-García JP, del Giorgio PA (2015) Terrestrial origin of bacterial communities in complex boreal freshwater networks. Ecol Lett 18:1198–1206. https://doi.org/10.1111/ele.12499

Savio D, Sinclair L, Ijaz UZ et al (2015) Bacterial diversity along a 2600 km river continuum. Environ Microbiol 17:4994–5007. https://doi.org/10.1111/1462-2920.12886

Schloss PD, Westcott SL, Ryabin T et al (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75:7537–7541. https://doi.org/10.1128/aem.01541-09

Schmera D, Árva D, Boda P et al (2018) Does isolation influence the relative role of environmental and dispersal-related processes in stream networks? An empirical test of the network position hypothesis using multiple taxa. Freshw Biol 63:74–85. https://doi.org/10.1111/fwb.12973

Stegen JC, Lin X, Fredrickson JK et al (2013) Quantifying community assembly processes and identifying features that impose them. ISME J 7:2069–2079. https://doi.org/10.1038/ismej.2013.93

Stegen JC, Lin X, Fredrickson JK, Konopka AE (2015) Estimating and mapping ecological processes influencing microbial community assembly. Front Microbiol 6:370–370. https://doi.org/10.3389/fmicb.2015.00370

Swanson FJ, James ME (1975) Geology and geomorphology of the H.J. Andrews Experimental Forest, Western Cascades, Oregon. U.S. Department of Agriculture, Forest Service, Pacific Northwest Forest and Range Experiment Station, Portland, OR

Swanson FJ, Jones JA (2002) Geomorphology and hydrology of the H.J. andrews experimental forest, blue river, oregon. Field Guide Geol Process Cascadia Dep Geol Miner Ind Spec Pap 36:289–314

Tonkin JD, Altermatt F, Finn DS et al (2018) The role of dispersal in river network metacommunities: patterns, processes, and pathways. Freshw Biol 63:141–163. https://doi.org/10.1111/fwb.13037

Tornwall BM, Swan CM, Brown BL (2017) Manipulation of local environment produces different diversity outcomes depending on location within a river network. Oecologia. https://doi.org/10.1007/s00442-017-3891-7

Vannote RL, Minshall GW, Cummins KW et al (1980) The river continuum concept. Can J Fish Aquat Sci 37:130–137. https://doi.org/10.1139/f80-017

Vellend M (2016) The theory of ecological communities. Princeton University Press, Princeton

Ward AS, Wondzell SM, Schmadel NM et al (2019) Spatial and temporal variation in river corridor exchange across a 5th-order mountain stream network. Hydrol Earth Syst Sci 23:5199–5225. https://doi.org/10.5194/hess-23-5199-2019

Westcott SL, Schloss PD (2017) OptiClust, an improved method for assigning amplicon-based sequence data to operational taxonomic units. mSphere 2:e00073–e117. https://doi.org/10.1128/mSphereDirect.00073-17

Wilhelm L, Singer GA, Fasching C et al (2013) Microbial biodiversity in glacier-fed streams. ISME J 7:1651–1660. https://doi.org/10.1038/ismej.2013.44

Wisnoski NI, Muscarella ME, Larsen ML et al (2020) Metabolic insight into bacterial community assembly across ecosystem boundaries. Ecology 101:e02968. https://doi.org/10.1002/ecy.2968

Zhou J, Ning D (2017) Stochastic community assembly: does it matter in microbial ecology? Microbiol Mol Biol Rev 81:e00002–17. https://doi.org/10.1128/MMBR.00002-17