The spatiotemporal dynamics of bacterial and fungal abundance in Great Plains non-perennial streams

by

Brett Adrian Nave Jr.

B.S, Kansas State University, 2019

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Biology
College of Arts and Sciences

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2024

Approved by:

Major Professor
Dr. Lydia H. Zeglin

Copyright

Abstract

Non-perennial streams comprise approximately 50% of global stream length and 59% across the continental U.S. Non-perennial streams are also predicted to become more common, since increased length and time of drying in rivers and streams is a widespread symptom of climate change. These substantial hydrologic fluctuations have the potential to impact the stream surface water and benthic microbiomes. Aquatic microorganisms are important drivers of carbon and nitrogen retention and immobilization, thus controlling both downstream water quality and integrated ecosystem metabolism. Surface flow connectivity to the local microbial habitat provides organic energy and dissolved nutrients that support microbial growth, and in turn, nutrient immobilization. However, the sensitivity of microbial populations to stream drying and rewetting is not well understood. Thus, learning more about the sensitivity of aquatic microbiomes to drying and rewetting cycles will improve our ability to assess future impacts to water quality.

We predicted that greater hydrological connectivity would support higher bacterial and fungal populations, to a plateau level where the growth response becomes saturated. Across fifty sampling locations in South Fork Kings Creek, Kansas, USA, microbial populations in surface waters, benthic (stream bed) sediments, epilithic biofilms (rock surfaces), and leaf surfaces were not related to the duration of flowing conditions before sampling, as expected, but did vary based on distance from the outlet of the watershed and differed between wet and dry sampling sites. During sequential hydroperiods (low flow, wet-up, dry-down, and disconnected) at three streams in Kansas and Oklahoma, USA, microbial abundance in the same four microhabitats changed during the wet-up and dry-down periods with patterns qualitatively consistent with spatial wet/dry differences. Specifically, epilithic biofilms tended to have higher microbial populations

in wet conditions, whereas microbial populations on leaves and in benthic sediments, unexpectedly, tended to be lower in wet than dry conditions.

These results suggest that as they grow, epilithic biofilm bacterial and fungal populations are more likely nutrient sinks during wet conditions. In contrast, larger sediment microbial populations can support more biogeochemical cycling, and provide potential refugia for stream microbiota, during dry conditions. Network continuum patterns also show greater surface water bacterial and fungal loads higher upstream, suggesting decreasing terrestrial dispersal pressure downstream in the stream network. This research establishes baselines on how microbial populations change in response to drying and rewetting over space and time, which will be used to inform water quality changes in non-perennial streams across the country. Future work should consider how bacterial, fungal, and protistan abundance, diversity, and activity can teach us more about the integrated functions of non-perennial stream ecosystems.

Table of Contents

List of Figures

List of Tables

List of Supplementary Figures

Acknowledgements

There are many people I would like to thank, but I would first like to begin by stating this research experience was one that will last a lifetime. Growing up in a single mother household, in a lower income neighborhood, never would I have thought to receive an amazing opportunity to collaborate across institutions and to receive the chance to network with other scientists from genuine, and diverse backgrounds. None of these experiences would have existed without my advisor and mentor, Dr. Lydia Zeglin. Thank you for the opportunity, guiding me through the research process, and returning me to the bigger picture when I began to drift. Her knowledge, passion and tenacity for science is an inspiration to many and why I aspire to conduct myself in scientific spaces in a similar manner. I would also like to thank my thesis committee members Dr. Matthew Kirk, and Dr. Charles Rice for aiding in project development, intellectual conversation and time spent editing my thesis. I would also like to thank past and present members of the Zeglin Lab for their assistance in the lab and field, and I especially would like to thank Janaye Figge for assistance in preparing field sampling kits and for being that someone to bounce ideas off when in a jam. I would also like to thank Dr. Matthew Nieland for working beside me and in aiding in my professional development as a scientist; and Nico Vega Anguiano, a lab member I started this journey with, for enthusiastic conversations on soil and biogeochemical cycling. I would also like to thank the affiliated institutions and people that allowed me to analyze samples from our many diligently managed sites: Kansas State University, University of Kansas, Oklahoma State University, University of Mississippi, University of Southern Mississippi, University of Alabama, and Idaho State University. I would also like to acknowledge that the land of these sites were home to indigenous people before European settlement, and still hold historical, and personal significance for Native Nations across the Great Plains. Last, but not least, is family. I would like to thank my mother and stepfather first and foremost. They believed in me, even during times when I struggled to believe in myself. Next, I would like to thank my father for the mental clarity and guidance he has provided that has allowed me to persist and go the distance. Lastly, I would like to thank my partner for her outpour of emotional support and for indulging in my tiresome tangents about microbes, but most importantly for remaining by my side throughout this adventure.

1 Chapter 1 – Introduction: Drivers of Microbial Abundance and Diversity in Non-Perennial Streams

Microorganisms, such as bacteria and fungi, are the nexus to downstream water quality and health, as they drive ecosystem nutrient cycles (Gionchetta et al., 2020; Arias-Real et al., 2020). These microbes are abundant in the waters, benthic sediments, epilithic biofilms and particulate organic matter of all stream types (Romaní et al., 2017). Important biogeochemical processes such as decomposition, denitrification, and nitrification are performed by both bacteria and fungi that occur in intermittent stream networks (Tomasek et al., 2017; Austin and Strauss, 2011; Six et al., 2006; Romaní et al., 2017). The growth and metabolism of microbes consume oxygen and various nutrients, contributing to nutrient retention and overall stream metabolism (Findlay, 2010; Battin et al., 2016). Despite the critical nature of microbes to ecosystem function, the relationship between microbial diversity and hydrological connectivity within intermittent stream networks is poorly understood. Non-perennial rivers are absent in many conceptual and predictive models of aquatic ecosystems, and this is problematic due to different biogeochemical cycling processes dominating during the dry phase compared to the wet phase (Zimmer et al., 2022). Since many intermittent headwater streams merge with larger bodies of water downstream, it is imperative to gain a finer resolution on their nutrient dynamics as they influence the microbial communities in the river network that then consequently affects biogeochemical cycling processes.

Intermittent and ephemeral stream network regimes are characterized by alternating wet and dry phases in the stream channel with various lateral, vertical, and longitudinal hydrological connections with neighboring ecosystems (Schiller et al., 2017). Many studies have shown that longitudinal connectivity is the primary driver of stream microbial distribution and abundance. This type of hydrological transport directly affects microbial composition and dispersal from original habitats into new habitats that can influence local species pools (Hassell et al., 2018; Ruiz-González et al., 2015). Intermittent and ephemeral streams comprise approximately 50% of global and 59% of continental U.S. stream length (Datry et al., 2014). More widespread and intense stream intermittency, as well as increasing frequencies and durations of drought, are

global consequences of climate change (Lohse and Gallo., 2020; Jaeger et al., 2014; Dodds et al., 2004).

Multiple environmental factors regulate microbial abundance, diversity, and extracellular enzymatic activity. Major factors such as microhabitat, seasonality, and hydrological regime are major drivers in cellular abundance and community composition differences (Zeglin, 2015). Minor, or local, factors that contribute to the oscillation of these microbial communities and their abundances are pH, dissolved oxygen concentrations and redox potential, conductivity, temperature, organic matter, inorganic nutrients, flow velocity, oxygen, and light (Febria et al., 2015; Webster and Benfield, 1986). Minor factors tailor the shape of the existing community that has been largely determined by the major factors. Compartment or microhabitat, the surface on which the microbes reside, also sets the stage for the growth and assembly of microbial populations and communities. The material surface of the microhabitat significantly contributes to the abundance and diversity of microbial species found within it. Romaní et al. (2013) found that grain size and organic matter content strongly influenced bacterial community composition, which suggest that substrates or environments with greater water-holding capacity and OM are potential places for microbial refugia during seasonal droughts and periods of desiccation.

Riparian tree cover plays a role in light and nutrient input, which subsequently affects the community composition and abundance of the stream microbial community. In drier and less perennial regions including shortgrass prairie, minimal riparian cover is present historically, even on rivers (Cross and Moss, 1987). Certain microbes are better at degrading leaf litter compounds than others and with limited riparian cover, richness and evenness of species are sure to be affected. In regions such as tallgrass prairie, more riparian tree cover develops in perennial reaches, leading to a downstream increase in leaf habitat and organic matter input to food webs, thus more detritivores, but a decrease in biofilm grazers. Decomposition of leaf litter that enters and is retained in prairie streams is influenced by inundation patterns, with generally slower decomposition in intermittent reaches compared with perennial reaches (Dodds et al., 2004; Hill et al., 1988). Differences in riparian tree cover from perennial reaches to intermittent ones can dictate different dissolved organic matter (labile or refractory) compounds entering the stream. With a diverse set of compounds in the stream we predict a diverse community of microbes capable of breaking down these compounds to be present. Since perennial streams are expected

to have more riparian tree cover, this could also reduce the abundance of photosynthetic organisms, like algae, on the epilithic biofilms that are hotspots for labile carbon input to stream food webs.

Redox gradients also play a key role in the structuring of microbial communities. The various available forms of carbon, nitrogen, phosphorus, and terminal electron acceptors, particularly dissolved oxygen, are a primary determinant of the identity and diversity of biogeochemical processes (Gómez-Gener et al., 2021). Strong redox gradients occur at the ecotone of surface water-groundwater interactions (i.e., the hyporheic zone), and concentrations of many biogeochemically important solutes vary widely as electron transport pathways are segregated by space, time, or both. A variety of electron acceptors such as oxygen, nitrate, ferric iron, manganic manganese, sulphate, and carbon dioxide may be used for organic matter oxidation depending on the redox potential of the environment. Fluctuating heights (flow state) of the water table caused by periodic flooding and drying change the dimensions of saturated and unsaturated areas within the stream riverbed. Subsequently, this affects nutrient concentrations, organic matter content, and redox potential of shallow groundwaters (Dahm et al., 1998). Biogeochemical cycling rates depend on the availability of limiting substrates both organic and inorganic, and redox gradients within non-perennial streams which depend on the flow state. Perennial microbial communities are noted for associations with generalist methanogens, in comparison to non-perennial streams, which appear to have more biogeochemical processes that are aerobic in nature relative to their perennial counterparts (Febria et al., 2015; Koch et al., 2015). This presumably reflects greater water saturation and connection to groundwater in perennial streams. Groundwater inputs can also mix surface microorganisms, such as those that perform photosynthesis, with anaerobic taxa associated with groundwater environments (methanogens, sulfur reducers, iron reducers), to promote microbial diversity and abundance. In a study conducted by Korbel et al., (2022) it was observed that the hyporheic zone and groundwater had a 44% similar microbial community as opposed to the 31% similarity with surface water to hyporheic zone. Some key microbial taxa responsible for that percent difference were *Cyanobacteria, Woesearchaeota, Nitrososphaerales*, and methanogens.

Hydrological regime or flow state is also a major driver of microbial abundance and community composition, as flowing water is the primary vector of microbial dispersal, and, pre-

existing drying conditions and soil moisture play a significant role in shaping microbial communities and abundances (Febria et al., 2015). Many studies illustrate that dry, moist, and saturated sediments (fragmented pools), as well as the overlaying flowing waters, have contrasting microbial community compositions and activity (Zeglin et al., 2011; Febria et al., 2012; Rees et al., 2006; Pohlon et al., 2013). Flow seasonality also affects microbes by altering major and minor drivers over time. When a non-perennial stream reaches the dry phase, it is common to see a decrease in microbial abundance and diversity and an increase in desiccant-tolerant bacterial taxa such as *Firmicutes* and *Actinobacteria*. The rationale that these two bacterial phyla are more abundant in non-perennial systems includes their possession of multiple rRNA operons within the genome, which is a proxy for maximum growth rate (Klappenbach et al., 2000) and the ability to acquire and convert resources to energy rather quickly, which allows for quick recovery after drought. These two bacteria are also gram-positive or have a thick peptidoglycan layer, and *Firmicutes* also includes many spore-forming populations, which can further explain desiccation-tolerant properties.

Fungal communities in non-perennial systems are most affected by temperature, pH, dissolved oxygen, dissolved nutrients, conductivity, physical abrasion and hydromorphological parameters (Webster and Benfield, 1986; Young et al., 2008; Tank et al., 2010). Low pH and low oxygen levels have a negative effect on fungal community composition and abundance while high conductivity (salinity) and high-temperature favor fungal development (Mendeiros et al., 2009; Mora-Gómez et al., 2015; Canhoto et al., 2016; Thiem et al., 2018). There are five fungal phyla typically found in freshwater: *Chytridiomycota, Cryptomycota, Blastocladiomycota, Ascomycota, and Basidiomycota* (Hibbett et al., 2007; Jones et al., 2014; Wurzbacher et al., 2015). However, most freshwater taxa belong to phylum *Ascomycota*, such as saprotrophic aquatic hyphomycete Ingoldians that participate primarily in the decomposition of leaf litter (Shearer et al., 2007; Bärlocher, 2016). In freshwater environments, fungal biomass accounts for 95 to >99 % of the total microbial biomass on decomposing plant detritus (Grossart et al., 2019; Gulis and Suberkropp, 2003; Krauss et al., 2011). As such, fungi play pivotal roles in maintaining multiple ecosystem functions and services, including, but not limited to global carbon processing, nutrient cycling, and energy transfer to higher trophic levels (Arias-Real, 2023). It has been observed that aquatic fungi are highly plastic and resistant microorganisms

(Coleine et al., 2022) and depend on aquatic habitats for at least part of their life cycle (Grossart et al., 2019; Grossart and Jiminez., 2016).

Evolution over time has led to aquatic fungi evolving numerous morphological and osmotic adaptations, such as hydrophobic cell walls, which are more efficient in reaching osmotic equilibrium during flow cessation and entering dormancy during drying (Canhoto et al., 2021; Gonçalves et al., 2019; Jones and Lennon, 2010). Previous studies also shed light on the notion that hyphae may cross air-filled sediment pores to access nutrients and water during drying (Gionchetta et al., 2019; Ghate and Sridhar, 2015). Given that aquatic fungi can present different drying strategies depending on their eco-physiology, morphology, and life-history (Crowther et al., 2014; Graça et al., 2023), the effects of drying may differ depending on which species in the community are most affected, subsequently altering rates of biogeochemical processes. In the present day, there are two assumptions that we must understand to interpret fungal diversity and the ecosystem services they provide: The first, is that fungal communities have functional plasticity and can adjust their performance in response to drying just as bacteria have been documented to do (Lipson et al., 2009; Allison and Martiny, 2008); and second, that different species of fungi with similar ecosystem roles can substitute for one another with no effects on ecosystem functioning, otherwise known as functional redundancy (Gionchetta et al., 2020; Allison and Martiny, 2008).

Arias-Real (2023) illustrated four niche-based survival strategies for aquatic fungi using an ecological niche conceptual model. They are the drying specialist, the generalist, the partly tolerant, and drying sensitive taxa. It is worth noting that the drying specialist, and the generalist will have less of an impact on ecosystem functioning due to eco-evolutionary tradeoffs, and the partly tolerant and drying-sensitive aquatic fungal taxa contribute the most to ecosystem functioning. There are limited comparisons of fungal community composition and abundance in non-perennial relative to perennial streams, but dissimilarities to expect are mainly in morphology (Romaní et al., 2017). Non-perennial fungi are expected to possess a filiform morphology to enable refugia mechanisms, while perennial fungi are compact or branched in nature to cope with higher flow velocities; and shear stress (Cornut, 2014).

Single-celled eukaryote (protist) community composition and abundance have also been rarely compared in non-perennial systems to perennial ones (Romaní et al., 2017). Abiotic

factors that are primarily responsible for shaping this microbial community are hydrology, conductivity, temperature, and dissolved organic matter (DOM) (Romaní et al., 2017). In streams and rivers, they are present in all microhabitat compartments, including the water column, sediment, biofilms on both organic and mineral surfaces, and the hyporheic zone (Franco et al., 1998; Cleven, 2004; Plebani et al., 2015).

