RUNNING TITLE: Spatiotemporal hyporheic assembly and physiology

# Deterministic assembly processes govern seasonal and spatial variation in microbiomes across hydrologically-connected hyporheic zones

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**Originality-Significance Statement.** Subsurface zones of groundwater and surface water mixing (hyporheic zones) are hotspots of biogeochemical activity and strongly influence carbon, nutrient and contaminant dynamics within riverine ecosystems. Hyporheic zone microbiomes are responsible for up to 95% of riverine ecosystem respiration, yet the ecology of these microbiomes remains poorly understood. While significant progress is being made in the development of microbially-explicit ecosystem models, poor understanding of hyporheic zone microbial ecology impedes development of such models in this critical zone. To fill the knowledge gap, we present a comprehensive analysis of hyporheic zone microbiomes through space and time. We quantify ecological drivers of microbiome change and identify taxa that may be particularly important to hyporheic zone biogeochemical function. Despite pronounced hydrologic connectivity throughout the hyporheic zone, we find that ecological selection deterministically governs microbiome composition within local environments and that comparatively high-organic C conditions during surface water intrusion into the hyporheic zone may support heterotrophic metabolisms, succumbing to autotrophy during time periods of groundwater discharge. These results provide new opportunities to develop microbially-explicit ecosystem models that incorporate the hyporheic zone and its influence over riverine ecosystem function.

**Keywords:** selection, dispersal, subsurface, microbial community structure, community assembly, freshwater biology, microbial ecology, stochastic assembly

**Summary.**

Subsurface zones of groundwater and surface water mixing (hyporheic zones) are regions of enhanced rates of biogeochemical cycling, but ecological processes influencing hyporheic microbiomes through space and time remain unknown. We sampled attached and planktonic microbiomes in the Columbia River hyporheic zone across seasonal hydrologic change within three hydrologically-connected, yet physicochemically-distinct geographic zones (inland, nearshore, river). Although microbiomes remained dissimilar through time across all zones and habitat types (attached vs. planktonic), consistent presence of certain heterotrophic taxa suggested dispersal and/or common selective pressures among all zones. We used statistical null models and co-occurrence network analysis, respectively, to demonstrate a pronounced impact of deterministic assembly on microbiomes in all data subsets and to elucidate taxa most affected by these processes. The composition of one network cluster of nearshore organisms exhibited a seasonal shift from heterotrophic to autotrophic microorganisms, and the abundance of taxa within this cluster also correlated positively with active microbial biomass and metabolism, possibly indicating that these taxa have strong influences over biogeochemical reactions within the hyporheic zone. Taken together, our research demonstrates a predominant role for deterministic assembly across highly-connected environments and provides insight into niche dynamics associated with seasonal changes in hyporheic microbiome composition and metabolism.

## Introduction.

Environmental transition zones exhibit elevated rates of biogeochemical cycling compared to other systems and experience extreme variation in physicochemical characteristics (Hedin et al., 1998; McClain et al., 2003). In particular, groundwater-surface water mixing (hyporheic) zones are characterized by temporally and spatially dynamic, redox-active environments associated with changes in river stage (Hancock et al., 2005; Boulton et al., 2010). Hyporheic zones regulate watershed-scale biogeochemistry by transporting and modifying carbon and nutrients between rivers and their catchments (Boano et al., 2014; Battin et al., 2016). Yet, sediment microbiomes mediating hyporheic biogeochemical cycling have rarely been examined (Robertson and Wood, 2010; Marmonier et al., 2012; Gonzalez and Bell, 2013). As such, microbial biogeography as well as ecological processes that impact microbiome composition and function throughout hyporheic zones constitute key knowledge gaps in understanding these important transition zones.

Interactions between hydrologic transport, local abiotic conditions, and biotic processes result in diverse hyporheic microbiomes and are essential to understanding major biogeochemical cycles (Boulton et al., 2010; Battin et al., 2016; Stegen et al., 2016). In particular, microbial community assembly processes are thought to be imperative in coupling microbiomes with ecosystem functioning across a range of environments (Ferrenberg et al., 2013; Nemergut et al., 2013; Graham et al., 2016a; Graham et al., 2016b). Determinism (*e.g.*, selection) may either sort species such that the microbiome is optimized for a given environment or exclude biodiversity that supports ecosystem functioning (Knelman and Nemergut, 2014; Graham et al., 2016a), while stochasticity (*e.g.*, dispersal, drift) can regulate microbial community membership via mechanisms such as priority effects (Fukami, 2004; Fukami et al.,

2010) and dispersal limitation (Lindström and Langenheder, 2012; Adams et al., 2013; Cline and Zak, 2014). In sediments, assembly processes may be particularly important for biogeochemical function because abiotic conditions and hydrologic flow vary over short (<1 mm) distances, generating diverse niches with distinct environmental filters as well as providing dispersal routes for microorganisms (Battin et al., 2016).

Selection has been widely shown to impact environmental microbiomes (Fierer and Jackson, 2006; Lauber et al., 2009), while stochastic processes can influence microbial community composition when environmental heterogeneity and/or species sorting is weak (Stegen et al., 2012; Wang et al., 2013; Woodcock et al., 2013; Battin et al., 2016). Both of these processes operate in concert to structure microbiomes, and recent work has focused on deciphering the balance of deterministic vs. stochastic assembly processes through space and time (Ferrenberg et al., 2013; Dini-Andreote et al., 2015; Graham et al., 2016a). Further, assembly processes function at an organismal level; determinism selects for traits contained within certain groups of organisms and dispersal disproportionately affects organisms expressing traits for motility (Martiny et al., 2006; Martiny et al., 2013). Understanding microbiome assembly at the sub-community level is therefore paramount in fully comprehending microbiome assembly and subsequent ecosystem functioning. Yet, influences of assembly processes on specific groups of microorganisms are poorly understood, and linking a process-based understanding of variation in microbiome composition to the metabolism of carbon and nutrients remains a vital unknown in ecosystem science, particularly within environmental transition zones that harbor a variety of complementary resources (Boulton et al., 2010; Stegen et al., 2016).

The Hanford Reach of the Columbia River constitutes a hyporheic zone with an extensive floodplain aquifer, presenting a prime opportunity for investigating spatiotemporal linkages

between community assembly processes and environmental microbiomes. We sampled geographically distinct but hydrologically-connected zones that lie in close proximity (<250m) – near the inland groundwater aquifer, in the nearshore subsurface environment, and in the Columbia River surface water – across a 9-month time period. Previous research has shown redox conditions as well as microbiomes to be variable across space and time in this system (Lin et al., 2012a; Lin et al., 2012b; Stegen et al., 2012; Graham et al., 2016a). Here, we use ecological modeling to infer assembly processes governing microbiome composition in planktonic and attached communities through time in these zones. Additionally, we use co-occurrence networks to infer relationships between deterministic assembly and specific groups of organisms; and we identify keystone taxa associated with seasonal changes in groundwater-surface water mixing. We also reveal relationships between the abundance of one cluster of nearshore organisms and changes in both active biomass and aerobic respiration, possibly denoting a central role for these organisms in hyporheic biogeochemical cycling. Together, our research furthers an understanding of spatiotemporal changes in hyporheic microbiomes and generates a conceptual model regarding the role of deterministic assembly in influencing shifts from heterotrophic to autotrophic organisms in hyporheic enviroments.

