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Spatiotemporal trends in bacterial diversity across three watersheds within the Platte River Basin, Nebraska

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SPATIOTEMPORAL TRENDS IN BACTERIAL DIVERSITY ACROSS THREE

WATERSHEDS WITHIN THE PLATTE RIVER BASIN, NEBRASKA

by

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River bacteria are understudied despite being critical components of river ecosystems. There are even fewer studies considering bacteria communities at large spatiotemporal scales, which may provide insight into drivers of community assembly. We investigated differences in bacterial diversity across environmental gradients within three sub-basins nested in the Platte River Basin, Nebraska. Surface water samples were collected weekly at 36 sites from May to September by the Nebraska Department of Environment and Energy (NDEE) in 2019. Bacterial communities were sequenced using the Illumina MiSeq platform. Sub-basins had similar counts of unique amplicon sequence variants (ASVs) but different community structures. These structural differences were partially driven by environmental factors influenced by climate, land-use, and geomorphology. Two sub-basins exhibited shifts in community structure between early and late summer, but the third exhibited no clear temporal pattern. Relative abundances of typical and common freshwater genera like *Flavobacterium* contributed the most to structural differences between sub-basins. The most abundant genera across all sub-basins included copiotrophs, suggesting that our study systems are nutrient-enriched. The trend in bacterial diversity observed in our study demonstrates the ecological relevance of considering bacterial diversity at large spatial and temporal scales.

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INTRODUCTION

Humans are dependent on rivers for drinking water, waste management, agriculture, transportation, and recreation (Dodds et al., 2004). Consequently, humans are also dependent on microorganisms such as bacteria for the functional processes that maintain river ecosystem health. Bacteria play a key role in cycling organic building blocks like carbon, nitrogen, phosphorus, and sulfur and are cornerstones of the aquatic food web (Porter et al., 1988; Cotner & Biddanda, 2002; Tank et al., 2010).

River ecologists have been accounting for bacterial processes in their studies and models for decades (Allison &Martiny, 2008). However, microbes are often simplified into kinetic constants representing functional processes rather than being treated as living themselves (Allison &Martiny, 2008). This simplification is in part due to our previous inability to characterize the function and dynamics of microbial communities. It is estimated that less than 1% of bacteria can be grown on culture media and until recently most studies have been reliant on culturing techniques to study bacterial diversity (Staley & Konopka, 1985; Givens et al., 2015). The advent of next-generation sequencing (NGS) has now allowed for cost-effective culture independent classification of microbes (Ghanbari et al., 2015). These technological advances have made it possible to conceptualize river microbes as dynamic communities whose function is dependent on taxonomic structure and composition, which are in turn directly influenced by environmental factors (Zeglin, 2015).

STABILITY AND FUNCTION AS INFLUENCED BY COMMUNITY COMPOSITION AND STRUCTURE

Bacterial community function is reliant on community composition and structure. Composition refers to the number of species in a community, whereas structure refers to the relative abundance of these species (Allison &Martiny, 2008; Shade et al., 2012). Composition and structure influence how a community responds to environmental disturbance, which is changes in the surrounding physical, chemical, and or biological environment (Pimm, 1984; Balmonte et al., 2016). The stability of a community, or how a community responds to environmental disturbance, is comprised of two concepts: resistance and resilience (Allison & Martiny, 2008).

Resistance is the extent to which a community composition and structure remains unaffected by an environmental disturbance (Shade et al., 2012). Resilience, sometimes referred to as recovery, is the rate at which a community assumes pre-disturbance composition and structure after a disturbance (Shade et al., 2012). Sometimes composition and structure remain permanently shifted after a disturbance event, but community functional process rates do not significantly change (Allison & Martiny, 2008; Louca et al., 2018). This phenomenon, known as functional redundancy, occurs when multiple taxa perform similar functional processes at similar rates so that when a taxon is lost or diminished, another can occupy its niche (Allison & Martiny, 2008; Louca et al., 2018).

The degree to which a community is resistant or resilient is dependent on community composition and structure. Generally, increasing species richness improves

community stability and function (Tilman, 1999; Zinger et al., 2012). Systems with low numbers of species across trophic levels are more likely to lose entire functional groups through stochastic events, resulting in reduced function (Morin & McGrady-Steed, 2004). Species richness alone, however, does not guarantee high functional rates or stability against environmental disturbance (Pimm, 1984). The types of species present in a community and their capacity to tolerate or take advantage of environmental change is also important (Sankaran & Mcnaughton, 1999; Griffiths et al., 2004; Bell et al., 2005). For example, Bell et al. (2015) found some species-poor microbial communities had similar respiration rates to species-rich ones in a study on semi-permanent rain pools. Likewise, Sankaran & McNaughton (1999) found that low-diversity plant communities could demonstrate high resistance to environmental disturbance if appropriate plant species were present. Evenness, or the proportions of constituent species, has also been found to influence resilience and resistance in communities of larger animals, yet this relationship is less apparent in microbial communities (Shade et al., 2012). Wittebolle et al. (2009) found that initial community evenness in denitrifying bacterial communities improved stability compared to communities with low evenness after salinity stress.