Heteronanoflagellates (HNF) and ciliate protozoans together are responsible for the removal of 40% to 77% of bacteria in freshwater streams (Weisse, 1991). The most detrimental factors to the community and abundance of protozoa are temperature and particular biogeochemical conditions. Temperature by itself is only expected to affect the respiration, growth, and feeding of the protist, not community composition in a significant sense, but with synergistic effects from biogeochemical conditions community structure will begin to change significantly (Müller and Geller, 1993; Kathol et al., 2009). Certain types of protozoa such as amoeba are more sensitive to changes in DOM or the form of nitrogen as ammonia or nitrate. At the same time, aquatic flagellates are surprisingly less sensitive to salt than their soil counterparts (Ekelund, 2002). During re-wet of non-perennial streams, the influx of labile organic matter is predicted to stimulate protozoan activity and production (Christensen et al., 1992).

With respect to multicellular eukaryotes, i.e., metazoans or animals, some invertebrates associated with freshwater streams reproduce in days to weeks, but fish and larger macroinvertebrates with longer generation times require sustained wet periods of weeks or months for successful reproduction in intermittent reaches; thus, refugia are particularly important for these species (Dodds et al., 2004). This suggests that fish and invertebrates have higher diversity and abundance in perennial streams compared to their intermittent counterparts. With a more diverse and abundant fish and invertebrate population, microbial diversity and abundance is also expected to diverge, due to stronger and more complex top-down pressures.

With multiple varying abiotic factors that contribute to the structure and abundance of microbial communities, it is pivotal to understand how habitat structure influences the microbiome in richness, diversity, and function due to differences in surface for colonization, as well as nutrient and organic matter provision. Hydrology is a driver that not only affects microorganisms through space, but over time, and subsequently affects nutrient quality in a similar context. The intensity and frequency of drying and rewetting cycles regulate both

microbial physiology, and physical habitat properties that consequently affect cellular growth and death, and nutrient mineralization and mobilization rates (Wang et al., 2017). This research will help improve the scientific understanding of how microhabitat, timing, and flow dynamics shape microbial abundance patterns on various stream substrata in Great Plains non-perennial streams.

1.1 References Cited

Allison, S. D., & Martiny, J. B. (2008). Resistance, resilience, and redundancy in microbial communities. *Proceedings of the National Academy of Sciences*, *105*(supplement_1), 11512-11519.

Arias-Real, R., Menéndez, M., Muñoz, I., & Pascoal, C. (2023). Drying shapes the ecological niche of aquatic fungi with implications on ecosystem functioning. *Science of the Total Environment*, *859*, 160374.

Arias-Real, R., Muñoz, I., Gutierrez-Cánovas, C., Granados, V., Lopez-Laseras, P., & Menéndez, M. (2020). Subsurface zones in intermittent streams are hotspots of microbial decomposition during the non-flow period. *Science of the Total Environment*, *703*, 135485.

Austin, B. J., & Strauss, E. A. (2011). Nitrification and denitrification response to varying periods of desiccation and inundation in a western Kansas stream. *Hydrobiologia*, *658*, 183-195.

Bärlocher, F. (2016). Aquatic hyphomycetes in a changing environment. *Fungal Ecology*, *19*, 14-27.

Canhoto, C., Bärlocher, F., Cañedo-Argüelles, M., Gómez, R., & Gonçalves, A. L. (2021). Salt modulates plant litter decomposition in stream ecosystems. *The ecology of plant litter decomposition in stream ecosystems*, 323-345.

Canhoto, C., Goncalves, A. L., & Baerlocher, F. (2016). Biology and ecological functions of aquatic hyphomycetes in a warming climate. *Fungal Ecology*, *19*, 201-218.

Christensen, S., Griffiths, B. S., Ekelund, F., & Rønn, R. (1992). Huge increase in bacterivores on freshly killed barley roots. *FEMS Microbiology Letters*, *86*(4), 303-309.

Cleven, E. J. (2004). Seasonal and spatial distribution of ciliates in the sandy hyporheic zone of a lowland stream. *European Journal of Protistology*, *40*(1), 71-84.

Coleine, C., Stajich, J. E., & Selbmann, L. (2022). Fungi are key players in extreme ecosystems. *Trends in ecology & evolution*.

Cornut, J., Chauvet, E., Mermillod-Blondin, F., Assemat, F., & Elger, A. (2014). Aquatic hyphomycete species are screened by the hyporheic zone of woodland streams. *Applied and Environmental Microbiology*, *80*(6), 1949-1960.

Cross F.B., Moss R.E. (1987). Historic changes in fish communities and aquatic habitats in plains streams of Kansas. Pages 155-165 in Matthews WJ Heins DC, eds. Community and Evolutionary Ecology of North American Stream Fishes. Norman: University of Oklahoma Press.

Crowther, T. W., Maynard, D. S., Crowther, T. R., Peccia, J., Smith, J. R., & Bradford, M. A. (2014). Untangling the fungal niche: the trait-based approach. *Frontiers in microbiology*, *5*, 579.

Dahm, C. N., Grimm, N. B., Marmonier, P., Valett, H. M., & Vervier, P. (1998). Nutrient dynamics at the interface between surface waters and groundwaters. *Freshwater biology*, *40*(3), 427-451.

Datry, T., Larned, S. T., & Tockner, K. (2014). Intermittent rivers: a challenge for freshwater ecology. *BioScience*, *64*(3), 229-235.

Dodds, W. K., Gido, K., Whiles, M. R., Fritz, K. M., & Matthews, W. J. (2004). Life on the edge: the ecology of Great Plains prairie streams. *BioScience*, *54*(3), 205-216.

Ekelund, F. (2002). Tolerance of soil flagellates to increased NaCl levels. *Journal of Eukaryotic Microbiology*, *49*(4), 324-328.

Febria, C. M., Beddoes, P., Fulthorpe, R. R., & Williams, D. D. (2012). Bacterial community dynamics in the hyporheic zone of an intermittent stream. *The ISME journal*, *6*(5), 1078-1088.

Febria, C. M., Hosen, J. D., Crump, B. C., Palmer, M. A., & Williams, D. D. (2015). Microbial responses to changes in flow status in temporary headwater streams: a cross-system comparison. *Frontiers in microbiology*, *6*, 522.

Findlay, S. (2010). Stream microbial ecology. Journal of the North American Benthological Society, 29(1), 170-181.

Franco, C., Esteban, G. and Téllez, C. (1998) Colonization and succession of ciliated protozoa associated with submerged leaves in a river. Limnologica 28, 275–283.

Ghate, S. D., & Sridhar, K. R. (2015). Diversity of aquatic hyphomycetes in streambed sediments of temporary streamlets of Southwest India. *Fungal Ecology*, *14*, 53-61.

Gionchetta, G., Oliva, F., Romaní, A. M., & Bañeras, L. (2020). Hydrological variations shape diversity and functional responses of streambed microbes. *Science of the Total Environment*, *714*, 136838.

Gionchetta, G., Oliva, F., Menéndez, M., Lopez-Laseras, P., & Romaní, A. M. (2019). Key role of streambed moisture and flash storms for microbial resistance and resilience to long-term drought. *Freshwater Biology*, *64*(2), 306-322.G.J.

Gómez-Gener, L., Siebers, A. R., Arce, M. I., Arnon, S., Bernal, S., Bolpagni, R., Datry, T., Gionchetta, G., Grossart, H.P., Mendoza-Lara, C., Pohl, V., Risse-Buhl, U., Shumilova, O., Tzoraki, O., Schiller, D., Weigand, A., Weigelhofer, G., Zak, D., & Zoppini, A. (2021). Towards an improved understanding of biogeochemical processes across surface-groundwater interactions in intermittent rivers and ephemeral streams. *Earth-Science Reviews*, *220*, 103724.

Graça, D., Fernandes, I., Cássio, F., & Pascoal, C. (2023). Eco-physiological responses of aquatic fungi to three global change stressors highlight the importance of intraspecific trait variability. *Microbial ecology*, *85*(4), 1215-1225.

Grossart, H. P., & Rojas-Jimenez, K. (2016). Aquatic fungi: targeting the forgotten in microbial ecology. *Current opinion in microbiology*, *31*, 140-145.

Grossart, H. P., Van den Wyngaert, S., Kagami, M., Wurzbacher, C., Cunliffe, M., & Rojas-Jimenez, K. (2019). Fungi in aquatic ecosystems. *Nature Reviews Microbiology*, *17*(6), 339-354.

Gulis, V., & Suberkropp, K. (2003). Leaf litter decomposition and microbial activity in nutrient-enriched and unaltered reaches of a headwater stream. *Freshwater biology*, *48*(1), 123-134.

Hassell, N., Tinker, K. A., Moore, T., & Ottesen, E. A. (2018). Temporal and spatial dynamics in microbial community composition within a temperate stream network. *Environmental microbiology*, *20*(10), 3560-3572.

Hibbett, D. S., Binder, M., Bischoff, J. F., Blackwell, M., Cannon, P. F., Eriksson, O. E., & Zhang, N. (2007). A higher-level phylogenetic classification of the Fungi. *Mycological research*, *111*(5), 509-547.

Hill B.H., Gardner T. J., Ekisola O. F. (1988). Breakdown of gallery forest leaf litter in intermittent and perennial prairie streams. Southwestern Naturalist. 33: 323-331.

Jaeger, K. L., Olden, J. D., & Pelland, N. A. (2014). Climate change poised to threaten hydrologic connectivity and endemic fishes in dryland streams. Proceedings of the National Academy of Sciences, 111(38), 13894-13899. doi: 10.1073/pnas.1320890111

Jones, E. G., Hyde, K. D., & Pang, K. L. (Eds.). (2014). *Freshwater fungi: and fungal-like organisms*. Walter de Gruyter GmbH & Co KG.

Jones, S. E., & Lennon, J. T. (2010). Dormancy contributes to the maintenance of microbial diversity. *Proceedings of the National Academy of Sciences*, *107*(13), 5881-5886.

Kathol, M., Norf, H., Arndt, H., & Weitere, M. (2009). Effects of temperature increase on the grazing of planktonic bacteria by biofilm-dwelling consumers. *Aquatic Microbial Ecology*, *55*(1), 65-79.

Klappenbach, J. A., Dunbar, J. M., & Schmidt, T. M. (2000). rRNA operon copy number reflects ecological strategies of bacteria. *Applied and environmental microbiology*, *66*(4), 1328-1333.

Koch, H., Lücker, S., Albertsen, M., Kitzinger, K., Herbold, C., Spieck, E., Nielsen, P.H., Wagner, M., & Daims, H. (2015). Expanded metabolic versatility of ubiquitous nitrite-oxidizing bacteria from the genus Nitrospira. *Proceedings of the national academy of sciences*, *112*(36), 11371-11376.

Korbel, K. L., Rutlidge, H., Hose, G. C., Eberhard, S. M., & Andersen, M. S. (2022). Dynamics of microbiotic patterns reveal surface water groundwater interactions in intermittent and perennial streams. *Science of The Total Environment*, *811*, 152380.

Krauss, G. J., Sole, M., Krauss, G., Schlosser, D., Wesenberg, D., & Baerlocher, F. (2011). Fungi in freshwaters: ecology, physiology and biochemical potential. *FEMS microbiology reviews*, *35*(4), 620-651.

Lipson, D. A., Monson, R. K., Schmidt, S. K., & Weintraub, M. N. (2009). The trade-off between growth rate and yield in microbial communities and the consequences for under-snow soil respiration in a high elevation coniferous forest. *Biogeochemistry*, *95*, 23-35.

Lohse, K. A., Gallo, E. L., & Meixner, T. (2020). Influence of climate and duration of stream water presence on rates of litter decomposition and nutrient dynamics in temporary streams and surrounding environments of southwestern USA. *Frontiers in Water*, *2*, 571044.

Mora-Gómez, J., Elosegi, A., Mas-Martí, E., & Romaní, A. M. (2015). Factors controlling seasonality in leaf-litter breakdown in a Mediterranean stream. *Freshwater Science*, *34*(4), 1245-1258.

Müller, H., & Geller, W. (1993). Maximum growth rates of aquatic ciliated protozoa: the dependence on body size and temperature reconsidered. *Archiv fur Hydrobiologie*, *126*, 315-315.

Plebani, M., Fussmann, K. E., Hansen, D. M., O'Gorman, E. J., Stewart, R. I., Woodward, G., & Petchey, O. L. (2015). Substratum-dependent responses of ciliate assemblages to temperature: a natural experiment in Icelandic streams. *Freshwater Biology*, *60*(8), 1561-1570.

Pohlon, E., Ochoa Fandino, A., & Marxsen, J. (2013). Bacterial community composition and extracellular enzyme activity in temperate streambed sediment during drying and rewetting. PloS one, 8(12), e83365.https://doi.org/10.1371/journal.pone.0083365

Rees, G. N., Watson, G. O., Baldwin, D. S., & Mitchell, A. M. (2006). Variability in sediment microbial communities in a semipermanent stream: impact of drought. Journal of the North American Benthological Society, 25(2), 370-378. https://doi.org/10.1899/0887"

Romaní, A. M., Amalfitano, S., Artigas, J., Fazi, S., Sabater, S., Timoner, X., & Zoppini, A. (2013). Microbial biofilm structure and organic matter use in mediterranean streams. *Hydrobiologia*, *719*, 43-58.

Romaní, A. M., Chauvet, E., Febria, C., Mora-Gómez, J., Risse-Buhl, U., Timoner, X., Weitere, M., & Zeglin, L. (2017). The biota of intermittent rivers and ephemeral streams: prokaryotes, fungi, and protozoans. In Intermittent rivers and ephemeral streams (pp. 161-188). Academic Press, https://doi.org/10.1016/B978-0-12-803835-2.00009-7

Ruiz-González, C., Niño-García, J. P., & Del Giorgio, P. A. (2015). Terrestrial origin of bacterial communities in complex boreal freshwater networks. Ecology letters, 18(11), 1198-1206. https://doi.org/10.1111/ele.12499

Shearer, C. A., Descals, E., Kohlmeyer, B., Kohlmeyer, J., Marvanová, L., Padgett, D., Porter, D., Raja, H.A., Schmidt, J.P., Thorton, H.A., & Voglymayr, H. (2007). Fungal biodiversity in aquatic habitats. *Biodiversity and Conservation*, *16*, 49-67.

Six, J., Frey, S. D., Thiet, R. K., & Batten, K. M. (2006). Bacterial and fungal contributions to carbon sequestration in agroecosystems. Soil Science Society of America Journal, 70(2), 555-569. https://doi.org/10.2136/sssaj2004.0347

Tank, J. L., Rosi-Marshall, E. J., Griffiths, N. A., Entrekin, S. A., & Stephen, M. L. (2010). A review of allochthonous organic matter dynamics and metabolism in streams. *Journal of the North American Benthological Society*, *29*(1), 118-146.

Thiem, D., Gołębiewski, M., Hulisz, P., Piernik, A., & Hrynkiewicz, K. (2018). How does salinity shape bacterial and fungal microbiomes of Alnus glutinosa roots?. *Frontiers in Microbiology*, *9*, 651.

Tomasek, A., Staley, C., Wang, P., Kaiser, T., Lurndahl, N., Kozarek, J. L., & Sadowsky, M. J. (2017). Increased denitrification rates associated with shifts in prokaryotic community

composition caused by varying hydrologic connectivity. Frontiers in microbiology, 8, 2304. https://doi.org/10.3389/fmicb.2017.02304

von Schiller, D., Bernal, S., Dahm, C. N., & Martí, E. (2017). Nutrient and organic matter dynamics in intermittent rivers and ephemeral streams. In *Intermittent rivers and ephemeral streams* (pp. 135-160). Academic Press.

Wang, Y., Jensen, C. R., & Liu, F. (2017). Nutritional responses to soil drying and rewetting cycles under partial root-zone drying irrigation. Agricultural Water Management, 179, 254-259.

Webster, J. R., & Benfield, E. F. (1986). Vascular plant breakdown in freshwater ecosystems. *Annual review of ecology and systematics*, *17*(1), 567-594.

Weisse, T. (1991). The annual cycle of heterotrophic freshwater nanoflagellates: role of bottom-up versus top-down control. *Journal of plankton research*, *13*(1), 167-185.

Wurzbacher, C., Grimmett, I. J., & Bärlocher, F. (2015). Metabarcoding-based fungal diversity on coarse and fine particulate organic matter in a first-order stream in Nova Scotia, Canada. *F1000Research*, *4*.