**Results.**

*Hydrology and Physicochemical Conditions.*

We observed variation in temporal dynamics of hydrologic mixing and physicochemistry among geographic zones (Fig. 1A-C, Table S1, Fig. S1). Increases in $Cl^-$ concentration in nearshore and inland zones at and beyond our July 22 sampling event indicated discharge of groundwater through the hyporheic zone (Fig. 1A), coincident with decreases in NPOC

131 concentration in all three zones (Fig. 1B). Temperature followed a seasonal trend in nearshore
132 and river zones but remained stable in the inland zone (Fig. 1C).
133
134 *Microbiome composition.*
135 River planktonic (PERMANOVA, $R^2 = 0.22$, $P = 0.001$), nearshore attached
136 (PERMANOVA, $R^2 = 0.44$, $P = 0.001$), nearshore planktonic (PERMANOVA, $R^2 = 0.44$, $P =$
137 0.001), and inland planktonic (PERMANOVA, $R^2 = 0.41$, $P = 0.001$) microbiomes changed
138 seasonally. Inland attached microbiomes (PERMANOVA, $P = 0.29$) were stable through time.
139 Comparing all 5 data subsets to each other revealed distinct microbiomes (PERMANOVA $R^2 =$
140 0.25, $P = 0.001$, Fig. S3), and within each zone the composition of planktonic and attached
141 communities differed (PERMANOVA, nearshore $R^2 = 0.19$, $P = 0.001$, inland $R^2 = 0.14$, $P =$
142 0.001).
143 The ten most abundant phyla and classes in each data subset are presented in Table 1.
144 Members of *Proteobacteria* were ubiquitous, and *Bacteroidetes* was highly abundant in all river
145 and nearshore subsets but had lower abundance in inland zone habitats. The *Planctomycetes-*
146 *Verrucomicrobia-Chlamydiae* (PVC) superphylum was prevalent, though the composition of
147 organisms within this phylum varied among data subsets. *Chloracidobacteria* was widespread in
148 attached microbiomes in both nearshore and inland zones but was not highly abundant in
149 planktonic microbiomes. In the inland zone, classes *PBS-25* and *koll11* of the *OP3* candidate
150 phylum were among the ten most abundant classes. Further, at the class level, the ammonia-
151 oxidizing *Thaumarcheaota* was highly abundant in both inland planktonic and attached
152 microbiomes, joined by a nitrite-oxidizer (*Nitrospira*) in attached microbiomes.
153

*Microbiome assembly.*

Deterministic assembly processes had more influence on microbiome composition than stochastic processes (Table 2). In attached microbiomes, both nearshore and inland, homogenous selection accounted for 82-100% of assembly processes, and in river microbiomes, homogenous selection account for >95% of assembly. While planktonic microbiomes in nearshore and inland zones exhibited higher stochasticity than their attached counterparts, selection still accounted for 59.8% and 35.7% of assembly, respectively.

Because we hypothesized dispersal mechanisms to have the greatest impact on planktonic microbiomes compared across geographic zones, we investigated assembly processes influencing across-zone comparisons within planktonic microbiomes only. Selection comprised 57-92% of across-zone assembly processes, with dispersal being of secondary importance. Variable selection was responsible for >90% of dissimilarity in microbiomes between nearshore and inland zones, while nearshore-to-river comparisons showed an even balance of variable (34.84%) and homogenous (29.18%) selection. The influence of spatial processes was most evident in dispersal limitation (26.76%) in inland-to-river comparisons and in homogenizing dispersal (15.30%) in nearshore-to-river comparisons.

*Spatiotemporal co-occurrence networks, environmental correlations, and keystone taxa.*

Properties of full networks at the family level are listed in Table S3, and the composition of clusters are listed in Table S4. In the nearshore zone, attached cluster 1 was negatively correlated with time, Cl-, and temperature (Fig. 2A-D); and planktonic cluster 9 was positively correlated with time, Cl-, and temperature and negatively correlated with NPOC (Fig. 2E-H). In the inland zone, clusters of attached organisms did not display consistent trends with time or

physicochemistry. Cluster 3 (Fig. 2I-L) and cluster 5 (Fig. 2M-P) of inland plankton exhibited contrasting relationships with time (positive/negative), $Cl^-$ (positive/negative), NPOC (negative/positive), and temperature (positive/negative). River planktonic cluster 2 was negatively correlated with day of year and positively correlated with $Cl^-$, with no evident relationships to NPOC or temperature (Fig. 2Q-T). Only one cluster – nearshore attached cluster 1 – exhibited correlations with ATP and Raz (Fig. 3).

Nearshore attached cluster 1 contained keystone families belonging to *Verrucomicrobia* and *Thaumarcheaota*, as well as families of *Gammaproteobacteria*, *Alphaproteobacteria*, and *Chloracidobacteria* with a secondary importance (Fig. 4A, Table S4). Nearshore planktonic cluster 9 contained two keystone families—one family of unassigned organisms and one belonging to the candidate phylum *OP3* (Fig. 4B). No keystone taxa were identified in inland planktonic cluster 5 (Fig. 4C), but inland planktonic cluster 3 contained two keystone families belonging to *Chloracidobacteria* and *Chloroflexi* as well as organisms with secondary importance (Fig. 4D). No keystone taxa were identified in river cluster 2 (Fig. 4E).

**Discussion.**

Our research elucidates seasonal changes in the hyporheic zone associated with shifts in groundwater-surface mixing conditions and physicochemistry. We observed a major influence of deterministic assembly processes both within and across geographic zones, indicating selection rather than dispersal as a primary driver of microbiome composition. Below we also detail phylogeny-inferred microbial physiologies in each zone and habitat type. We further identify clusters of organisms, and keystone taxa therein, that are most effected by deterministic assembly processes, and we propose one cluster of organisms as foundational to metabolism in

the nearshore hyporheic environment. Finally, we present a conceptual model describing assembly processes and shifts in metabolism within hyporheic environments.

*Hydrologic and physicochemical dynamics.*

We observed a gradual decline in water stage beginning in June and continuing through mid-September (Fig. S1). This seasonal hydrologic change drove shifts in groundwater-surface water mixing and associated physicochemistry across all geographic zones (Fig. 1). Hydrodynamics can be a driver for both stochastic and deterministic assembly processes, as advection can physically transport microorganisms across geographic barriers as well as generate changes in physicochemical selective environments. These potential influences are parsed and discussed in the following sub-sections.

*Microbiome biogeography and physiology.*

Biogeographical patterns in microbiomes suggest localized processes as key structuring forces in our system. While dispersal potential within hyporheic zones remains unclear (Bärlocher et al., 2006; Cornut et al., 2014), microbiomes were distinct across geographic zones in our system and physical filtering of particulates as well as steep physicochemical grandients may inhibit microbial dispersal across zones (Brunke, 1999; Hartwig and Borchardt, 2014). Additionally, planktonic and attached microbiomes were distinct from each other despite physical co-location, supporting little overlap between porewater and sediment microbiomes in hyporheic zones (Febria et al., 2012) and suggesting substrate mineralogy and/or traits for attachment as selective filters (Carson et al., 2007; Jorgensen et al., 2012).

In particular, we observed higher abundances of *Chloracidobacteria* in attached vs. planktonic microbiomes within both nearshore and inland zones (Table 1). Biofilm communities, common to hyporheic zones, consist of a mixture of eukaryotic and prokaryotic organisms embedded in a porous extracellular matrix and contain diverse organisms with complementary metabolisms (Battin et al., 2016). *Chloracidobacteria* were prevalent in attached microbiomes (*i.e.*, within biofilms) and are thought to be photoheterotrophs (Bryant et al., 2007; Tank and Bryant, 2015) that can utilize non-visible near-infrared light as an energy source for growth (Behrendt et al., 2012). While these properties may confer advantages in subsurface sediment conditions with low levels of visible light, our environments experience virtually no light, and their prevalence suggests possible alternative physiologies for these organisms in subsurface habitats.