Young, R. G., Matthaei, C. D., & Townsend, C. R. (2008). Organic matter breakdown and ecosystem metabolism: functional indicators for assessing river ecosystem health. *Journal of the North American Benthological Society*, *27*(3), 605-625.

Zeglin, L. H. (2015). Stream microbial diversity in response to environmental changes: review and synthesis of existing research. *Frontiers in microbiology*, *6*, 454.

Zeglin, L. H., Dahm, C. N., Barrett, J. E., Gooseff, M. N., Fitpatrick, S. K., & Takacs-Vesbach, C. D. (2011). Bacterial community structure along moisture gradients in the parafluvial sediments of two ephemeral desert streams. *Microbial ecology*, *61*, 543-556.

Zimmer, M. A., Burgin, A. J., Kaiser, K., & Hosen, J. (2022). The unknown biogeochemical impacts of drying rivers and streams. *nature communications*, *13*(1), 7213.

2 Chapter 2 - Spatiotemporal Dynamics of Bacterial and Fungal Abundance to Great Plains Stream Intermittency

2.1 Abstract

Non-perennial streams comprise approximately 50% of global stream length and 59% across the continental U.S. Non-perennial streams are also predicted to become more common, since increased length and time of drying in rivers and streams is a widespread symptom of climate change. These substantial hydrologic fluctuations have the potential to impact the stream surface water and benthic microbiomes. Aquatic microorganisms are important drivers of carbon and nitrogen retention and immobilization, thus controlling both downstream water quality and integrated ecosystem metabolism. Surface flow connectivity to the local microbial habitat provides organic energy and dissolved nutrients that support microbial growth, and in turn, nutrient immobilization. However, the sensitivity of microbial populations to stream drying and rewetting is not well understood. Thus, learning more about the sensitivity of aquatic microbiomes to drying and rewetting cycles will improve our ability to assess future impacts to water quality.

We predicted that greater hydrological connectivity would support higher bacterial and fungal populations, to a plateau level where the growth response becomes saturated. Across fifty sampling locations in South Fork Kings Creek, Kansas, USA, microbial populations in surface waters, benthic (stream bed) sediments, epilithic biofilms (rock surfaces), and leaf surfaces were not related to the duration of flowing conditions before sampling, as expected, but did vary based on distance from the outlet of the watershed and differed between wet and dry sampling sites. During sequential hydroperiods (low flow, wet-up, dry-down, and disconnected) at three streams in Kansas and Oklahoma, USA, microbial abundance in the same four microhabitats changed during the wet-up and dry-down periods with patterns qualitatively consistent with spatial wet/dry differences. Specifically, epilithic biofilms tended to have higher microbial populations in wet conditions, whereas microbial populations on leaves and in benthic sediments, unexpectedly, tended to be lower in wet than dry conditions.

These results suggest that as they grow, epilithic biofilm bacterial and fungal populations are more likely nutrient sinks during wet conditions. In contrast, the larger sediment microbial populations can support more biogeochemical cycling, and provide potential refugia for stream

microbiota, during dry conditions. Network continuum patterns also show greater surface water bacterial and fungal loads higher upstream, suggesting decreasing terrestrial dispersal pressure downstream in the stream network. This research establishes baselines on how microbial populations change in response to drying and rewetting over space and time, which will be used to inform water quality changes in non-perennial streams across the country. Future work should consider how bacterial, fungal, and protistan abundance, diversity, and activity can teach us more about the integrated functions of non-perennial stream ecosystems.

2.2 Introduction

Non-perennial, intermittent, and ephemeral streams comprise approximately 50% of global and 59% of continental U.S. stream length (Datry et al., 2014). More widespread and intense stream intermittency is a global consequence of climate change (Lohse and Gallo, 2020; Jaeger et al., 2014; Dodds et al., 2004). Intermittent and ephemeral stream network regimes are generally characterized by alternating wet and dry phases in the stream channel with various lateral, vertical, and longitudinal hydrological connections with neighboring ecosystems (Schiller et al., 2017). Non-perennial rivers are absent in many conceptual and predictive models of aquatic ecosystems, and this is problematic for our understanding of ecosystem health and water quality, because different physiological constraints on organismal function, and different biogeochemical cycling processes, dominate during the dry phase compared to the wet phase (Zimmer et al., 2022). Since many intermittent headwater streams merge with larger bodies of water downstream, it is imperative to gain a finer resolution on their nutrient dynamics as they influence the microbial communities in the river network that consequently affect biogeochemical cycling processes and water quality.

Microorganisms, such as bacteria and fungi, are the nexus to downstream water quality and health, because they drive ecosystem nutrient cycles (Gionchetta et al., 2020; Arias-Real et al., 2020). The growth and metabolism of microorganisms cycles oxygen and consumes nutrients, contributing to stream metabolism and nutrient retention, and sustaining metazoan food webs (Findlay, 2010; Battin et al., 2016). Microbes are abundant in the waters, benthic sediments, epilithic biofilms, and leaves of all stream types (Zeglin, 2015), and they perform important biogeochemical processes such as respiration, decomposition, denitrification, and

nitrification (Tomasek et al., 2017; Austin and Strauss, 2011; Six et al., 2006; Romaní et al., 2017). Despite the critical nature of microbes to ecosystem function, the relationship between microbial diversity and hydrological connectivity is poorly understood, particularly within intermittent stream networks (Romaní et al., 2017).

Multiple environmental factors regulate microbial abundance, diversity, and function in all streams, including non-perennial streams. These include large-scale factors such as seasonality and hydrological regime; and local factors like pH, dissolved oxygen and redox potential, conductivity, temperature, organic matter (OM), inorganic nutrients, and light (Febria et al., 2015; Webster and Benfield, 1986). Local factors tailor the shape of the existing community that has been largely determined by the major factors. The microhabitat within a stream reach also constrains the growth and structure of microbial populations and communities through the particular physical surface characteristics and nutrient availability of different stream compartments (i.e., surface water, epilithic biofilm, benthic sediment, or leaves) (Zeglin, 2015). Further, the compartment type influences bacterial community composition in non-perennial streams, such that microhabitats with greater water holding capacity and OM, such as finer-grained sediments, are more likely locations of microbial refugia and activity during seasonal droughts and periods of desiccation (Romaní et al., 2013). However, the richness and abundance of microbes in both epilithic biofilms and stream sediments have been observed to decline during dry periods (Rees et al., 2006; Amalfitano et al., 2008; Febria et al., 2012; Sabater et al. 2016).

Hydrological regime or flow state is also a major driver of microbial abundance and community composition, as flowing water is a primary vector of microbial dispersal (Crump et al., 2012; Ruiz-González et al., 2015), and also, pre-existing drying conditions and soil moisture play a significant role in shaping microbial communities and abundances (Febria et al., 2015). Many studies illustrate that dry, moist, and saturated sediments (fragmented pools), as well as the overlaying flowing waters, have contrasting microbial community compositions and activity (Zeglin et al., 2011; Febria et al., 2012; Rees et al., 2006; Pohlon et al., 2013). Flow seasonality also affects microbes by altering major and minor drivers over time. Biogeochemical cycling rates depend on the availability of limiting substrates both organic and inorganic, and redox gradients within non-perennial streams vary depending on the flow state. Perennial microbial communities are noted for having microbial associations with generalist methanogens, in

comparison to non-perennial streams, which appear to have more biogeochemical processes that are aerobic relative to their perennial counterparts (Febria et al., 2015; Koch et al., 2015). Groundwater inputs can also produce mixing of surface microorganisms, such as those that perform photosynthesis, with anaerobic taxa associated with groundwater environments (methanogens), to promote microbial diversity and abundance.

Microbial abundance within stream ecosystems is a parameter of key interest, because it represents the direct connection between water quality and the microbiome, through nutrient immobilization in microbial biomass. The growth of stream biofilms largely relies on the delivery of inorganic nutrients from surface water (Findlay, 2010; Battin et al., 2016). Nutrient immobilization from surface waters to benthic sediments and epilithon can contribute significantly to whole-stream biogeochemical cycles and metabolism (Mulholland et al. 2009, Bernot et al. 2010), and nutrient pollution of streams stimulates production and biomass in both autotrophic and heterotrophic stream microorganisms and food webs globally (Ardón et al. 2020). In flowing conditions, bacterial and algal abundance of epilithic biofilms in non-perennial streams can increase orders of magnitude within weeks, immobilizing nutrients, fixing carbon, and driving ecosystem metabolism (Besemer, 2015; Veach et al., 2016). Upon rewet of dry sediments, there can be rapid increases in microbial respiration rates and nutrient immobilization (McIntyre et al., 2009). Even on leaves, environmental controls on immobilization are less well understood, due to nutrient demand while decomposing low-nutrient organic matter, fungi and bacteria acquire nitrogen and phosphorus from the surface water (Costello et al., 2022). However, during times of drying, the reduced transport of nutrients and microbial dispersal and colonization can contribute to both decreasing microbial activity and nutrient immobilization on all stream microhabitats (Mora-Gómez et al., 2018; Bruder et al., 2011; Langhans and Tockner, 2006). Within biofilms, drying can also favor heterotrophic over autotrophic processes due to the positive relationship between photosynthetic efficiency and water content (Timoner et al., 2012; Sabater et al., 2016), which can promote net nutrient mineralization rather than immobilization.

Because stream flow removes waste products and delivers nutrients that promote population growth, we predicted that greater hydrological connectivity would support higher bacterial and fungal populations, to a plateau level where the growth response was saturated. Hydrological connectivity was estimated differently in spatial and temporal contexts: Across a

small stream network, connectivity was measured as the duration of flowing conditions preceding sample collection; over time in three different stream catchments, connectivity level was categorized into hydroperiods that differentiated connected, disconnected, increasing, and decreasing flow conditions. Bacterial and fungal abundance was estimated using quantitative PCR (qPCR) of marker genes, and other catchment and water chemistry attributes were also quantified to assess the strength of covarying relationships with population size. Finally, because growth dynamics are expected to differ among microhabitat types, we evaluated our predictions using microbial population data collected from water column, epilithic biofilms, leaves, and benthic sediment substrates.

2.3 Methods

2.3.1 Site and Experimental Design

Spatial and temporal sampling was conducted at Kings Creek, located at Konza Prairie Biological Station, near Manhattan, KS, USA (39.092281, -96.58719). This site lies within the Flint Hills ecoregion, which has 835 mm annual precipitation, with high interannual variability (Hayden 1998). The average air temperature of Konza Prairie is 13ºC. It is estimated that 75% of annual precipitation occurs during late spring and early summer (Hayden 1998). The underlying bedrock of the Flint Hills ecotone is characterized as limestone, mudstone, and shale with predominately silty clay loam soils that rest atop (Hayden 1998, Briggs et al 2016, Vero et al. 2018). The riparian vegetation consists of deciduous forest trees such as mature bur oak (*Quercus macrocarpa*), chinquapin oak (*Q. muehlenbergii*), hackberry (*Celtis occidentalis*) and beyond the riparian area, tallgrass prairie dominates the area (Samson and Knopf 1994) with dominant grass species of big bluestem (*Andropogon gerardii*), little bluestem (*Schizachyrium scoparium*), Indian grass (*Sorghastrum nutans*), and switchgrass (*Panicum virgatum*), although riparian woody encroachment has occurred in most subwatersheds (Veach et al. 2015).

The second site is the south branch of the Elk River headwaters located within Youngmeyer Ranch, east of Wichita, KS (37.545022, -96.489850). Youngmeyer Ranch is a 1902-hectare field station that is actively cattle grazed and burned every 1-2 years. Sections of Elk River run perennially and are fed by natural springs. This site has a mean annual temperature and precipitation of 13.7ºC and 979 mm (Aurell et al. 2023), and is geologically constructed of Permian age limestone and shale with layers of chert below silty clay loam soils. This site is

predominantly grassland composed of big blue stem (*A. gerardii*), little bluestem (*A. scoparium*), Indian grass (*S. nutans*), and switchgrass (*P. virgatum*), with scattered black oaks (*Q. veluntina)* along the creeks (Houseman et al. 2016).

The third site is Anoatubby Creek, a first order stream located in the 3600-hectare Oka'Yanahli National Preserve near Johnston, OK (34.436567, -96.651805). This site has grazing rotations, prescribed burns, and woody shrub removal. Mean annual temperature and mean annual precipitation is 17ºC and 1410 mm (Buthod and Hoagland, 2020). The Oka'Yanahli National Preserve is part of the cross timbers ecoregion, which is a hybrid ecotone of deciduous forests and grasslands (Woods et al. 2005). This ecoregion is a mixture of woodland, prairie, and savannah on coarse sandy soil and the geomorphic composition is limestone from the Ordovician age (Premathilake, 2018). Tree species commonly observed are post-oak (*Q. stellata*), blackjack oak (*Q. marilandica*), American elm (*Ulmus americana*) and eastern red cedar (*Juniperus virginiana*) in wooded areas (Kasparian et al. 2004). Dominant grasses across the Reserve are silver bluestem (*A. saccharoides*), little bluestem (*A. scoparius*), broomsedge bluestem (*A. virginicus*), oldfield threeawn (*Aristida oligantha*), and buffalo grass (*Bouteloua dactyloides*).

In July 2021, we conducted a coordinated synoptic sampling campaign at fifty sites distributed throughout the South Fork Kings Creek watershed (Figure S 2.1). Sampling locations were chosen to cover a range of contributing drainage areas and topographical wetness index (TWI) values, because TWI was previously shown to be a significant predictor of flow permanence within non-perennial streams (Warix et al. 2021). TWI is a unitless physiographic variable that integrates drainage area and local slope, and in this study was used as a proxy of how likely a site is to be wet (Riihimäki et al. 2021). To measure connectivity at each sampling point, we used sensors for stream temperature, intermittency, and conductivity (STICs), which record the presence or absence of water every 20 minutes in a binary format (Chapin et al., 2014). Using this information, a percent duration of wet conditions preceding the sampling time (since the STIC sensors were installed in March 2021) was derived.

Between March and November 2022, a comparison of dynamics during different hydroperiods at each of the three study watersheds (South Fork Kings Creek, Youngmeyer Ranch, and Oka'Yanahli). Following seasonal wetting and drying patterns, samples were collected during the dry phase preceding wet conditions (March, Kings Creek only), wet-up

phase (May), dry-down phase (July), and dry phase following wet conditions (November, Kings Creek only) (Figure S 2.2). Within each sampling catchment, seven long term monitoring sites were identified to cover the range of positions within the stream network and a range of TWI values, as with the intensively sampled watershed. These critical junctures within each non-perennial stream network integrate distinct upstream contributing watershed areas and were all sampled during each hydroperiod.

2.3.2 Sample Collection

To collect each sample, we used sterile techniques with composite grab sampling across 3 transects across the channel width for leaf litter, biofilm on rock surface (i.e., epilithon), benthic sediment (to 2 cm depth), and the stream water column itself, when water was present (Zeglin et al. 2022). Water was collected first to prevent benthic disturbance from contaminating the sample: Up to 120 mL of water was filtered through a sterile 0.22 µM filter (hydrophilic cellulose acetate, GVS Life Sciences 1213124). Epilithon was scraped from the surface of three rocks per transect using a wire brush and an aerial template (25 cm^2) into a pre-sterilized container, then up to 10 mL of the biofilm slurry (suspended in stream water or sterile DI water if the stream was dry at that location) was filtered onto another sterile 0.22 µM filter. Leaves that were fully submerged, not freshly fallen but still holding their shape, were collected and cut into smaller pieces using flame-sterilized scissors into a sterile 15 mL conical vial. Finally, a sterile 50mL conical vial was used as a coring device to collect the top 2 cm of benthic sediment at three subsample locations, and 5 mL of the composite sample was transferred using a sterile scoopula to a sterile 15 mL conical vial. All samples were held on ice for < 4 h while transported from the field, then flash-frozen in liquid nitrogen, and stored at -80ºC until further processing.

To enable field-scale quantitative population size estimates, we standardized and recorded the volume of water filtered, mass of leaf and sediment collected, and area of eplilithon scraped. If rocks did not fit within the areal template used for eplilithon collection, then rock outlines were traced onto field data sheets, images taken with scale bar included, and ImageJ software (LOCI, University of Wisconsin) was used to calculate the surface area of rocks for total epilithon enumeration (Rueden et al., 2017).

2.3.3 DNA Extraction and microbial abundance estimates

DNA extraction of collected samples was performed using the DNeasy PowerSoil® DNA Pro Kit (Qiagen, Germany) following the manufacturer's protocol with adapted alterations (Dunham-Cheatham and You, 2019). After DNA extraction and purification, the DNA yield was measured using the Quant-iT PicoGreen dsDNA Pico Assay Kits with a Filtermax Pro fluorometric microplate reader. For estimation of DNA yield across the four substrate types at a field-scale level, DNA concentration by purification volume was normalized by the mass of leaf or sediment, the area biofilm scraped, or the volume of water filtered.

For estimation of bacterial and fungal abundance, quantitative PCR (qPCR) assays were performed using an Applied Biosystems 7500 Real-Time PCR instrument with Universal SYBR Green Supermix. The bacterial and archaeal community target for abundance estimation is the V4 hypervariable region of 16S rRNA gene and for fungal estimation is the ITS region of the rRNA gene. The primers used for bacterial abundance are EUB338 and EUB518, and for fungal abundance are ITS1f and 5.8s. DNA was amplified using established primer sequences and thermal cycler programs (Fierer et al., 2005). Gene copy number was expressed both per mass of DNA extracted, and for field-scale relevance, normalized by the mass, volume, or area of leaf or sediment, water, or epilithic biofilm, respectively.

Chlorophyll-a (chl-a) concentration, a proxy for primary producer abundance, was collected from the water column and epilithon on rocks. Following composite grab sampling of the water and biofilm slurry, filtrate from a GF/F filter (Whatman, 1825-025) was collected and stored at -20°C until analysis. Chl-a pigments were extracted from filters in 90% ethanol at 80ºC for 5 minutes, steeped overnight at 4ºC in darkness, then concentrations were analyzed on a high-performance liquid chromatograph using fluorescence detection at the University of Southern Mississippi (Meyns et al., 1994; Halvorson et al., 2019).

2.3.4 Stream hydrological and water chemistry variables

Numerous attributes of stream physiochemistry were collected concurrently to facilitate predictive understanding between stream hydrology, water chemistry, and microbiology. In addition to flow duration preceding the synoptic sampling, and TWI, both described above, the watershed area and distance from the outlet were used to quantify the position of each sampling

location within the drainage network (higher distance from outlet, in m, means a more upstream position). Canopy cover was measured using a densiometer, as the proportion of closed squares on the densiometer grid averaged over four cardinal perspectives at the center of the stream channel at each sampling location. Stream water pH and conductivity were collected using a YSI ProDSS meter (YSI, Yellow Springs, OH). Stream water chemistry grab sampling bottles were triple rinsed with site water, filtered through Whatman GF/F filters, and analyzed for dissolved organic carbon (DOC), nitrogen as nitrate (NO_3^--N), and soluble reactive phosphorus (SRP) using standard procedures. The molar ratio of NO_3^--N:SRP was also tested as a possible covariate with microbial abundances.

2.3.5 Statistical analysis

All statistical analysis was run in RStudio (R Software Core team version 4.1.3). To fairly compare biofilm abundance with the other compartments we performed Tukey HSD post-hoc analysis on DNA per unit copy number instead of DNA per unit field habitat. This is largely due to the inequivalence of the unit cm^2 across other habitat substrates. Correlations were used to test if there were statistically significant differences between various explanatory variables and bacterial and fungal copy numbers. For temporal analysis, a two-way ANOVA was used in which copy number per habitat was compared to hydroperiod and site. This not only allowed for within site analysis, but across site analysis as well. All gene copy number data were log-transformed to approach assumptions of normality of the ANOVA and correlation analyses, as were some continuous explanatory variables, such as watershed area and nitrate concentration.

2.4 Results

2.4.1 Spatial sampling: Synoptic survey

Using units comparable among different substrates (gene copy numbers ng^{-1} of total genomic DNA yielded from each sample; Table 2.1, Figure 2.1), the water column carried the least bacteria of the four microhabitats, but also had a high ratio of fungi:bacteria. Leaves supported the highest bacterial abundance and the lowest fungal:bacterial ratio. In sediment, fungal abundance was highest and fungal:bacterial ratio was also high, while abundance and relative abundance of bacteria and fungi was intermediate in biofilms relative to the other microhabitats (Table 2.1, Figure 2.1).

When comparing dry to wet conditions, the DNA yield was almost ten times higher from wet than dry biofilms (18.8 ± 3.2 > 2.1 ± 0.6 ug DNA cm^{-2} biofilm (mean ± SE)), and almost three times higher in dry than wet sediments (21.0 ± 7.9 > 7.9 ± 1.1 ug DNA g^{-1} sediment) (Table 2.1). Bacterial and fungal abundance cm^{-2} biofilm was also an order of magnitude higher in wet than in dry conditions; in benthic sediments, bacterial abundance ug^{-1} DNA was three times higher, and g^{-1} sediment was ten times higher in dry than wet conditions (Table 2.1, Figure 2.2). Neither leaf DNA yield nor microbial abundance varied between wet and dry conditions, nor did biofilm chlorophyll-a (Table 2.1).

Flow duration preceding sample collection at each location was not correlated with any microbial abundance metric (Table 2.2). Other correlates that were tested but showed weak or no relationships with microbial abundance included canopy cover percentage, pH, conductivity, and dissolved organic carbon, NO_3^--N, and SRP concentrations (Table 2.2). Instead, distance from the study catchment outlet was the best correlate with surface water DNA yield, bacterial and fungal abundance, and chlorophyll-a concentration, all of which tended to be higher at a further distance upstream (Table 2.2, Figure 2.3). In contrast, epilithic biofilm bacterial abundance tended to be lower at greater distances upstream, and sediment DNA yield, bacterial and fungal abundances, and leaf fungal abundances tended to be higher at locations with lower TWI (Table 2.2, Figure 2.3). Also, DNA yield and bacterial and fungal abundances in benthic sediments tended to be higher in locations where stream water NO_3^--N:SRP ratios were higher (Table 2.2, Figure 3). These patterns covary in that NO_3^--N concentrations and NO_3^--N:SRP ratios also tended to decrease downstream, such that inorganic N is increasingly depleted relative to inorganic P lower in the drainage network (correlation between log(NO_3^--N:SRP), log(NO_3^--N), and log(SRP) and distance from outlet, respectively: R, P = 0.71, < 0.001; 0.56, <0.001; and -0.32, 0.038).

2.4.2 Cross-site and temporal sampling

Among-site and temporal differences in microbial populations were both apparent, but no post-hoc tests of the weak interactive effects shown by 2-way ANOVA were significant (Table 2.3). DNA yields were highest in Oka'Yanahli leaves and sediments relative to the same substrates at other sites, and lowest during the dry sampling period in biofilms and leaves; also,

biofilm and leaf DNA yields were lowest in dry-phase conditions at Kings Creek (Table 2.3, Figure S 2.2).

Bacterial populations in the water column and in leaf litter, and fungal populations in benthic sediments, were higher during dry-down than wet-up conditions. In comparison biofilm fungal populations were lower during dry-down than wet-up conditions (Table 2.3, Figure 2.4). Water column bacterial and fungal populations were higher at the Youngmeyer and Oka'Yanahli sites than at Kings Creek, and sediment bacterial and fungal populations were highest at Kings Creek (Table 2.3, Figure 2.4). The fungal:bacterial ratio was higher during wet-up than dry-down conditions in biofilms and leaves, and lower during wet-up than dry-down conditions in sediments, but no post-hoc differences in the interactive site by hydroperiod effect on fungal:bacterial ratio were significant (Table 2.3, Figure S 2.2).

2.5 Discussion

Microbial abundance in benthic sediment, leaves, and epilithic biofilms of non-perennial study streams differed by an order of magnitude or more between hydroperiods, by up to an order of magnitude between dry and wet locations within the intensively sampled network, and less than an order of magnitude among the three different study sites. Microbial abundance in the water column varied by more than an order of magnitude among sites, within the sampling network, and between hydroperiods. It was not surprising to learn that microbial abundance differed among the four microhabitat substrates. However, the differential responses of microbiota in biofilms and sediments to both seasonal and spatial drying were unexpected. Also, microbial abundance within the stream network was not predictable based on preceding flow conditions; instead, position within the network (as distance from the stream outlet), the topographic wetness index, and nitrate relative to SRP concentrations were the best predictors of bacterial and fungal abundance. Overall, results suggest that the direct effects of stream drying on microbiota may be strongest in biofilm habitats, while populations of bacteria and fungi in sediments are more likely driven by factors that are indirectly moderated by drying.

Hydrological effects

Microbial population size tended to change between the rising and falling limbs of the hydrograph (wet-up and dry-down hydrophases), and to differ between wet and dry locations

within a stream network, but was not correlated with the duration of wet conditions preceding sampling time (Tables 2.1-2.2, Figures 2.2-2.3). A similar qualitative response to wetting and drying conditions emerged from both spatial and temporal perspectives, in that epilithic microbial populations tended to be higher in wet locations and during the rising limb of the seasonal hydrograph (Figure 2.4). In contrast, microbial populations in benthic sediments and on leaf surfaces tended to be higher in dry locations and during the falling limb of the hydrograph. As spatial fragmentation of a non-perennial stream occurs over time, heterotrophic bacteria and fungi may either persist longer, or actively seek refuge, in microhabitats that have higher water-holding capacity and organic matter content, such as leaves and sediments (Romaní et al., 2013). Non-exclusively, saturation of sediments and leaves under wet conditions may restrict oxygen diffusion and thus reduce the amount of energy available for cellular respiration and growth (Gómez et al., 2017).

In contrast, bacterial and fungal populations in the biofilm compartment increased during wet conditions. As spatial connectivity through a stream network increases along the rising limb of the hydrograph, conditions for biofilm growth are likely promoted through the delivery of nutrients in flowing water, following the rationale for our original predictions. Also, while high flow can reduce biomass from some biofilms via physical shear stress, it is possible that epilithon in our non-perennial study streams was resistant to this force due to the production of extracellular polymeric substances (EPS). EPS, composed of polysaccharides, alginate, nucleic acids, and lipids (Flemming et al., 2010), can be secreted by bacteria and fungi within the biofilm to increase resistance against both hydrodynamic shear stress and changes in osmotic pressure associated with drying (Wilking et al., 2011; Karimi et al., 2015). EPS allows specific populations to resist the death phase and continue reproducing within the biofilm and also creates potential for organic matter produced from the lysis of microbes from water pressure upstream to be retained within biofilms in the lower portions of the stream. In addition to reduced influx of growth-limiting nutrients during periods of decreasing flow, it is possible that demand for epilithic biomass as food for organisms at higher trophic levels increases, reducing net biofilm growth and decreasing standing population sizes (Sabater et al., 2016).

While fungal:bacterial abundance ratio was not different among wet and dry samples collected at the same time within the Kings Creek network (Table 2.1), this ratio decreased

during the dry-down hydrophase period on leaves and in biofilms, but increased during the same period in sediments (Figure 2.4). As a stream dries, fungi that are specialized to thrive on saturated leaf surfaces or within biofilm communities may undergo population reductions due to reallocation of biomass or hyphal networks due to nutrient-depleted substrates to colonize new habitats (Crowther et al., 2014; Graça et al., 2023). Also, this pattern could result if detected populations in biofilms and leaves included a significant proportion of fungal spores that traveled down the stream continuum. In contrast, the growth of fungi that thrive in unsaturated conditions (i.e., the majority of fungal taxa) may be promoted as sediments dry and begin to more closely resemble a soil habitat (Arias-Real, 2023). It is also worth noting that due to the chitin cell wall morphology of fungi are much more suited to deal with drying stresses, followed by gram-negative bacteria with their peptidoglycan cell wall structure, and gram-negative bacteria being the most vulnerable to such stresses (Csonka, 1989). Also, due to the importance of fungi in producing exoenzymes that can hydrolyze and oxidize complex organic compounds (Sinsabaugh et al., 1992), we were surprised that the fungal:bacterial ratio on leaves was relatively low. Other studies have found that bacterial colonization of leaves is greater in aquatic and intermittent than terrestrial environments (Folquier et al., 2015).

Surprisingly, the duration of flow preceding the sampling time did not predict microbial abundance; rather, the topographical wetness index (TWI) was negatively correlated with sediment bacterial and fungal abundance and leaf fungal abundance, and positively correlated with biofilm fungal abundance. The TWI was used for site selection because it could be calculated *a priori*, with no real-time information on stream flow state yet in hand, but we did not expect it to be related to microbial dynamics because it does not convey proximate microhabitat information. Rather, it is an index of how likely a location is to be wet at any point in time, based on the watershed area above and the hillslope at that point in space. Mechanistically, TWI best helps explain lower microbial populations in sediments, because zones of anoxia are more likely to develop and persist within the sediment matrix at lower portions of the stream and more likely to collect water (higher TWI). So, if our rationale for oxygen availability promoting microbial growth in dry sediments is accurate, then the negative TWI correlation makes sense. However, it is also possible that relationships between microbial populations and TWI reflect generalized network continuum influences, because TWI is derived from and positively correlated with watershed area.

Network continuum effects

While microbial populations across the intensively sampled non-perennial stream network, Kings Creek, were influenced by hydrological factors (as wet or dry conditions), there was also a clear correlation between population size and position within the stream network, as measured by distance from the outlet of the study catchment. Many studies have found that the longitudinal river continuum is intimately related to multiple environmental variables including stream temperature, oxygen, and riparian vegetation (Doretto et al. 2020). The classic River Continuum Concept (RCC) states that as light availability to the channel increases with stream width further downstream, biomass of autotrophs and their consumers should also increase (Vannote et al. 1980). The RCC framework suggests that down the study stream network, there could be less shade and more photosynthesizing algae in biofilms that produce labile carbon substrates for bacterial consumption both within the biofilm and the whole stream ecosystem, which is consistent with the observed higher epilithon bacterial abundance closer to the catchment outlet (Figure 2.3). However, there was no relationship between biofilm microbial abundance and canopy cover, a more direct index of light availability (Table 2.2), which directly contradicts RCC predictions. The incompatibility of the RCC with stream dynamics at this study site is already well established (Dodds et al., 1998): Headwaters here tend to be more open while downstream reaches are increasingly shaded, exemplifying a baseline expectation for streams in grasslands and semi-arid to arid ecosystems, i.e., many non-perennial catchments.

Instead of canopy cover, dissolved NO_3^--N:SRP ratio was correlated with the biofilm bacterial abundance increases downstream (Table 2.1), a pattern driven by decreases in NO_3^--N downstream. The decrease in nutrient content with an increase in 16S rRNA gene copies in biofilm descending the stream could suggest lower nutrient content due to microbial metabolic activity and growth, or immobilization of inorganic N, within epilithic biofilms, as has been observed in many nutrient enrichment experiments globally (Ardón et al., 2021). Non-exclusively, as mentioned above, the higher TWI downstream could be associated with a higher probability of anaerobic conditions. Anoxia also promotes NO_3^--N removal through denitrification, as NO_3^--N is the most favorable electron acceptor for bacterial respiration after oxygen is depleted (Burgin & Hamilton, 2007). However, because of the covariance among these variables, we cannot discern which mechanisms are most important.

Notably, the abundance of water column bacteria, fungi, and primary producers (measured using chl-a concentration) were all higher at distances further from the outlet of the study catchment, i.e., higher upstream (Table 2.1). Many heterotrophic bacteria and fungi disperse from the soil into the stream, which could cause a higher bacterial propagule or fungal spore load higher in the network (Chauvet et al., 2016). It is plausible that higher dispersal would be detected by our methods, because DNA abundance could increase because of either growth or a higher inactive cell load from upstream (Wisnoski et al. 2020). However, chl-a increases only with activity or growth of photosynthesizing bacteria or protists. While, like biofilm microbial abundance, water column chl-a was not correlated with canopy cover, the pattern of higher chl-a upstream is directly inconsistent with the traditional RCC and underscores the need for a different framework to understand microbial population, community, and ecosystem nutrient dynamics in grassland and non-perennial streams.