Inland communities, characterized by a low-C, high ionic strength environment, also contained many unique taxa relative to river and nearshore microbiomes (Table 1). Stegen *et al.* (2012) have proposed stochastic assembly processes as a major influence in this zone, and a smaller influence of homogenous selection in the inland zone relative to other zones may be reflective of niche diversification (Table 2). In particular, we observed members of the candidate phylum *OP3* in high abundance in the inland zone. *OP3* has been shown to be abundant in other subsurface communities with high inorganic C concentrations (Emerson et al., 2015) and appears to thrive in low oxygen environments, potentially utilizing anaerobic metabolisms reliant on iron, manganese, and/or sulfur cycling (Glöckner et al., 2010). Our inland zone is characterized by high levels of inorganic C but remains oxygenated year round (Table S1), and our research suggests that *OP3* may be able to persist under a broader range of redox conditions than currently recognized. Further, nitrifying organisms *Thaumarcheaota* and *Nitrospira* were

prevalent in the inland zone. These organisms are not dependent on external organic material, and instead fix $CO_2$ through autotrophic pathways involving $NH_4^+$ and $NO_2^-$ oxidation. The unique physicochemical environment of the inland zone may therefore exclude heterotrophic organisms that are dominant in the nearshore and river zones and favor organisms with physiologies that align with oligotrophic conditions.

Despite pronounced biogeographical patterns in microbiomes, our results indicate some common selective features and/or dispersal routes across geographic zones and habitat types. We observed high abundances of *Proteobacteria* and members of the PVC superphyla in all data subsets, and *Bacteroidetes* was among the most abundant phyla in both planktonic and attached microbiomes in nearshore and river zones (Table 1). *Proteobacteria* are prominent community members in other aquatic systems (Cottrell and Kirchman, 2000; Zwart et al., 2002; Battin et al., 2016) and exhibit pronounced metabolic flexibility (Brenner et al., 2005). The ability of these organisms to assimilate low-energy substrates, such as sulfate, nitrate, and inorganic C, in addition to a range of organic C compounds may contribute to their abilities to thrive across physicochemically-distinct geographic zones. For example, *Alphaproteobacteria* can degrade chemically-complex humic substances as well as monomers (Newton et al., 2011; Battin et al., 2016), and *Betaproteobacteria* exhibit diverse heterotrophic metabolisms (Amakata et al., 2005; Yang et al., 2005; Sato et al., 2009; Battin et al., 2016). *Gammaproteobacteria* can fix inorganic C in light-limited environments (Dyksma et al., 2016) as well as process organic C (Nikrad et al., 2014). Likewise, *Deltaproteobacteria* utilize organic molecules as carbon sources and as electron donors for sulfate- and iron-reduction (Lovley et al., 1998; Torres et al., 2010), reflecting adaptations to both high- and low-C environments. Microbiomes with a diverse community of *Proteobacteria* may therefore be well-adapted to fluctuations in organic C

availability, facilitating their persistence across all geographic zones. Moreover, *Bacteriodetes* has been shown to be prevalent in both planktonic and biofilm microbiomes in other systems—preferentially degrading recalcitrant C compounds and recycling biomass (Cottrell and Kirchman, 2000; Fernández-Gómez et al., 2013; Martin et al., 2015; Bennke et al., 2016). The presence of *Bacteroidetes* in addition to *Proteobacteria* within nearshore and river microbiomes may therefore indicate niche complementarity within these higher-C environments.

Additionally, members of the PVC superphyla were abundant in all data subsets and possess distinctive physiologies. Many of these organisms have a proteinaceous membrane conveying antibiotic resistance (Fuerst and Sagulenko, 2011; Speth et al., 2012) and/or metabolize C1 compounds such as methane (Dunfield et al., 2007; Fuerst and Sagulenko, 2011; Sharp et al., 2013). Although the biogeochemical implications of selection for these organisms are unexplored, their unique ecology, ability to consume methane, and abundance within our system merits future investigating into their role in C cycling in hyporheic environments.

*Microbiome assembly and spatiotemporal dynamics.*

Given variation among microbiomes, we investigated assembly processes structuring microbiomes and found a predominant role for deterministic assembly processes in all data subsets. In particular, attached communities were nearly entirely assembled by homogeneous selective pressures and displayed network topologies indicating a highly-structured microbiome (*i.e.*, high clustering coefficient and low heterogeneity, Table S3). Our results are consistent with a recent review that noted the prevalence of deterministic processes over stochasticity in structuring stream-associated biofilm microbiomes (Battin et al., 2016). Battin *et al.* (2016) suggest micro-niches in biofilms select for specific organisms and repel poorly-adapted

291 immigrating species. Indeed, substrate features may impose strong environmental filters on
292 microbiomes—sediment geochemistry (Carson et al., 2007; Jorgensen et al., 2012) and matrix
293 structure (Vos et al., 2013; Breulmann et al., 2014) can select for traits that enhance attachment
294 on a particular substrate.

295 Likewise, river planktonic microbiomes were assembled via homogeneous selection
296 across time but were differentiated from nearshore and inland zone microbiomes via dispersal
297 limitation (Table 2). Network structure was also consistent with a highly-organized river
298 microbiome (*i.e.*, high clustering coefficient and low heterogeneity, Table S3). A relatively
299 stable geochemical environment in the river may provide a steady selective environment despite
300 seasonal changes in temperature (Table S1, Fig. 1C). In particular, NPOC concentration was
301 approximately 2-5 times higher in river water than the nearshore and inland aqueous
302 environments. Furthermore, organic C associated with surface water intrusion is correlated with
303 short-term increases in deterministic assembly processes and changes in microbiome
304 composition in our nearshore environment (Stegen et al., 2016). Thus, consistently high NPOC
305 concentration in the river may impose environmental filters for free-living heterotrophic
306 organisms within river bacterioplankton, resulting in homogeneous selection through time.
307 Moreover, distinct river physicochemistry, as compared to nearshore and inland zones (Table
308 S1), may limit successful colonization of planktonic river-associated taxa within other zones.

309 Although deterministic assembly processes were prevalent forces in structuring
310 microbiomes, nearshore and inland planktonic microbiomes showed a greater influence of
311 stochastic assembly processes than other zones. Within the nearshore, significant fluxes of both
312 groundwater and surface water may contribute to spatial assembly processes. For example, we
313 observed the impact of both dispersal limitation and homogenizing dispersal on nearshore

planktonic microbiomes, suggesting that this zone may experience periods of comparatively low and high rates of hydrologic transport. Furthermore, sampling locations for inland planktonic microbiomes were distributed across a broader spatial extent than any other zone, perhaps facilitating limited dispersal across the sampled domain.

When we examined assembly processes differentiating planktonic microbiomes across zones, we anticipated more impact from spatial processes due to an increase in geographic scale. In contrast to this expectation, selection was responsible for more than 50% of community dissimilarity in all comparisons. While we have suggested physical inhibition of microbial dispersal as a possible mechanism generating microbiome dissimilarity across geographic zones, our analyses indicate that an overarching influence of selection generally outweighed influences of spatial processes. Our results do not necessarily indicate that spatial processes play no role in shaping microbiomes, but rather that local physicochemical environments limit the ability of physically-transported microorganisms to outcompete local biota. Indeed, nearshore-to-inland zones comparisons suggested that distinct selective pressures in each environment were the dominant cause (>90%) of differences among planktonic microbiomes.