Implications for water quality at stream reach scales

The dynamic but contrasting responses within different microhabitats may be largely attributed to the differences in surface area and texture for microbial growth, and organic matter inputs that each microhabitat receives (Zeglin, 2015; Romaní et al., 2017). At the coarsest level, epilithic biofilms growing on inorganic surfaces are dominated by autotrophic algae, diatoms, and cyanobacteria, and the heterotrophic bacteria and fungi that use carbon fixed by these organisms, whereas organic-matter laden sediments and leaves support predominantly heterotrophic microorganisms (Findlay, 2010; Battin et al., 2016). It may be that growth of bacteria and fungi in consortia that depend primarily on energy from algal-fixed carbon is more directly dependent on water flow, or flow-transported nutrients, than the growth of microorganisms in consortia that depend primarily on energy from the organic matter held in sediments and leaves. In turn, biofilm bacterial and fungal populations are more likely nutrient sinks during wet conditions. In contrast, larger sediment and leaf microbial populations can support more biogeochemical cycling, and provide potential refugia for stream microbiota, during dry conditions.

However, because the stream channel benthos is a mosaic of these microhabitats, the contrasting dry-down/re-wet population dynamics on autotrophic and heterotrophic substrates makes it difficult to predict the net impact on water quality that may emerge at the whole-stream

scale. In this study, the benthic surface coverage of our stream sampling sites ranged between 0-65% leaves (median 4%), 1-95% exposed sediments (median 19%), and 5-96% rock surfaces (median 75%). Also, at the whole-stream level, food web dynamics come into play in regulating nutrient retention and ecosystem metabolism. During dry periods, the concentration of metazoa into isolated pools increases the grazing pressure on all benthic biofilms, and leads to major shifts in consumer-driven nutrient recycling feedbacks to water quality (Hopper et al., 2020). Also, numerous studies show high mortality of stream animals and vegetation, in addition to microbes, during drying conditions, which in sum would tend to lower immobilization and release large amounts of organic matter (Schiller et al., 2017; Amalfitano et al., 2008; Zoppini and Marxsen, 2011) that could be mobilized quickly upon re-wet of a dry channel. Therefore, the entire consortia of micro- and macro-organisms within streams is important to overall ecosystem function.

Overall, this research establishes baselines on how microbial populations change in response to drying and rewetting over space and time, which can be used to inform water quality changes in non-perennial streams across the country and world. The relative paucity of information about non-perennial streams contributes to the lack of regulation of waterways that are disconnected from downstream channels in the USA. Our work illustrates that spatiotemporal dynamics of microorganisms in non-perennial streams can be higher than variation among streams, thus suggesting that when connected to downstream channels, non-perennial catchments can be major contributors to water quality, in both positive or negative ways. Future work should also consider how bacterial, fungal, and protistan abundance, diversity, and activity can teach us more about the integrated functions of non-perennial stream ecosystems.

2.6 References Cited

Allison, S. D., & Martiny, J. B. (2008). Resistance, resilience, and redundancy in microbial communities. *Proceedings of the National Academy of Sciences*, *105*(supplement_1), 11512-11519.

Amalfitano, S., Fazi, S., Zoppini, A., Barra Caracciolo, A., Grenni, P., & Puddu, A. (2008). Responses of benthic bacteria to experimental drying in sediments from Mediterranean temporary rivers. *Microbial Ecology*, *55*, 270-279.

Ardón, M., Zeglin, L. H., Utz, R. M., Cooper, S. D., Dodds, W. K., Bixby, R. J., Burdett, A.S., Shah, J.F., Griffiths, N.A., Harms, T.K., Johnson, S.L., Jones, J.B., Kominoski, J.S., Mcdowell, W.H., Rosemond, A.D., Trentman, M.T., Van Horn, D., & Ward, A. (2021). Experimental nitrogen and phosphorus enrichment stimulates multiple trophic levels of algal and detrital-based food webs: a global meta-analysis from streams and rivers. *Biological Reviews*, *96*(2), 692-715.

Arias-Real, R., Menéndez, M., Muñoz, I., & Pascoal, C. (2023). Drying shapes the ecological niche of aquatic fungi with implications on ecosystem functioning. *Science of the Total Environment*, *859*, 160374.

Arias-Real, R., Muñoz, I., Gutierrez-Cánovas, C., Granados, V., Lopez-Laseras, P., & Menéndez, M. (2020). Subsurface zones in intermittent streams are hotspots of microbial decomposition during the non-flow period. *Science of the Total Environment*, *703*, 135485.

Aurell, J., Gullett, B., Grier, G., Holder, A., & George, I. (2023). Seasonal emission factors from rangeland prescribed burns in the Kansas Flint Hills grasslands. *Atmospheric Environment*, *304*, 119769.

Austin, B. J., & Strauss, E. A. (2011). Nitrification and denitrification response to varying periods of desiccation and inundation in a western Kansas stream. *Hydrobiologia*, *658*, 183-195.

Battin, T. J., Besemer, K., Bengtsson, M. M., Romani, A. M., & Packmann, A. I. (2016). The ecology and biogeochemistry of stream biofilms. *Nature Reviews Microbiology*, *14*(4), 251-263.

Bernot, M. J., Sobota, D. J., Hall Jr, R. O., Mulholland, P. J., Dodds, W. K., Webster, J. R., Tank, J.L., Ashkenas, L.R., Cooper, L.W., Dahm, C.N., Gregory, S.V., Grimm, N.B., Hamilton, S.K., Johnson, S.L., Mcdowell, W.H., Meyer, J.L., Peterson, B., Poole, G.C., Valett, H.M., Arango, C., Beaulieu, J.J., Burgin, A.J., Crenshaw, C., Helton, A.M., Johsnon, L., Merriam, J., Niederlehner, B.R., O'Brien, J.M., Potter, J.D., Sheibley, R.W., Thomas, S.M., & Wilson, K. Y. M. (2010). Inter-regional comparison of land-use effects on stream metabolism. *Freshwater Biology*, *55*(9), 1874-1890.

Besemer, K. (2015). Biodiversity, community structure and function of biofilms in stream ecosystems. Research in microbiology, 166(10), 774-781.

Briggs, J. M., Blair, J. M., & Horne, E. A. (2016). Ecohydrological and climate change studies at the Konza Prairie Biological Station. Transactions of the Kansas Academy of Science (1903-), 119(1), 5–11. http://www.jstor.org/stable/24887836

Bruder, A., Chauvet, E., & Gessner, M. O. (2011). Litter diversity, fungal decomposers and litter decomposition under simulated stream intermittency. Functional Ecology, 25(6), 1269-1277.

Burgin, A. J., & Hamilton, S. K. (2007). Have we overemphasized the role of denitrification in aquatic ecosystems? A review of nitrate removal pathways. *Frontiers in Ecology and the Environment*, *5*(2), 89-96.

Buthod, A. K., & Hoagland, B. W. (2020). A floristic inventory of The Nature Conservancy's Oka'Yanahli Preserve, Johnston County, Oklahoma. *Oklahoma Native Plant Record*, *20*(1).

Chapin, T. P., Todd, A. S., & Zeigler, M. P. (2014). Robust, low-cost data loggers for stream temperature, flow intermittency, and relative conductivity monitoring. *Water Resources Research*, *50*(8), 6542-6548.

Chauvet, E., Cornut, J., Sridhar, K. R., Selosse, M. A., & Bärlocher, F. (2016). Beyond the water column: aquatic hyphomycetes outside their preferred habitat. *Fungal Ecology*, *19*, 112-127.

Costello, D. M., Tiegs, S. D., Boyero, L., Canhoto, C., Capps, K. A., Danger, M., ... & Zwart, J. A. (2022). Global patterns and controls of nutrient immobilization on decomposing cellulose in riverine ecosystems. *Global Biogeochemical Cycles*, *36*(3), e2021GB007163.

Crowther, T. W., Maynard, D. S., Crowther, T. R., Peccia, J., Smith, J. R., & Bradford, M. A. (2014). Untangling the fungal niche: the trait-based approach. *Frontiers in microbiology*, *5*, 579.

Crump, B. C., Amaral-Zettler, L. A., & Kling, G. W. (2012). Microbial diversity in arctic freshwaters is structured by inoculation of microbes from soils. *The ISME journal*, *6*(9), 1629-1639.

Csonka, L. N. (1989). Physiological and genetic responses of bacteria to osmotic stress. *Microbiological reviews*, *53*(1), 121-147.

Datry, T., Larned, S. T., & Tockner, K. (2014). Intermittent rivers: a challenge for freshwater ecology. *BioScience*, *64*(3), 229-235.

Dodds, W. K., Gido, K., Whiles, M. R., Fritz, K. M., & Matthews, W. J. (2004). Life on the edge: the ecology of Great Plains prairie streams. *BioScience*, *54*(3), 205-216.

Dodds, W. K., Jones, J. R., & Welch, E. B. (1998). Suggested classification of stream trophic state: distributions of temperate stream types by chlorophyll, total nitrogen, and phosphorus. *Water research*, *32*(5), 1455-1462.

Doretto, A., Piano, E., & Larson, C. E. (2020). The River Continuum Concept: lessons from the past and perspectives for the future. Canadian Journal of Fisheries and Aquatic Sciences, 77(11), 1853-1864.

Dunham-Cheatham, S. M., You, Y. (2019). General Geochemistry and Microbiology Techniques. In Analytical Geomicrobiology: A Handbook of Instrumental Techniques, JPL Kenney, H Veeramani & DS Alessi, (Eds.), Cambridge University Press.

Febria, C. M., Beddoes, P., Fulthorpe, R. R., & Williams, D. D. (2012). Bacterial community dynamics in the hyporheic zone of an intermittent stream. *The ISME journal*, *6*(5), 1078-1088.

Febria, C. M., Hosen, J. D., Crump, B. C., Palmer, M. A., & Williams, D. D. (2015). Microbial responses to changes in flow status in temporary headwater streams: a cross-system comparison. *Frontiers in microbiology*, *6*, 522.

Fierer, N., Jackson, J. A., Vilgalys, R., and Jackson, R. B. (2005). Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. Appl. Environ. Microbiol. 71, 4117–4120. doi: 10.1128/aem.71.7.4117-4120.2005

Findlay, S. (2010). Stream microbial ecology. Journal of the North American Benthological Society, 29(1), 170-181.

Flemming, H. C., & Wingender, J. (2010). The biofilm matrix. *Nature reviews microbiology*, *8*(9), 623-633.

Gionchetta, G., Oliva, F., Menéndez, M., Lopez-Laseras, P., & Romaní, A. M. (2019). Key role of streambed moisture and flash storms for microbial resistance and resilience to long-term drought. *Freshwater Biology*, *64*(2), 306-322.G.J.

Gionchetta, G., Oliva, F., Romaní, A. M., & Bañeras, L. (2020). Hydrological variations shape diversity and functional responses of streambed microbes. *Science of the Total Environment*, *714*, 136838.

Gómez, R., Arce, M. I., Baldwin, D. S., & Dahm, C. N. (2017). Water physicochemistry in intermittent rivers and ephemeral streams. In Intermittent rivers and ephemeral streams (pp. 109-134). Academic Press.

Graça, D., Fernandes, I., Cássio, F., & Pascoal, C. (2023). Eco-physiological responses of aquatic fungi to three global change stressors highlight the importance of intraspecific trait variability. *Microbial ecology*, *85*(4), 1215-1225.

Halvorson, H. M., Barry, J. R., Lodato, M. B., Findlay, R. H., Francoeur, S. N., & Kuehn, K. A. (2019). Periphytic algae decouple fungal activity from leaf litter decomposition via negative priming. Functional Ecology 33: 188-201.

Hayden, B. P. (1998). Regional climate and the distribution of tallgrass prairie. ln: Knapp, AK, Briggs, JM, Hartnett, DC, Collins, SL (Eds.), Grassland Dynamics: Long-Term Ecological Research in Tallgrass Prairie.

Hopper, G. W., Gido, K. B., Pennock, C. A., Hedden, S. C., Guinnip, J. P., Fisher, M. A., Tobler, C.M., Heden, C.K., & Bruckerhoff, L. A. (2020). Biomass loss and change in species dominance shift stream community excretion stoichiometry during severe drought. *Freshwater Biology*, *65*(3), 403-416.

Houseman, G. R., Kraushar, M. S., & Rogers, C. M. (2016). The Wichita State University biological field station: bringing breadth to research along the precipitation gradient in Kansas. *Transactions of the Kansas Academy of Science*, *119*(1), 27-32.

Jaeger, K. L., Olden, J. D., & Pelland, N. A. (2014). Climate change poised to threaten hydrologic connectivity and endemic fishes in dryland streams. Proceedings of the National Academy of Sciences, 111(38), 13894-13899. doi: 10.1073/pnas.1320890111

Karimi, A., Karig, D., Kumar, A., & Ardekani, A. M. (2015). Interplay of physical mechanisms and biofilm processes: review of microfluidic methods. *Lab on a Chip*, *15*(1), 23-42.

Kasparian, M. A., E. C. Hellgren, S. M. Ginger, L. P. Levesque, J. E. Clark, D. L. Winkelman, and D. M. Engle. (2004). *Population characteristics of Virginia opossum in the Cross Timbers during raccoon reduction*. The American Midland Naturalist 151:154-163.

Koch, H., Lücker, S., Albertsen, M., Kitzinger, K., Herbold, C., Spieck, E., Nielsen, P.H., Wagner, M., & Daims, H. (2015). Expanded metabolic versatility of ubiquitous nitrite-oxidizing bacteria from the genus Nitrospira. *Proceedings of the national academy of sciences*, *112*(36), 11371-11376.

Langhans, S. D., & Tockner, K. (2006). The role of timing, duration, and frequency of inundation in controlling leaf litter decomposition in a river-floodplain ecosystem (Tagliamento, northeastern Italy). Oecologia, 147, 501-509.

Lohse, K. A., Gallo, E. L., & Meixner, T. (2020). Influence of climate and duration of stream water presence on rates of litter decomposition and nutrient dynamics in temporary

streams and surrounding environments of southwestern USA. *Frontiers in Water*, *2*, 571044.

McIntyre, R. E., Adams, M. A., Ford, D. J., & Grierson, P. F. (2009). Rewetting and litter addition influence mineralisation and microbial communities in soils from a semi-arid intermittent stream. Soil Biology and Biochemistry, 41(1), 92-101.

Meyns, S., Illi, R., & Ribi, B. (1994). Comparison of chlorophyll-a analysis by HPLC and spectrophotometry: Where do the differences come from? Archiv für Hydrobiologie: 132(2), 129-139.

Mora-Gómez, J., Duarte, S., Cássio, F., Pascoal, C., & Romaní, A. M. (2018). Microbial decomposition is highly sensitive to leaf litter emersion in a permanent temperate stream. Science of the Total Environment, 621, 486-496.

Mulholland, P. J., Hall Jr, R. O., Sobota, D. J., Dodds, W. K., Findlay, S. E., Grimm, N. B., Hamilton, S.K., McDowell, W.H., O'Brien, J.M., Tank, J.L., Ashkenas, L.R., Cooper, L.W., Dahm, C.N., Gregory, S.V., Johnson, S.L., Meyer, J.L., Peterson, B.J., Poole, G.C., Valett, H.M., Webster, J.R., Arango, J.P., Beaulieu, J.J., Bernot, M.J., Burgin, A.J., Crenshaw, C.L., Helton, A.M., Johnson, L.T., Niederlehner, B.R., Potter, J.D., Sheibley, R.W., & Thomasn, S. M. (2009). Nitrate removal in stream ecosystems measured by 15N addition experiments: Denitrification. *Limnology and Oceanography*, *54*(3), 666-680.

Pohlon, E., Ochoa Fandino, A., & Marxsen, J. (2013). Bacterial community composition and extracellular enzyme activity in temperate streambed sediment during drying and rewetting. PloS one, 8(12), e83365.https://doi.org/10.1371/journal.pone.0083365

Premathilake, E. D. (2018). Integrating camera trap data and occupancy modeling to estimate seasonal variations in occurrence, detection, and activity patterns of mesocarnivores in Southcentral Oklahoma. University of Central Oklahoma.