Likewise, spatial processes were overwhelmed by selection in nearshore-to-river comparisons. Differences among these microbiomes were due to relatively even proportions of homogenous and variable selection, with no evident seasonal trends in the balance between these processes. Our system is characterized by pronounced geomorphic heterogeneity in the subsurface environment that creates preferential flow paths for groundwater-surface water mixing (Johnson et al., 2015). The presence of both homogeneous and variable selection may indicate spatial variation in surface water intrusion or local biogeochemical conditions across our sampling locations (distributed parallel to the river across a distance of ~150m). Homogenizing

337 dispersal also had a small but detectable influence in nearshore-to-river comparisons, supporting
338 some hydrologic transport of microorganisms between these zones.
339 Lastly, assembly relationships between the inland and river zones provide the strongest
340 evidence for spatial processes in our system (42.5% stochasticity). In particular, inland-to-river
341 comparisons yielded the largest impact of dispersal limitation (26.8%), supporting a decay of
342 community similarity across increasing spatial distances (Green and Bohannan, 2006). Dispersal
343 limitation is, therefore, likely to play a more significant role in subsurface microbiome assembly
344 at larger spatial scales.
345
346 *Spatiotemporal environmental correlations and keystone taxa.*
347 Given pervasive deterministic assembly, we examined physicochemical properties related
348 to changes in microbiome composition in each data subset. Co-occurrence networks fragment
349 into clusters that are sensitive to variation in hydrological regimes (Widder et al., 2014; Febria et
350 al., 2015) and have been used to identify keystone taxa (González et al., 2010; Vick-Majors et
351 al., 2014; Banerjee et al., 2015; Banerjee et al., 2016). Here, we examined relationships between
352 temporal co-occurrence networks and aqueous physicochemistry to identify organisms
353 associated with seasonal environmental and hydrologic change.
354 In attached microbiomes, no relationships were present in the inland zone, but we found
355 that nearshore attached cluster 1 was favored during early season conditions with pronounced
356 surface water intrusion and low temperature (Fig. 2A-D). Clusters of organisms can denote both
357 positive and negative co-occurrence patterns, signifying similar or opposite (respectively)
358 ecological dynamics influencing taxa. For instance, organisms sharing a cluster may constitute
359 an ecological niche, whereby organisms either co-occur in similar environments or have

beneficial interactions (e.g., mutualisms)(Shi et al., 2016). Alternatively, clusters may contain organisms that are favored under opposing environmental conditions or have negative interactions (e.g., competition, predation). Nearshore attached cluster 1 contained two anticorrelated groups of organisms—group 1 consisted of heterotrophic organisms (*Oxalobacteraceae*, *Comamonadaceae*, *Verrucomicrobiaceae*, and *Flavobacteriaceae*) that decreased through time, while organisms in group 2 were primarily autotrophic oligotrophs (*Crenarchaeaceae*, *Ectothiorhodospiraceae*, *Pelagibacteraceae*, and families of *Alphaproteobacteria*) that increased through time. Because of the abundance of organisms in group 1 compared to group 2 (4498 vs. 1987), group 1 dictated correlations between this cluster and the environment, supporting an association between heterotrophic organisms and periods experiencing relatively high-organic C surface water intrusion. However, as the abundance of cluster 1 declined in concert with groundwater intrusion into the nearshore environment (Fig. 3A-D), organisms in group 2 increased, putatively outcompeting heterotrophic organisms in group 1 under more oligotrophic conditions. The co-association of heterotrophic and autotrophic organisms within a single cluster may indicate comparatively strong tradeoffs between these lifestyles and merits further investigation into niche dynamics and competitive interactions between these taxa. Further, we identified primary keystone taxa as *Verrucomicrobiaceae* and *Crenarchaeaceae*, possibly denoting the principal heterotrophic and autotrophic organisms in groups 1 and 2, respectively.

In planktonic microbiomes, we observed relationships between clusters of organisms in all zones with hydrologic mixing and physicochemistry. Nearshore planktonic cluster 9 displayed a seasonal association with groundwater discharging conditions and high temperature (Fig. 2E-H). Organisms were assigned to families highly abundant in the inland zone (*e.g.*,

383 *Thaumarchaeota*, candidate phylum *OP3*, Table 1, Table S4), and keystone taxa, in particular,
384 belonged to *OP3*. Trends in the relative abundance of this cluster may therefore denote enhanced
385 dispersal from the inland zone during groundwater discharge and/or a change in selection
386 favoring these organisms corresponding to physicochemical shifts in nearshore environment. The
387 inland planktonic microbiome also revealed two clusters related to environmental change.
388 Cluster 3 was present in low abundance, correlated with higher groundwater contribution and
389 temperature (Fig. 2I-L), and contained facultative anaerobic microorganisms (*Lentisphaeria*,
390 *Gemmatimonadetes*, *Anaerolineae, Alphaproteobacteria*, *Gammaproteobacteria*, *Chloroflexi*,
391 *Clostridia*, *Spirochaetes*) and methylotrophs (*Betaproteobacteria*, Table S4). Such organisms are
392 largely distinct from organisms found in the nearshore and river environments, supporting the
393 inference that physical and geochemical isolation drives differences between microbiomes. In
394 contrast, cluster 5 was associated with surface water intrusion and low temperature (cluster 5,
395 Fig. 2M-P). An influx of surface water-associated NPOC (Fig. 1B) should favor heterotrophic
396 microorganisms, and cluster 5 contained methanotrophs (*Methylacidiphilae, Betaproteobacteria)*
397 and diverse *Actinobacteria* and *Acidimicrobiia* involved in C cycling, possibly denoting a shift
398 towards heterotrophy under comparatively high-organic C conditions. While no keystone taxa
399 were found in cluster 5, *Chloracidobacteria* and a family of *Chloroflexi* were identified in cluster
400 3, meriting further investigation into their role in the hyporheic environment.
401 Finally, we identified one cluster of diverse river organisms that was positively
402 associated with $Cl^-$ concentration, which may indicate significant groundwater discharge into the
403 river (Fig. 2Q-T, Table S4). In particular, *Cytophagia* was the most abundant organism in this
404 cluster and was common in the nearshore and river zones, but all other families in this cluster
405 were primarily found in the inland zone. These inland taxa were, however, in low abundance in

406 the river and may therefore indicate a small contribution of the inland microbiome to river
407 communities under discharging condtions. No keystone taxa were found in this cluster.
408
409 *Functional implications.*
410 We further investigated the involvement of each cluster in microbial metabolism and
411 found only one cluster—cluster 1of nearshore attached microorganisms—that correlated with
412 active biomass (ATP) and aerobic respiration (Raz). Positive correlations between cluster 1 and
413 both ATP and Raz (Fig. 3) indicate that organisms within this cluster are either responsible for
414 biomass growth and aerobic nutrient cycling or are facilitated by conditions under which these
415 processes are promoted. Physical heterogeneity within sediments increases the rate and diversity
416 of compounds metabolized in biofilms (Singer et al., 2010), an effect that has been attributed to
417 complementary resource use and niche diversification (Battin et al., 2016). In particular, the
418 spatial organization of biofilms and the ability of microorganisms to excrete extracellular
419 enzymes can allow for complementarity that enhances rates of metabolism (Loreau et al., 2001;
420 Naeem et al., 2012). Nearshore attached cluster 1 contains organisms with a broad range of
421 metabolic capabilities and may therefore constitute the foundation (or portion thereof) of this
422 complementary resource structure.
423
424 *Conclusions*
425 Our results generate new hypotheses regarding spatiotemporal dynamics in hyporheic
426 environments (Fig. 5A-B). We advance that spatial and temporal patterns in ecological selection
427 impose strong environmental filters in hyporheic zones, limiting successful immigration of
428 organisms across physicochemical gradients (Fig. 5A). However, despite largely distinct

429 microbiomes, we reveal common taxa across a geographic continuum from groundwater- to
430 surface water-dominated habitats. We also identify clusters of organisms, and keystone taxa
431 therein, that change through seasonally dynamic groundwater-surface water mixing conditions,
432 providing insight into microbial physiologies in each geographic zone. In particular, one cluster
433 of attached organisms in the nearshore environment suggests a seasonal tradeoff between
434 heterotrophic and autotrophic organisms in our system. We therefore propose that comparatively
435 high-organic C conditions during surface water intrusion into the hyporheic zone support
436 heterotrophy, succumbing to autotrophy during time periods of groundwater discharge (Fig. 5B).
437 This cluster also correlated with enhanced rates of microbial metabolism, suggesting a critical
438 biogeochemical role for taxa within this cluster. Together, our results provide evidence for
439 deterministic assembly processes in hyporheic environments, despite pronounced hydrologic
440 connectivity, and further a process-based understanding of spatiotemporal patterns in
441 microbiomes in a critical environmental transition zone.
442
443 **Experimental Procedures.**
444 *Study Design.*
445 This study was conducted in three geographic zones (inland, nearshore, river) within the
446 Hanford 300A (approximately 46$^{o}$ 22’ 15.80”N, 119$^{o}$ 16’ 31.52”W) in eastern Washington State
447 (Slater et al., 2010; Zachara et al., 2013; described in Graham et al., 2016a). The inland zone lies
448 within 250m of the Columbia River and is characterized by an unconfined groundwater aquifer
449 in the Hanford formation. It displays relatively stable temperatures (~15$^{o}$C) and elevated
450 concentrations of anions and inorganic carbon relative to other zones (Table S1). In contrast, the
451 Columbia River contains higher organic carbon and lower ion concentrations with seasonally

variable temperatures. Surface water from the river and groundwater from the inland zone mix in the nearshore hyporheic zone. At high river stages, surface water intrudes into the inland zone; and at low river stages, groundwater discharges into the Columbia River. To monitor groundwater-surface water mixing, we employed $Cl^-$ as a conservative groundwater tracer per Stegen *et al.* (2016).

Detailed sampling and analytical methods are in the Supplemental Material. Briefly, attached (nearshore and inland only) and planktonic (all zones) microbiomes were obtained from deployed colonization substrate and aqueous samples, respectively. Colonization substrate was incubated *in situ* six weeks prior to removal. Samples were collected at three-week intervals from March through November 2014, with the first planktonic samples collected in March and the first attached samples collected in April after a six-week incubation period. Attached samples were obtained from fully screened stainless steel piezometers installed to 1.2m depth below the riverbed (nearshore, 5.25cm inside diameter (MAAS Midwest, Huntley, IL)) or from established groundwater wells (inland). Planktonic samples were obtained from galvanized piezometers located <1m from the piezometers used to sample attached microbiomes. For each inland location, a single well was used for both planktonic and attached samples. River water was sampled adjacent to the piezometers. DNA was extracted from each sample using the MoBio PowerSoil kit (MoBio Laboratories, Inc., Carlsbad, CA), and the 16S rRNA gene was sequenced on the Illumina MiSeq platform as described in the Supplemental Material. Physicochemical properties, active biomass (ATP), and aerobic respiration (Raz) were determined as per the Supplemental Material.

*Statistical analysis.*

All analyses were conducted in *R* software (http://cran.r-project.org/) unless otherwise noted. Relative abundances of major microbial taxa at phylum- and class- levels were calculated in each of five data subsets (nearshore attached, nearshore planktonic, inland attached, inland planktonic, river planktonic). An apparent breakpoint in hydrology at July 22 was assessed by comparing $Cl^-$ and NPOC concentration pre- and post- July 22 with one-sided Mann Whitney U tests. Temporal changes in temperature in each subset were assessed with quadratic regressions. Microbiome dissimilarities were estimated as Bray-Curtis distance and analyzed through time and across data subsets with PERMANOVA in QIIME (Caporaso et al., 2010).

*Modeling assembly processes.*

We implemented null modeling methods developed by Stegen *et al.* (2013; 2015) to disentangle community assembly processes. The approach uses pairwise phylogenetic turnover between communities, calculated using the mean-nearest-taxon-distance (βMNTD) metric (Webb et al., 2008; Fine and Kembel, 2011), to infer the strength of selection. Communities were evaluated for significantly less turnover than expected (βNTI < -2, homogeneous selection) or more turnover than expected (βNTI > 2, variable selection) by comparing observed βMNTD values to the mean of a null distribution of βMNTD values—and normalizing by its standard deviation—to yield βNTI (Stegen et al., 2012). Pairwise community comparisons that did not deviate from the null βMNTD distribution were evaluated for the influences of dispersal limitation and homogenizing dispersal by calculating the Raup-Crick metric extended to account for species relative abundances ($RC_{bray}$), as per Stegen *et al.* (2013; 2015). Observed Bray-Curtis dissimilarities were compared to the null distribution to derive $RC_{bray.}$ $RC_{bray}$ values that were > 0.95, > -0.95 and < 0.95, or < -0.95 were interpreted as indicating dispersal limitation, no

498 dominant assembly process, or homogenizing dispersal, respectively. Significance levels for
499 βNTI and $RC_{bray}$ are respectively based on standard deviations—|βNTI| = 2 denotes two standard
500 deviations from the mean of the null distribution—and alpha values—$|RC_{bray}| = 0.95$ reflects
501 significance at the 0.05 level. Inferences from both βNTI and $RC_{bray}$ have previously been
502 shown to be robust (Dini-Andreote et al., 2015; Stegen et al., 2015).
503
504 *Co-occurrence network analysis.*
505 Co-occurrence networks were constructed using Spearman's correlation at four
506 taxonomic levels – class, order, family, and OTU (singletons removed). To examine taxa with
507 moderate to strong co-occurrence patterns, correlations with *rho* > +/- 0.6 and FRD-corrected *P*
508 < 0.01 were imported into Cytoscape v3.3 for visualization and calculation of network
509 parameters. Networks were examined for deviations from randomness by comparing network
510 parameters to random networks generated with Network Randomiser 1.1 in Cytoscape (Barabasi-
511 Albert model with same number of nodes). Keystone taxa were identified using the Betweenness
512 Centrality metric (BC > 0), whereby increasing BC values indicate greater contribution of nodes
513 to network structure (González et al., 2010; Vick-Majors et al., 2014; Banerjee et al., 2016).
514 We identified clusters in Cytoscape using MCODE, using default parameters as per
515 Banerjee *et al.* (2015; 2016). We then calculated Pearson's momentum correlation between the
516 relative abundance of each cluster and selected parameters ($Cl^-$, NPOC, temperature, ATP, and
517 Raz) to screen for clusters of interest ($P < 0.05$). Regression models (linear and quadratic, as
518 appropriate) were then fit between cluster abundance (dependent variable) and the selected
519 parameters (independent variables). Analyses were conducted at all taxonomic levels, but we
520 present family-level correlations of microbiomes with time and physicochemistry due to the

consistency of patterns. For correlations with microbiome activity and aerobic respiration, results with were similar at family- and class-levels (Fig. S2 and Table S2), and we present class-level results that exhibited better model fit.