Rees, G. N., Watson, G. O., Baldwin, D. S., & Mitchell, A. M. (2006). Variability in sediment microbial communities in a semipermanent stream: impact of drought. Journal of the North American Benthological Society, 25(2), 370-378. https://doi.org/10.1899/0887"

Riihimäki, H., Kemppinen, J., Kopecký, M., & Luoto, M. (2021). Topographic wetness index as a proxy for soil moisture: The importance of flow-routing algorithm and grid resolution. *Water Resources Research*, *57*(10), e2021WR029871.

Romaní, A. M., Amalfitano, S., Artigas, J., Fazi, S., Sabater, S., Timoner, X., & Zoppini, A. (2013). Microbial biofilm structure and organic matter use in mediterranean streams. *Hydrobiologia*, *719*, 43-58.

Romaní, A. M., Chauvet, E., Febria, C., Mora-Gómez, J., Risse-Buhl, U., Timoner, X., Weitere, M., & Zeglin, L. (2017). The biota of intermittent rivers and ephemeral streams: prokaryotes, fungi, and protozoans. In Intermittent rivers and ephemeral streams (pp. 161-188). Academic Press, https://doi.org/10.1016/B978-0-12-803835-2.00009-7

RStudio Team (2022). RStudio: Integrated Development for R. RStuido, Inc., Boston, MA, URL http: //www.rstudio.com/.

Rueden, C. T., Schindelin, J., Hiner, M. C., DeZonia, B. E., Walter, A. E., Arena, E. T., & Eliceiri, K. W. (2017). ImageJ2: ImageJ for the next generation of scientific image data. *BMC bioinformatics*, *18*, 1-26.

Ruiz-González, C., Niño-García, J. P., & Del Giorgio, P. A. (2015). Terrestrial origin of bacterial communities in complex boreal freshwater networks. Ecology letters, 18(11), 1198-1206. https://doi.org/10.1111/ele.12499

Sabater, S., Timoner, X., Borrego, C., & Acuña, V. (2016). Stream biofilm responses to flow intermittency: from cells to ecosystems. *Frontiers in Environmental Science*, *4*, 14.

Samson, F., & Knopf, F. (1994). Prairie conservation in north america. *BioScience*, *44*(6), 418-421.

Sinsabaugh, R. L., Weiland, T., & Linkins, A. E. (1992). Enzymic and molecular analysis of microbial communities associated with lotic particulate organic matter. Freshwater Biology, 28(3), 393-404.

Six, J., Frey, S. D., Thiet, R. K., & Batten, K. M. (2006). Bacterial and fungal contributions to carbon sequestration in agroecosystems. Soil Science Society of America Journal, 70(2), 555-569. https://doi.org/10.2136/sssaj2004.0347

Timoner, X., Acuña, V., von Schiller, D., and Sabater, S. (2012). Functional responses of stream biofilms to flow cessation, desiccation and rewetting. Freshw. Biol. 57, 1565–1578. doi: 10.1111/j.1365-2427.2012.02818.x

Tomasek, A., Staley, C., Wang, P., Kaiser, T., Lurndahl, N., Kozarek, J. L., & Sadowsky, M. J. (2017). Increased denitrification rates associated with shifts in prokaryotic community composition caused by varying hydrologic connectivity. Frontiers in microbiology, 8, 2304. https://doi.org/10.3389/fmicb.2017.02304

Vannote, R. L., Minshall, G. W., Cummins, K. W., Sedell, J. R., & Cushing, C. E. (1980). The river continuum concept. *Canadian journal of fisheries and aquatic sciences*, *37*(1), 130-137.

Veach A.M., Dodds W.K., Skibbe, A. (2015) Correction: Fire and Grazing Influences on Rates of Riparian Woody Plant Expansion along Grassland Streams. PLOS ONE 10(5): e0129409. https://doi.org/10.1371/journal.pone.0129409

Veach, A. M., Stegen, J. C., Brown, S. P., Dodds, W. K., & Jumpponen, A. (2016). Spatial and successional dynamics of microbial biofilm communities in a grassland stream ecosystem. Molecular ecology, 25(18), 4674-4688.

Vero, S., Macpherson, G., Sullivan, P., Brookfield, A., Nippert, J., Kirk, M., Datta, S., & Kempton, P. (2018). Developing a Conceptual Framework of Landscape and Hydrology on Tallgrass Prairie: A Critical Zone Approach. Vadose Zone Journal : VZJ., 17(1), 1–11. https://doi.org/10.2136/vzj2017.03.0069

von Schiller, D., Bernal, S., Dahm, C. N., & Martí, E. (2017). Nutrient and organic matter dynamics in intermittent rivers and ephemeral streams. In *Intermittent rivers and ephemeral streams* (pp. 135-160). Academic Press.

Warix, S. R., Godsey, S. E., Lohse, K. A., & Hale, R. L. (2021). Influence of groundwater and topography on stream drying in semi-arid headwater streams. *Hydrological Processes*, *35*(5), e14185.

Webster, J. R., & Benfield, E. F. (1986). Vascular plant breakdown in freshwater ecosystems. *Annual review of ecology and systematics*, *17*(1), 567-594.

Wilking, J., Angelini, T., Seminara, A., Brenner, M., & Weitz, D. (2011). Biofilms as complex fluids. *MRS Bulletin, 36*(5), 385-391. doi:10.1557/mrs.2011.71

Wisnoski, N. I., Muscarella, M. E., Larsen, M. L., Peralta, A. L., & Lennon, J. T. (2020). Metabolic insight into bacterial community assembly across ecosystem boundaries.

Woods, A. J., Omernik, J. M., Martin, W. H., Pond, G. J., Andrews, W. M., Call, S. M., ... & Taylor, D. D. (2005). Ecoregions of Kentucky (2 Sided Color Poster with Map, Descriptive Text, Summary Tables, and Photographs, Map Scale 1: 1,000,000). *US Geological Survey, Reston, Virginia.(Available from: ftp://ftp. epa. gov/wed/ecoregions/ky/ky_eco_lg. pdf)*.

Ylla, I., Sanpera-Calbet, I., Vázquez, E., Romaní, A. M., Munoz, I., Butturini, A., & Sabater, S. (2010). Organic matter availability during pre-and post-drought periods in a Mediterranean stream. *Global Change and River Ecosystems—Implications for Structure, Function and Ecosystem Services*, 217-232.

Zeglin, L. H. (2015). Stream microbial diversity in response to environmental changes: review and synthesis of existing research. *Frontiers in microbiology*, *6*, 454.

Zeglin, L. H., Dahm, C. N., Barrett, J. E., Gooseff, M. N., Fitpatrick, S. K., & Takacs-Vesbach, C. D. (2011). Bacterial community structure along moisture gradients in the parafluvial sediments of two ephemeral desert streams. *Microbial ecology*, *61*, 543-556.

Zeglin, L. H., Thomas, S., Richards, B., Burgin, A., Zipper, S., Brooks-Kieffer, J., Babbit, A., & Seybold, E. (2022, December 13). *AIMS Microbial Field Sampling SOP*. OSF. https://osf.io/g3msz

Zimmer, M. A., Burgin, A. J., Kaiser, K., & Hosen, J. (2022). The unknown biogeochemical impacts of drying rivers and streams. *nature communications*, *13*(1), 7213.

Zoppini, A., & Marxsen, J. (2011). Importance of extracellular enzymes for biogeochemical processes in temporary river sediments during fluctuating dry–wet conditions. *Soil enzymology*, 103-117.

2.7 Tables and Figures

Figure 2.1. Box and whisker plots that illustrate a comparison of microbial populations among substrate microhabitats oriented by color, comparing gene copy numbers ng-1 of total genomic DNA yielded from each sample with A. Bacterial 16S rRNA gene copy number, B. Fungal ITS copy number and C. ITS/16S Ratios. Tukey's post hoc tests were conducted to denote differences among substrate type ($P < 0.05$)

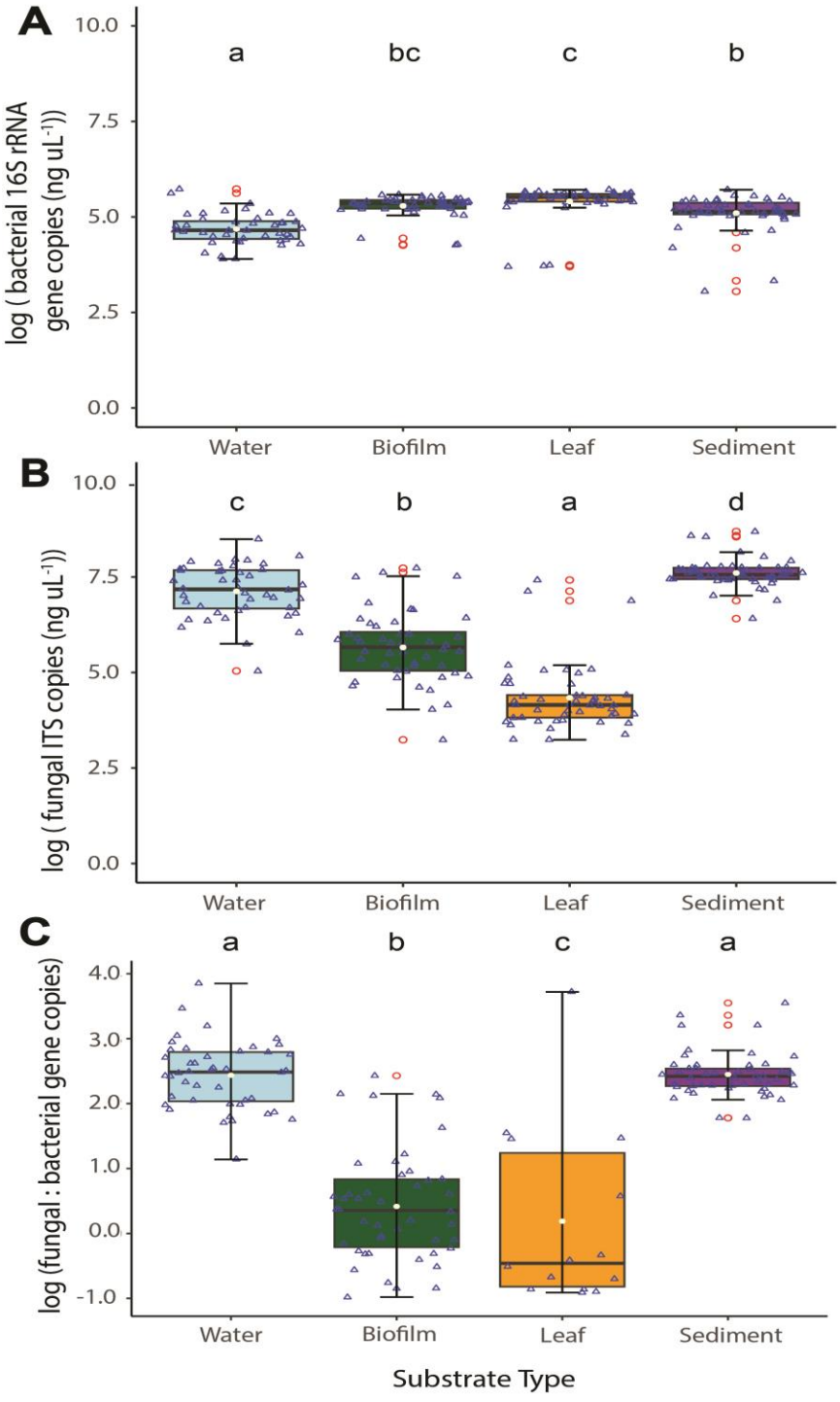


Figure 2.2. Box and whisker plots representing microbial populations in flowing (wet) versus dry locations within the stream network, for A. Biofilms, B. Leaves, C. Sediments. Saturated colors are wet substrates, and the unsaturated colors are dry substrates. Differences between wet and dry samples were detected by Tukey's post-hoc test ($P < 0.05$), and are denoted by asterisks.

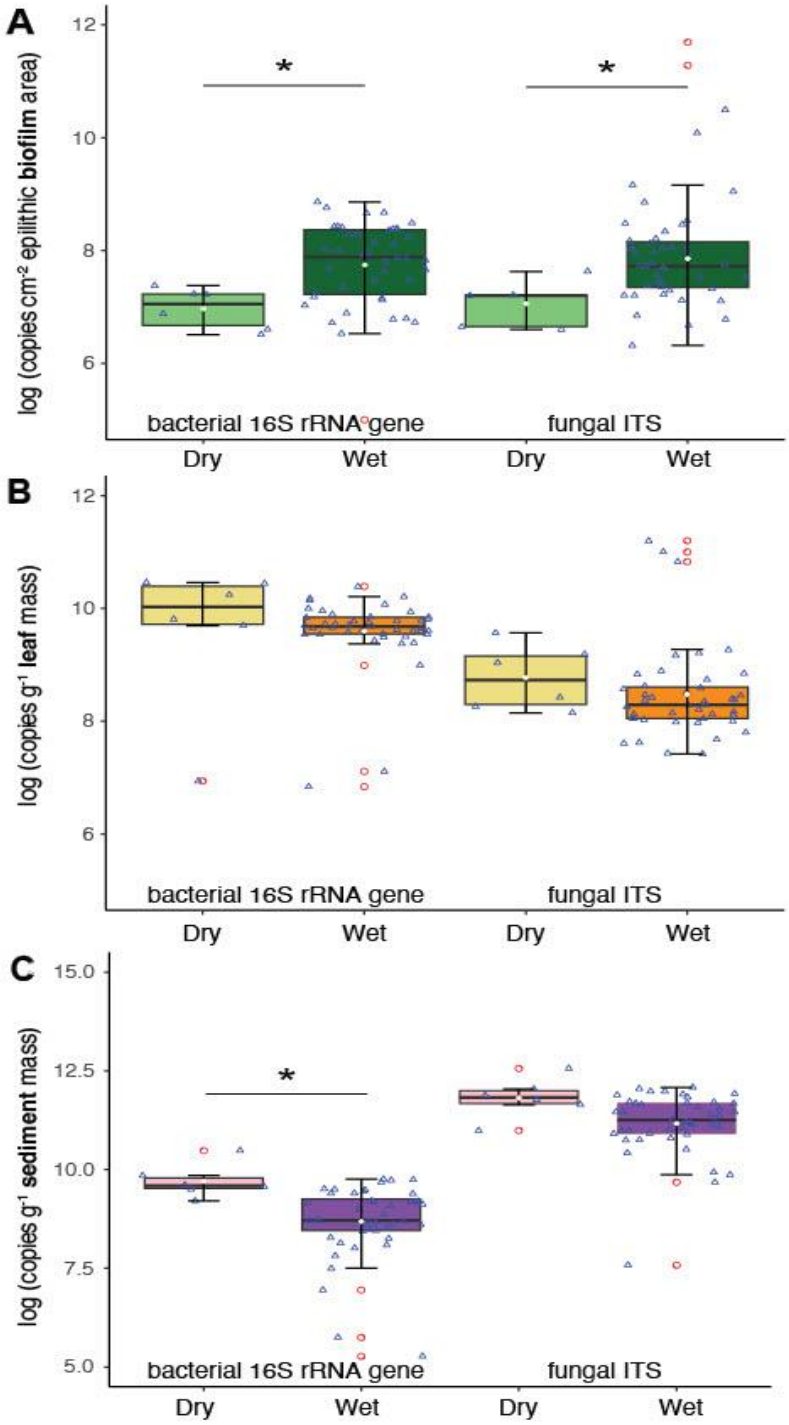


Table 2.1: Statistical results (F, P) for one-way analysis of variance on difference by substrate type, and wet/dry status (for biofilm, leaf, and sediment substrates, with values expressed g-1, g-1, and cm-2, respectively) on DNA yield, bacterial 16S rRNA gene copy number, fungal ITS copy number, and 16S:ITS ratio across all synoptic sampling sites. Superscript "l" notes variables that were log-transformed to meet assumptions of normality. Probability of null hypothesis (no effect) of < 0.05 is noted by bold font.