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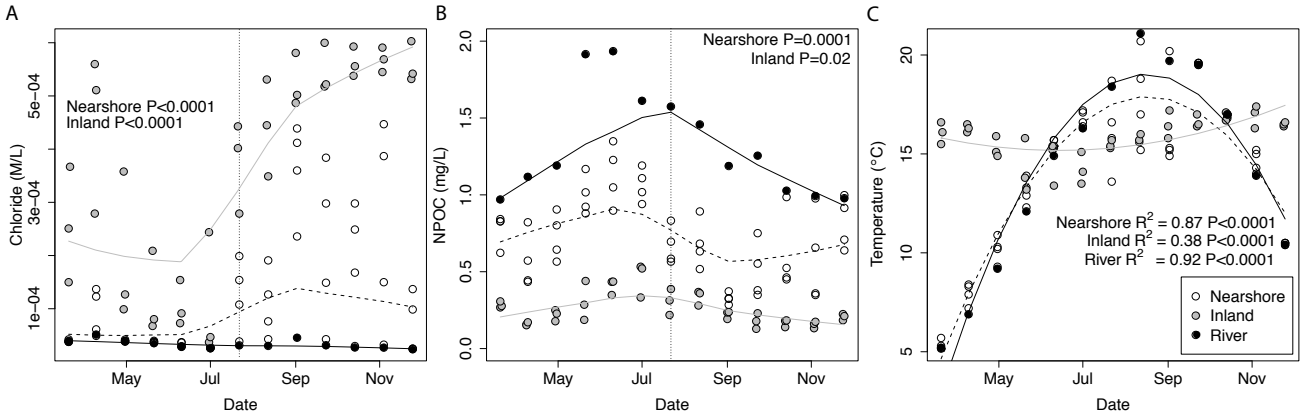
771 **FIGURES AND TABLES.**

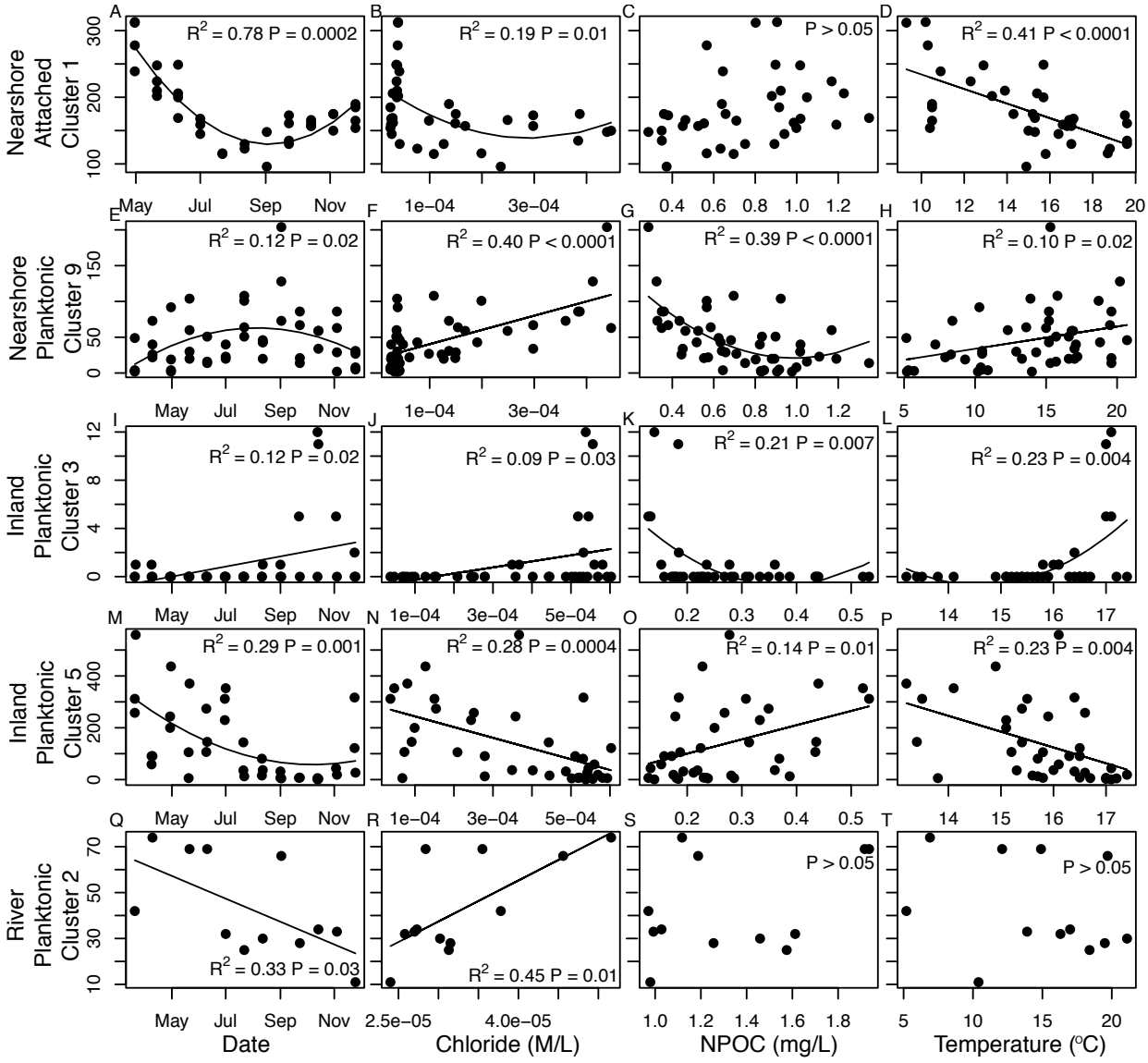



772

773 **Figure 1.** Changes in aqueous physicochemistry through time in each geographic zone are
774 presented in Figure 1. In the nearshore and inland zones, (A) $Cl^-$ concentration was higher and
775 (B) NPOC concentration was lower at and beyond our July 22 sampling event, indicating
776 groundwater discharging conditions from July 22 onward. Temperature (C) followed a smooth
777 temporal trajectory in the nearshore and river zones and was comparatively stable in the inland
778 zone. Lowess smoothers are plotted in panels (A) and (B) and quadratic regressions are plotted in
779 (C) to aid in visualization. Data from nearshore, inland, and river zones are plotted with open,
780 gray, and black circles, respectively. River stage dynamics are presented in Figure S1.

781

782




783

784 **Figure 2.** Regressions between the abundance of selected clusters and day of year (column 1),

785 $Cl^-$ concentration (column 2), non-purgable organic carbon (NPOC) (column 3), and temperature

786 (column 4). Nearshore attached cluster 1 is presented in row 1 (panels A-D), nearshore

787 planktonic cluster 9 is in row 2 (E-H), inland planktonic cluster 3 is in row 3 (I-L), inland

788 planktonic cluster 5 is in row 4 (M-P), and river cluster 2 is in row 5 (Q-T).