F, P	**Substrate Type**	**Biofilm** (wet/dry)	**Leaf** (wet/dry)	**Sediment** (wet/dry)
DNA yield (ug unit^{-1} substrate)	.	**5.59, 0.022**	0.86, 0.36	**5.84, 0.020**
16S rRNA gene copy^{l} (ng^{-1} DNA)	**47.5, < 0.001**	0.47, 0.50	0.52, 0.47	**5.24, 0.027**
16S rRNA gene copy^{l} (unit^{-1} substrate)	.	**5.88, 0.019**	0.00, 0.99	**6.84, 0.012**
ITS copy^{l} (ng^{-1} DNA)	**191.5, < 0.001**	0.03, 0.86	0.11, 0.74	0.00, 0.98
ITS copy^{l} (unit^{-1} substrate)	.	**4.52, 0.039**	0.60, 0.44	3.68, 0.061
ITS:16S ratio	**220.2, < 0.001**	0.10, 0.75	0.38, 0.54	2.89, 0.096
Chlorophyll a (ug cm^{-2} epilithon)	.	0.00, 0.99	.	.

Figure 2.3. Correlation relationships between hydrophysical variables and bacterial and fungal populations on each substrate, with color denoting microhabitat type. Regression lines through datapoints indicate correlation trends ($P < 0.05$) for the associated substrate. Graph frames A, B show flow duration; C, D show distance from outlet; E, F show topographic wetness index (TWI); and G, H show NO3--N:SRP ratio relationships.

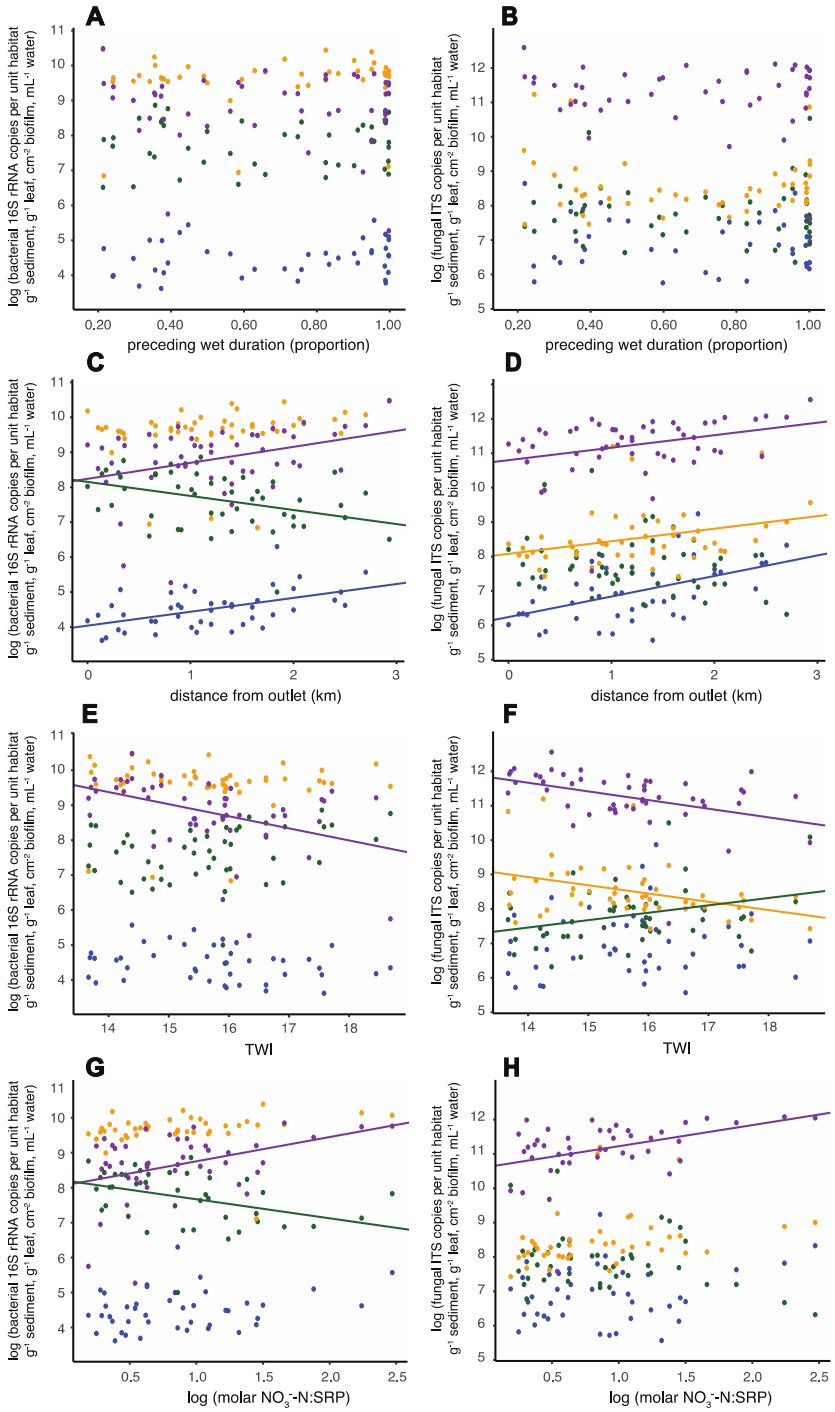


Table 2.2. Correlation test results (n, R, P) matrix for relationships between water, biofilm, leaf, and sediment DNA yield, bacterial 16S gene copy number, fungal ITS copy number, and ng chlorophyll-a (for water, biofilm, leaf, and sediment substrates, with values expressed mL-1, g-1, g-1, and cm-2, respectively) with hydrophysical and biogeochemical attributes at synoptic sampling points. Superscript "l" notes variables that were log-transformed to meet assumptions of normality. Probability of null hypothesis (no effect) of < 0.05 is noted by bold font. Flow dur. = proportion of time preceding sampling with flow, WS area = watershed area, Dist. from outlet = distance from outlet (km), TWI = topographical wetness index, Can. cover = canopy cover, pH = pH, DOC = dissolved organic carbon, NO3- - N = nitrogen in the form of nitrate, SRP = soluble reactive phosphorus, NO3- - N: SRP = Molar ratios of NO3- - N: SRP.

	Flow dur.	WS area^{l}	Dist. from outlet	TWI	Can. cover	pH	Cond.	DOC^{l}	NO_3^- -N	SRP^{l}	NO_3^- -N: SRP^{l}
R, P	**log DNA yield (ug DNA unit^{-1} substrate)**										
Water	0.06, 0.73	-0.20, 0.18	**0.36, 0.02**	-0.16, 0.29	0.13, 0.39	0.10, 0.54	-0.09, 0.56	0.01, 0.96	0.26, 0.09	-0.13, 0.40	0.23, 0.14
Biofilm	0.05, 0.75	0.25, 0.09	-0.27, 0.06	0.21, 0.14	0.03, 0.84	0.12, 0.43	0.05, 0.75	0.10, 0.54	0.03, 0.87	0.21, 0.19	-0.29, 0.06
Leaf	0.08, 0.58	-0.15, 0.30	0.22, 0.14	-0.09, 0.52	0.04, 0.77	-0.07, 0.67	-0.10, 0.54	-0.04, 0.79	**0.31, 0.04**	-0.04, 0.79	0.28, 0.07
Sediment	-0.07, 0.65	**-0.42, 0.003**	**0.30, 0.04**	**-0.44, 0.001**	**-0.30, 0.03**	0.07, 0.66	-0.24, 0.12	0.18, 0.25	0.27, 0.07	-0.26, 0.09	**0.35, 0.02**
R, P	**log bacterial abundance (16S rRNA gene copy unit^{-1} substrate)**										
Water	0.18, 0.25	**-0.32, 0.04**	**0.51, <.001**	-0.18, 0.23	0.03, 0.86	0.17, 0.28	-0.16, 0.32	0.07, 0.64	0.29, 0.06	-0.20, 0.21	0.26, 0.10
Biofilm	0.08, 0.58	**0.32, 0.03**	**-0.38, 0.006**	0.26, 0.07	-0.02, 0.87	0.05, 0.75	0.04, 0.80	0.12, 0.44	-0.14, 0.37	0.21, 0.18	**-0.37, 0.02**
Leaf	0.10, 0.50	0.01, 0.94	0.19, 0.20	0.05, 0.73	0.12, 0.42	-0.15, 0.34	-0.10, 0.54	-0.08, 0.63	0.17, 0.27	0.01, 0.96	0.12, 0.45
Sediment	-0.07, 0.63	**-0.42, 0.002**	**0.35, 0.01**	**-0.46, <.001**	-0.25, 0.08	0.02, 0.89	-0.21, 0.17	0.14, 0.36	0.28, 0.07	-0.27, 0.08	**0.40, 0.009**
R, P	**log fungal abundance (ITS copy unit^{-1} substrate)**										
Water	-0.03, 0.83	-0.07, 0.66	**0.51, <.001**	0.06, 0.71	0.05, 0.77	0.24, 0.13	-0.21, 0.18	-0.02, 0.92	0.29, 0.06	-0.22, 0.16	0.19, 0.22
Biofilm	0.01, 0.93	**0.36, 0.01**	-0.25, 0.08	**0.33, 0.02**	0.13, 0.38	-0.21, 0.18	0.24, 0.12	-0.03, 0.87	**-0.34, 0.03**	0.24, 0.13	-0.28, 0.08
Leaf	-0.08, 0.61	**-0.34, 0.02**	**0.33, 0.02**	**-0.38, 0.007**	-0.29, 0.05	0.01, 0.93	0.03, 0.87	-0.06, 0.71	0.17, 0.29	-0.04, 0.82	0.31, 0.05

Sediment	-0.09, 0.53	**-0.38, 0.007**	**0.33, 0.02**	**-0.40, 0.004**	-0.19, 0.19	-0.01, 0.94	-0.20, 0.20	0.10, 0.54	0.30, 0.05	-0.24, 0.12	**0.41, 0.006**
R, P	**log chlorophyll a (ug unit^{-1} substrate)**										
Water	-0.07, 0.66	-0.29, 0.05	**0.40, 0.007**	-0.05, 0.73	-0.22, 0.14	-0.01, 0.93	0.03, 0.87	0.03, 0.82	0.29, 0.06	**-0.37, 0.02**	0.28, 0.08
Biofilm	-0.15, 0.33	0.04, 0.80	0.13, 0.39	0.09, 0.53	-0.06, 0.66	0.04, 0.78	-0.10, 0.53	0.14, 0.35	-0.13, 0.41	-0.24, 0.12	-0.20, 0.21

Figure 2.4. Box and whisker plots displaying seasonal microbial populations in each of four hydrophases across the three sampling sites in A, B water column; C, D epilithic biofilm; E, F. leaf; and G, H. sediment. Colors represent distinct hydrophases over time. Significant differences were detected by Tukey's post-hoc test ($P < 0.05$): Lower-case letter annotations below the boxplots represent differences between hydrophases (temporal), while annotations above the boxplots indicate among sites (spatial).

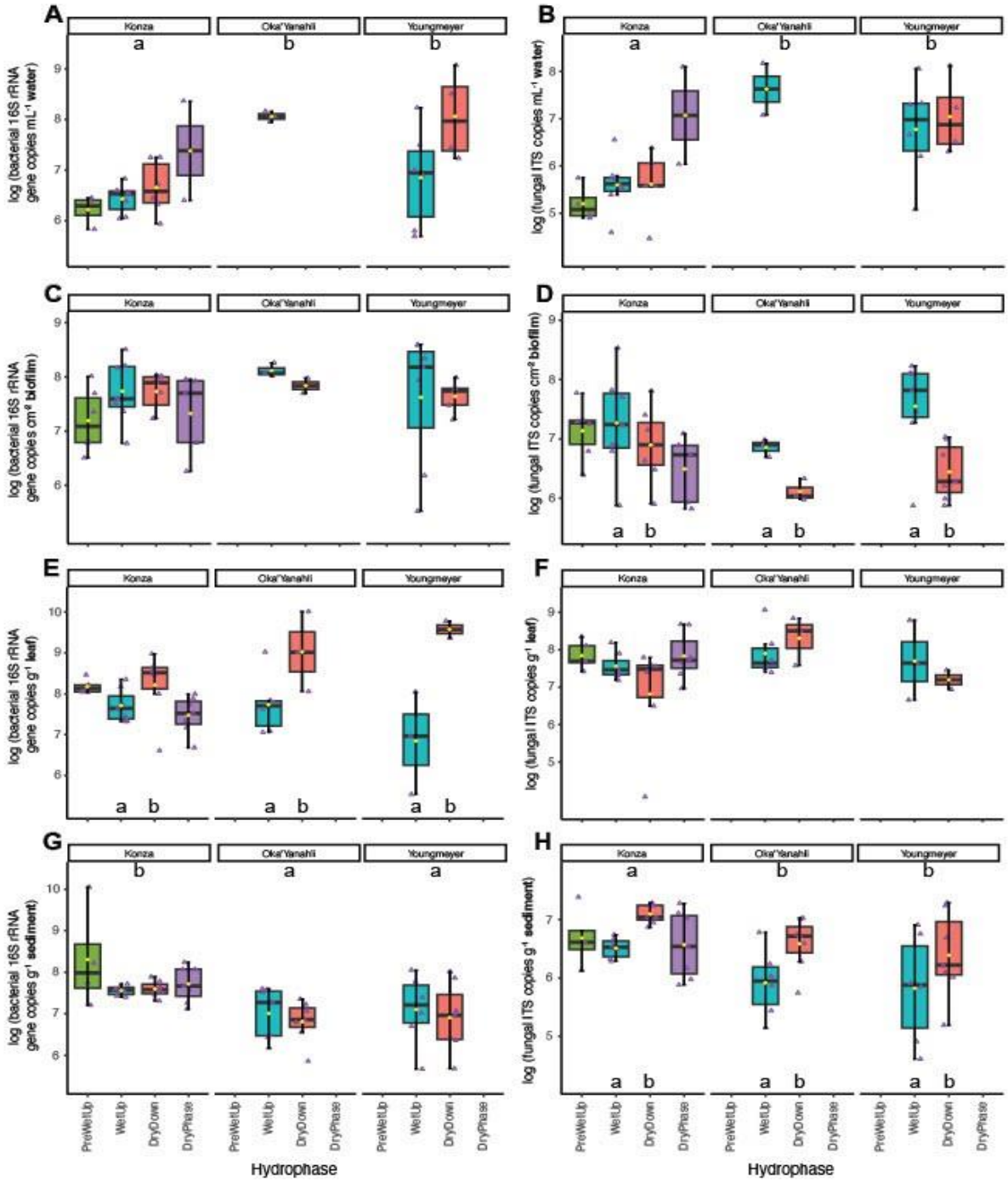


Table 2.3. Statistical results (F, P) for two-way analysis of variance on the direct and interactive effects of site and hydroperiod (for water, biofilm, leaf, and sediment substrates, with values expressed mL-1, g-1, g-1, and cm-2, respectively) on DNA yield, bacterial 16S gene copy number, fungal ITS copy number, and 16S:ITS ratio across seasonal sampling points. Superscript "l" notes variables that were log-transformed to meet assumptions of normality. Probability of null hypothesis (no effect) of < 0.05 is noted by bold font.

	Hydro-period	Site	Site * Hydro-period	Hydro-period	Site	Site * Hydro-period
F, P	**log DNA yield (ug DNA unit^{-1} substrate)**			**log ITS:16S gene copy ratio**		
Water	0.11, 0.95	3.1, 0.06	0.01, 0.91	**3.1, 0.04**	3.4, 0.05	1.6, 0.22
Biofilm	**6.2, 0.002**	0.81, 0.46	0.60, 0.55	**7.7, <0.001**	**6.9, 0.003**	1.6, 0.21
Leaf	**4.6, 0.009**	**6.0, 0.006**	1.1, 0.36	**10.8, <0.001**	**3.6, 0.04**	**3.6, 0.04**
Sediment	0.73, 0.54	**46.7, <0.001**	3.0, 0.06	**16.2, <0.001**	0.35, 0.71	0.94, 0.40
F, P	**log bacterial abundance (16S rRNA gene copy unit^{-1}substrate)**			**log fungal abundance (ITS copy unit^{-1} substrate)**		
Water	**3.1, 0.04**	**10.1, <0.001**	3.0, 0.10	2.6, 0.08	**9.9, 0.001**	0.14, 0.72
Biofilm	1.0, 0.40	0.50, 0.61	0.08, 0.92	**5.1, 0.005**	2.0, 0.16	1.2, 0.31
Leaf	**7.9, <0.001**	0.85, 0.44	**5.0, 0.01**	1.7, 0.19	2.6, 0.09	1.4, 0.27
Sediment	1.4, 0.24	**4.3, 0.02**	0.15, 0.87	**4.0, 0.01**	**5.4, 0.008**	0.03, 0.97

Figure S 2.1. Topographical wetness index (TWI) maps that illustrate TWI at (a) all 50 synoptic sampling sites across the South Fork Kings Creek watershed, (b) 7 long term monitoring sites at Kings Creek, (c) 7 long term monitoring sites at Youngmeyer Ranch, and (d) 7 long term monitoring sites at Oka'Yanahli National Preserve. Sites in darker colors are more likely to be wet. In contrast, lighter colors represent sites that are least likely to be wet.