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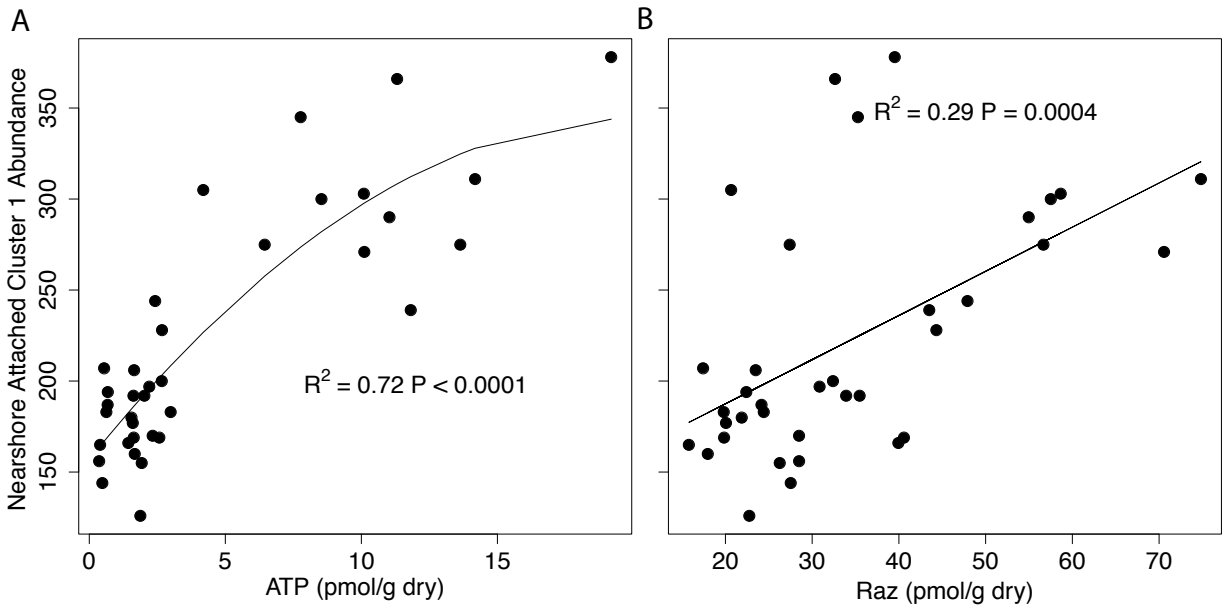


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792 **Figure 3.** The abundance of attached cluster 1 was positively associated with (A) active

793 microbial biomass and (B) aerobic respiration. Class-level analyses are presented here; family

794 level-analyses are presented in Figure S2.

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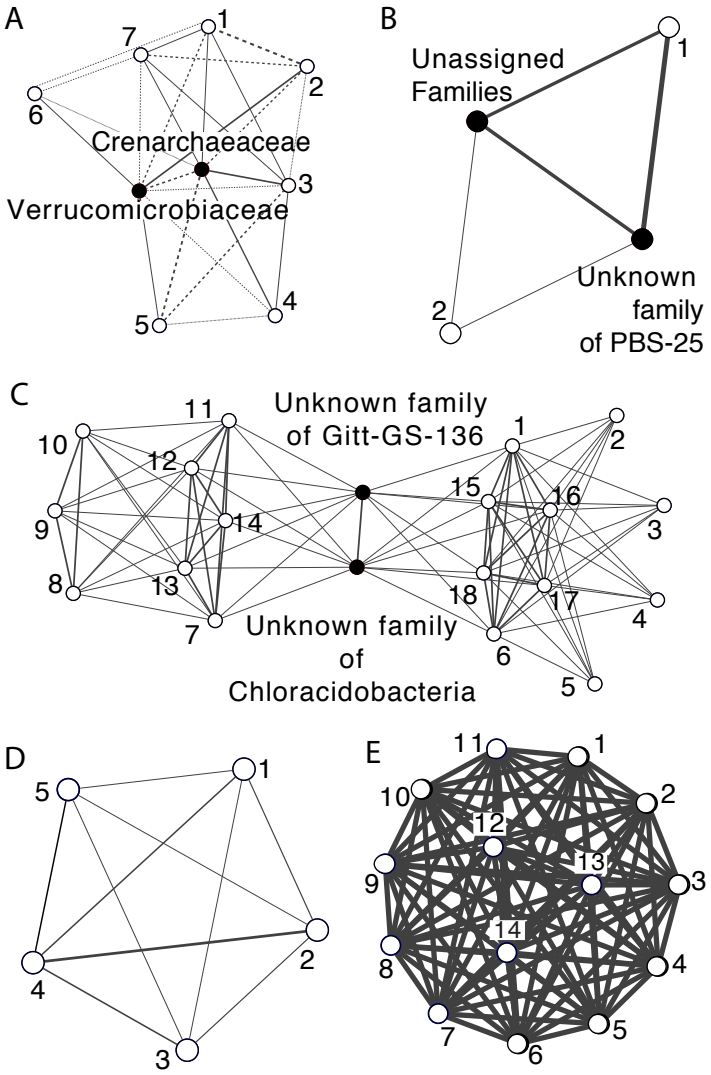


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798 **Figure 4.** Network topology of clusters of organisms that correlated with time, $Cl^-$, NPOC,

799 temperature, ATP, and/or Raz (see Figure 3) are presented in (A) nearshore attached cluster 1,

800 (B) nearshore planktonic cluster 9, (D) inland planktonic cluster 3, (E) inland cluster 5, and (E)

801 river cluster 2. Edge thickness denotes the strength of Spearman's correlation between two nodes

802 (ranging from 0.6 to 1.0), and keystone taxa are denoted as black nodes with labels. All other

803 nodes are numbered according to Table S4, and BC values for all nodes are also listed in table

804 S4. Solid edges represent positive correlations and dashed edges represent negative correlations.

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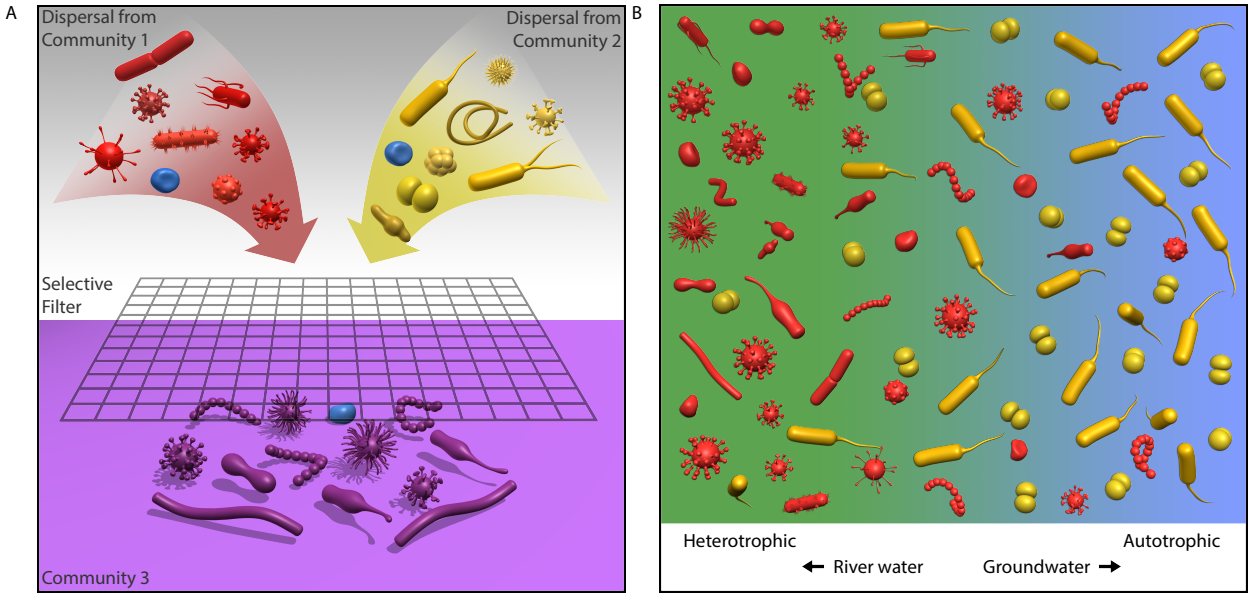