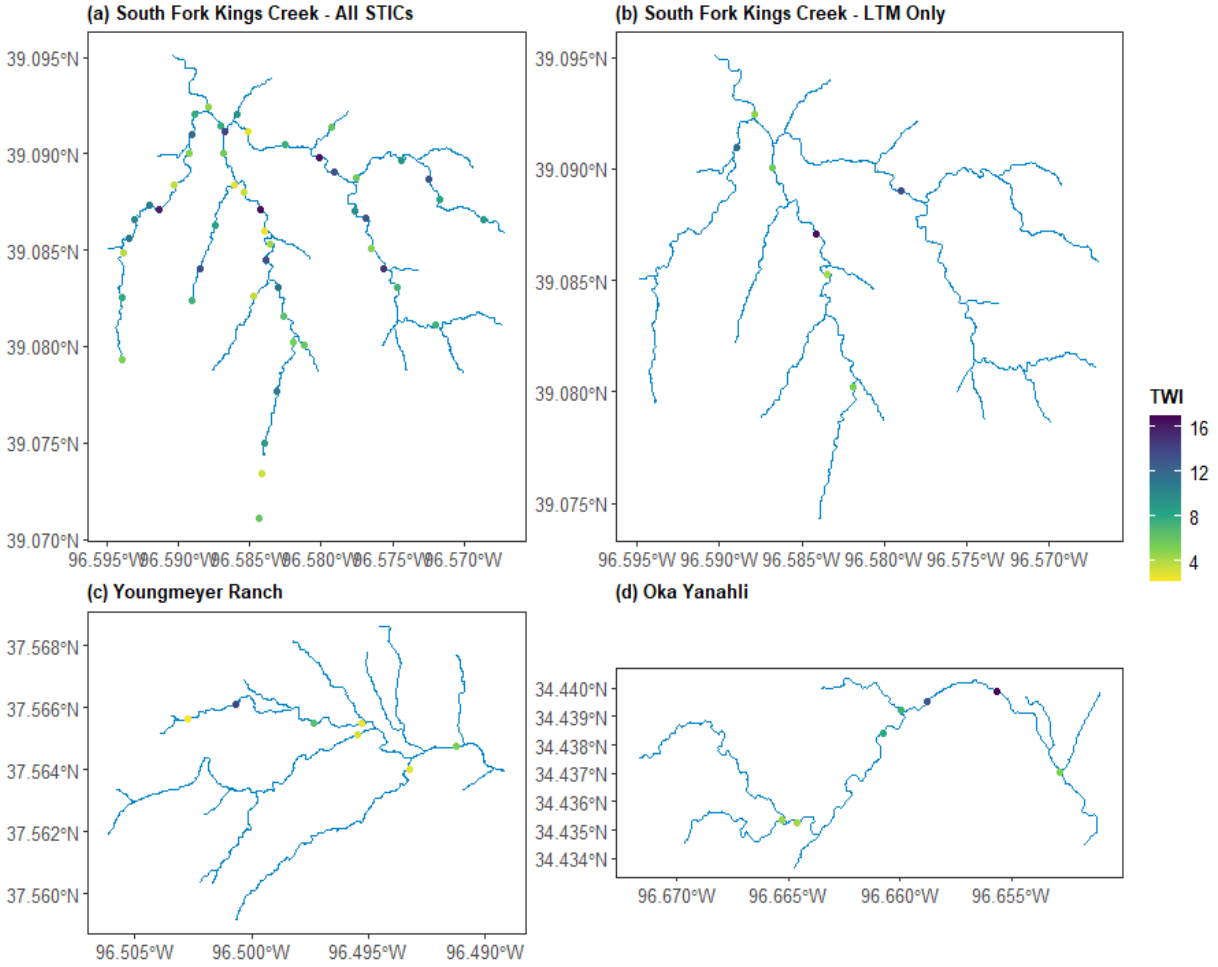


Figure S 2.2. Hydrograph between March and December 2022 that shows respective sampling periods and illustrates how the hydrophase variable was defined for use in temporal analysis. March is classified as pre-wet up, with wet-up, dry-down, and dry phase subsequently occurring during the following sampling months along the rising limb, falling limb, and no-flow portions of the hydrograph, respectively.

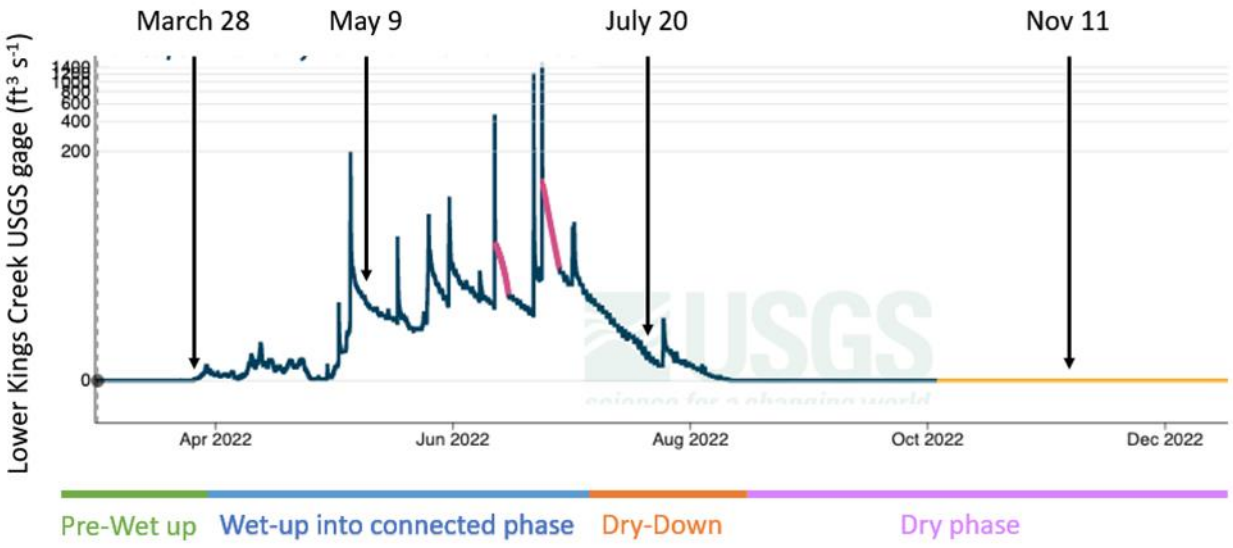
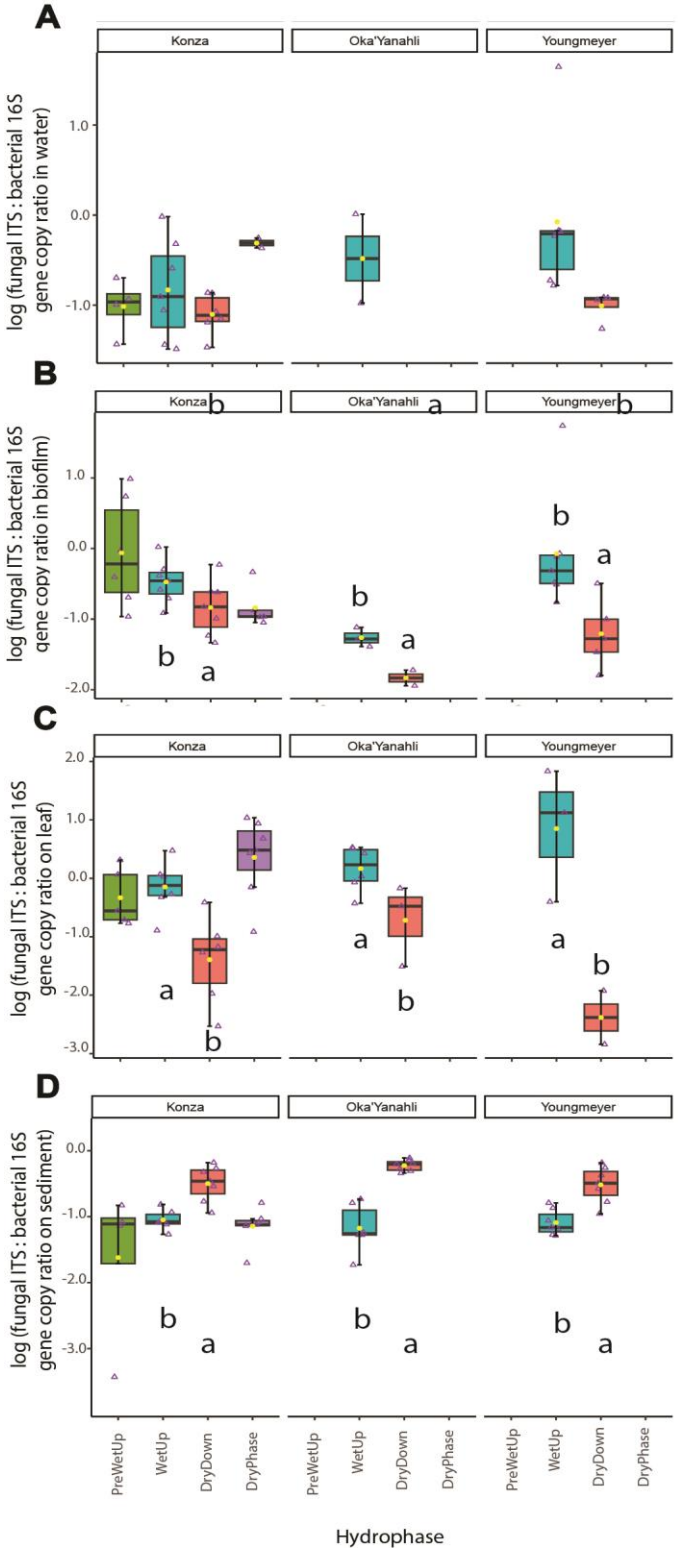


Figure S 2.3. Log transformed ITS:16S ratios in (A) the water column, (B) epilithon on rocks, (C) leaf litter, and (D) sediments over time across the three study sites. Post-hoc annotations next to the site locations are indicative of site differences, and annotations directly above or below the boxplots reflect differences between hydrophases, with Tukey's post hoc test results of $P < 0.05$.



3 Chapter 3 – Concluding Remarks

Across space and time in the study watersheds, microorganisms in water, leaves, sediments, and epilithic biofilms all exhibited dynamic responses to the hydrophysical and hydrochemical predictor variables that we evaluated. However, spatiotemporally, the divergent biofilm and sediment trends in both fungal and bacterial population dynamics proved most intriguing, largely because epilithon may exemplify autotroph-driven responses to non-perenniality while sediments exemplify heterotroph-driven responses. Microbial populations on epilithic biofilms tend to be lower, while in contrast, sediment populations tend to be higher in sites further upstream from the outlet, dry at the time of sampling, during dry-down hydrophases, and less likely to be wet at any time. In addition to interpretations about these contrasting patterns, discussed in Chapter 2, it is interesting to consider interactions between sediment and epilithon biomass changes. For example, higher duration of wetness is likely to induce anoxia within sediments. Anoxia at the sediment-water surface interface stimulates the release of sediment phosphorus, nitrogen, hydrogen sulfide, manganese, iron, and other elements into the overlaying waters (Preece et al., 2019), and this nutrient release has the potential to stimulate algal growth (Beutel and Horne, 1999; Nürnberg, 1984). This rationale could help explain the observed biofilm and sediment gene copy patterns. Sediments at sites more likely to be wet, with relatively no flow, are potentially releasing and suspending sedimentary nutrients that are then taken up by the algal constituents, resulting in reduction of nutrients in sediments and contributing to anoxia in sites near the outlet.

Also, future work conceptualizing controls over stream microbial abundance should be developed as a departure from the River Continuum Concept (RCC) framework (Vannote et al., 1980). The stream order of the Kings Creek study catchment varied from 1 to 3, and the RCC states that the zone from which the stream shifts from heterotrophic to autotrophic is dependent on canopy cover and that in deciduous forests it is typically in stream order 3 (Vannote et al., 1980). In our study system this was not the case. Since there was no correlation between canopy cover and bacterial abundance, fungal abundance, water column or epilithon chlorophyll, it seems that Kings Creek does not fit the RCC model. In fact, a synthesis by Dodds et al. (2004) found that stream ecosystem and community variation in Kings Creek is more characteristic of a desert stream, another non-perennial system. Regarding the RCC, Kings Creek may be affected by continuous catchment scale elements, but does not follow trends of traditional perennial

stream systems. Many other streams diverge from the RCC model (Doretto et al. 2020), and at drier times of year than the sampling time, Kings Creek likely exhibits more discontinuity in its network scale patterns and dynamics.

Overall, the presented research shows highly variable microbial population sizes on different microhabitat substrates within freshwater Great Plains non-perennial stream networks. Using quantitative PCR, analysis of the bacterial 16S rRNA gene and fungal ITS amplicons allowed us to quantify bacterial and fungal populations in water, biofilm, sediments, and leaves under fluctuating hydrological conditions. This research also allowed us to test an array of hydrological predictors, and distance from the outlet, topographical wetness index, and nitrate-N to SRP ratio, emerged as the most significant predictor variables across the catchment, while the re-wetting and dry-down phases of the hydrograph also sustained different microbial populations. This research expands our understanding of spatiotemporal dynamics on microbial population sizes on a myriad of substrates at varying hydrological regimes. Future directions for this area of research should encompass new methods of hydrological connectivity, and expand upon existing microbial analysis to include protistan abundance and community data to understand how grazing dynamics on bacteria (Weisse, 1991) affect biogeochemical cycling, ecosystem metabolism, and greenhouse gas emissions. Lastly, understanding the legacy effects of intermittent regimes on microbial populations across microhabitats could help characterize spatiotemporal microbial and nutrient patterns that could allow for a better understanding of downstream water quality in non-perennial freshwater systems.

3.1 References Cited

Beutel, M. W., & Horne, A. J. (1999). A review of the effects of hypolimnetic oxygenation on lake and reservoir water quality. *Lake and Reservoir Management*, *15*(4), 285-297.

Dodds, W. K., Gido, K., Whiles, M. R., Fritz, K. M., & Matthews, W. J. (2004). Life on the edge: the ecology of Great Plains prairie streams. *BioScience*, *54*(3), 205-216.

Doretto, A., Piano, E., & Larson, C. E. (2020). The River Continuum Concept: lessons from the past and perspectives for the future. Canadian Journal of Fisheries and Aquatic Sciences, 77(11), 1853-1864.

Nürnberg, G. K. (1984). The prediction of internal phosphorus load in lakes with anoxic hypolimnia 1. *Limnology and oceanography*, *29*(1), 111-124.

Preece, E. P., Moore, B. C., Skinner, M. M., Child, A., & Dent, S. (2019). A review of the biological and chemical effects of hypolimnetic oxygenation. *Lake and Reservoir Management*, *35*(3), 229-246.

Vannote, R. L., Minshall, G. W., Cummins, K. W., Sedell, J. R., & Cushing, C. E. (1980). The river continuum concept. *Canadian journal of fisheries and aquatic sciences*, *37*(1), 130-137.

Weisse, T. (1991). The annual cycle of heterotrophic freshwater nanoflagellates: role of bottom-up versus top-down control. *Journal of plankton research*, *13*(1), 167-185.