**Figure 5.** Conceptual diagrams depicting (A) influences of assembly processes on micriobiomes in our system and (B) microbiome shifts associated with hydrologic change in the nearshore hyporheic zone. In (A), hydrologic processes transport microorganisms from two communities to a third location. Each community consists of largely distinct organisms denoted by color (red vs. yellow), but contains a small proportion of similar organisms represented by the blue organism common to both dispersing communities. The establishment of dispersing organisms in the third location (purple zone) is strongly impacted by selective filters that limit successful immigration of organisms across environments. The local environment therefore selects for a unique microbiome (purple organisms) with the exception of a blue organism common to all three communities. Panel (B) depicts changes in microbiomes from heterotrophic to autotrophic metabolisms as hydrologic mixing conditions shift from surface water-dominated to groundwater-dominated. Mixing conditions are displayed as the background color, with green indicating surface water and blue indicating groundwater. Heterotrophic organisms are displayed in red, and autotrophic organisms are displayed in yellow.

823 **Table 1.** Phyla and classes of high abundance microorganisms are listed in Table 1.

***Phyla***

| ***Nearshore attached*** | | ***Nearshore planktonic*** | | ***Inland attached*** | | ***Inland planktonic*** | | ***River planktonic*** | |
|---|---|---|---|---|---|---|---|---|---|
| Proteobacteria | 34.82% | Proteobacteria | 29.35% | Proteobacteria | 60.66% | Proteobacteria | 27.66% | Bacteroidetes | 28.82% |
| Bacteroidetes | 19.12% | Bacteroidetes | 17.38% | Bacteroidetes | 8.13% | Unassigned | 17.32% | Actinobacteria | 23.35% |
| Planctomycetes | 13.74% | Actinobacteria | 14.67% | Planctomycetes | 5.27% | OP3 | 12.91% | Proteobacteria | 23.31% |
| Verrucomicrobia | 11.91% | Verrucomicrobia | 8.16% | Acidobacteria | 4.41% | Actinobacteria | 7.45% | Verrucomicrobia | 10.07% |
| Acidobacteria | 6.03% | Planctomycetes | 6.68% | Crenarchaeota | 4.07% | Bacteroidetes | 5.98% | Planctomycetes | 5.18% |
| Crenarchaeota | 2.68% | Unassigned | 5.13% | Verrucomicrobia | 2.93% | Planctomycetes | 4.43% | Cyanobacteria | 4.66% |
| Unassigned | 2.50% | Acidobacteria | 4.11% | Actinobacteria | 2.76% | Crenarchaeota | 4.00% | Acidobacteria | 1.37% |
| Actinobacteria | 1.59% | OP3 | 3.15% | Unassigned | 2.44% | Verrucomicrobia | 2.87% | Armatimonadetes | 7.51% |
| Nitrospirae | 1.56% | Cyanobacteria | 2.44% | Nitrospirae | 2.29% | Acidobacteria | 2.58% | Unassigned | 6.08% |
| Chloroflexi | 1.28% | Crenarchaeota | 2.10% | Chloroflexi | 1.72% | Nitrospirae | 2.17% | Chloroflexi | 5.40% |
| Remainder | 4.76% | Remainder | 6.83% | Remainder | 5.32% | Remainder | 12.65% | Remainder | 1.35% |

***Class***

| ***Nearshore attached*** | | ***Nearshore planktonic*** | | ***Inland attached*** | | ***Inland planktonic*** | | ***River planktonic*** | |
|---|---|---|---|---|---|---|---|---|---|
| Gammaproteobacteria | 12.49% | Actinobacteria | 12.06% | Betaproteobacteria | 30.50% | Unassigned | 17.32% | Actinobacteria | 21.19% |
| Planctomycetia | 12.07% | Betaproteobacteria | 10.22% | Gammaproteobacteria | 17.78% | Betaproteobacteria | 8.68% | Betaproteobacteria | 10.95% |
| Betaproteobacteria | 9.20% | Alphaproteobacteria | 9.98% | Alphaproteobacteria | 9.40% | Alphaproteobacteria | 7.71% | Alphaproteobacteria | 9.80% |
| Alphaproteobacteria | 9.11% | Saprospirae | 5.81% | Planctomycetia | 4.37% | PBS-25 | 7.34% | Flavobacteriia | 9.46% |
| Pedosphaerae | 6.93% | Planctomycetia | 5.18% | Thaumarchaeota | 4.07% | Actinobacteria | 6.41% | Saprospirae | 9.45% |
| Saprospirae | 5.79% | Unassigned | 5.13% | Chloracidobacteria | 3.09% | Gammaproteobacteria | 5.53% | Sphingobacteriia | 5.94% |
| Flavobacteriia | 5.05% | Flavobacteriia | 4.73% | Unassigned | 2.44% | koll11 | 5.52% | Planctomycetia | 4.55% |
| Cytophagia | 4.15% | Gammaproteobacteria | 4.44% | Deltaproteobacteria | 2.35% | Deltaproteobacteria | 5.45% | Synechococcophycideae | 4.10% |
| Chloracidobacteria | 3.84% | Deltaproteobacteria | 4.42% | Saprospirae | 2.29% | Thaumarchaeota | 3.85% | Cytophagia | 3.77% |
| Deltaproteobacteria | 3.81% | Sphingobacteriia | 4.01% | Nitrospira | 2.29% | Planctomycetia | 2.59% | Opitutae | 3.65% |
| Remainder | 27.54% | Remainder | 34.02% | Remainder | 21.43% | Remainder | 29.58% | Remainder | 17.15% |

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828 **Table 2.** Assembly processes influencing microbiome composition are listed as percentage of

829 assembly mechanisms in Table 2.

| | Variable Selection | Homogeneous Selection | Total Selection | Dispersal Limitation | Homogenizing Dispersal | Undominated | Total Stochasticity |
|---|---|---|---|---|---|---|---|
| *Within Geographic Zone* | | | | | | | |
| Nearshore attached | 0 | 100 | 100 | 0.0 | 0.0 | 0.0 | 0 |
| Nearshore planktonic | 6.8 | 52.9 | 59.8 | 6.6 | 14.9 | 18.7 | 40.2 |
| Inland attached | 0.4 | 82.1 | 82.5 | 1.7 | 1.7 | 14.0 | 17.5 |
| Inland planktonic | 14.3 | 21.5 | 35.7 | 22.1 | 7.2 | 35.6 | 64.3 |
| River planktonic | 0 | 95.5 | 95.5 | 0.0 | 3.0 | 1.5 | 4.5 |
| | | | | | | | |
| *Across Geographic Zones* | | | | | | | |
| Inland-to-nearshore planktonic | 91.96 | 0 | 91.96 | 1.79 | 1.79 | 4.46 | 8.04 |
| Nearshore-to-river planktonic | 34.84 | 29.18 | 64.02 | 2.83 | 15.3 | 17.85 | 35.98 |
| Inland-to-river planktonic | 51.17 | 6.33 | 57.5 | 26.76 | 1.64 | 14.1 | 42.5 |

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