MICROBIAL COMMUNITY RESPONSE TO ENVIRONMENTAL CHANGE

Bacterial communities may resist or recover from environmental disturbance, but compositional and structural changes are also a natural part of a bacterial community. Bacteria are sensitive to environmental change and have short generation times, making rapid compositional, structural, and functional community shifts possible (Prosser et al., 2007). Salinity, temperature, pH, nutrients, and dissolved oxygen are environmental factors that have commonly been found to influence freshwater bacterial community

structure and composition (Nold & Zwart, 1998, Zeglin, 2015).

Salinity

Transition of microorganisms between fresh and saline environments is rare as it requires multiple adaptations for osmoregulation (Zwart et al., 2002). The rarity of these adaptations often results in freshwater and saline-associated species being phylogenetically distant, with some taxonomic groups being entirely confined to fresh or saltwater (Zwart et al., 2002). Bacterial community composition has thus been observed to change across salinity gradients (Crump et al., 2004; Fortunato et al., 2012). A study on multiple Tibetan lakes ranging from freshwater to hypersaline observed that bacterial community compositions between saltwater and freshwater lakes were nearly entirely different (Wu et al., 2006). For example, while Betaproteobacteria was only present in low salinities, Gammaproteobacteria thrived in saline environments. Class Betaproteobacteria appears to be a class specialized for freshwater, as it is rarely found in marine environments (Zwart et al. 2002). Notable freshwater bacteria within Betaproteobacteria that prefer low salinities are *Polynucleobacter* and *Limnohabitans*.

Water pH

Enzymatic activity within prokaryotes often operates within a specific pH range and conditions outside this range can have negative effects on cell function (Zhalnina et al., 2015). Chamier et al. (1987) found that in leaf litter communities increased acidity was associated with decreasing rates of bacterial degradation of organic matter. Bacteria vary in their range of pH tolerance (Lauber et al., 2009), and so pH may have a strong

effect on lotic bacterial community composition. For example, Fierer et al. (2007) found in a study of an acidic, forested stream that pH was the best predictor of benthic bacterial community composition. Relationships between pH and bacteria can vary between members at the genus and sub-species levels (Newton & Mclellan, 2015). For example, pH appears important in selecting for different sub-clusters within Genus *Polynucleobacter*, with *P. necessarius* preferring more acidic conditions and *P.acidiphobus* preferring more alkaline conditions (Wu & Hahn, 2006).

Temperature

Increasing temperatures are often tied to increased bacterial metabolism and consequently bacterial abundance (Shiah & Ducklow, 1994). An experiment investigating temperature and substrate regulation of bacterial abundance found that bacterial growth rates were exponentially and positively correlated with incubation temperature (Shiah & Ducklow, 1994). Many studies have identified temperature as a strong predictive factor of bacterial community composition and structure (Crump & Hobbie, 2005; Hullar et al., 2006; T. Liu et al., 2018). Liu et al. (2018) observed that temperature was the primary factor influencing bacterial abundance in the Songhua River, China. Increasing bacterial abundance in response to temperature, however, does not necessarily result in increased bacterial diversity. Liu et al. (2018) also found that bacterial diversity decreased with temperature from a combination of heat and oxygen stress. Furthermore, the positive effect that temperature has on bacterial growth rates can result in intra- and interspecific competition for resources as populations grow, limiting the abundances of some species (Mayo et al. 1996). Temperature along with nutrient

enrichment is an important factor in the proliferation of Cyanobacteria blooms, which are ecologically significant events in freshwater systems (Scott, J.T., and Marcarelli, 2012).

Nitrogen and phosphorus

Nitrogen and phosphorus are critical nutrients for growth and metabolic upkeep of a bacterial cell. These nutrients can be limiting in freshwater systems (Smith & Prairie, 2004; Elser et al., 2007) and so nutrient additions can trigger a rapid functional and compositional community response (Haukka et al., 2006; Van Horn et al., 2011; Yang et al., 2018). Some bacterial taxa such as Betaproteobacteria are nutrient-loving and increase in abundance when nutrient levels are high (Yang et al., 2018). Others such as freshwater Alphaproteobacteria are oligotrophic and predominate in low nutrient concentrations (Salcher et al. 2011). High abundance of nutrient-loving bacteria in lotic systems can be indicative of nutrient pollution from human activities like agriculture, animal husbandry, and wastewater treatment (Wang et al., 2015; Li et al., 2016; Yang et al., 2018). For example, a study on the North Canal River, China, and that the abundance of *Polynucleobacter*(Betaproteobacteria) and *Hydrogenophaga*(Betaproteobacteria) was highest directly downstream of a wastewater treatment plant and decreased with improving water quality (Yang et al., 2018).

Organic Carbon

Sources of organic carbon come from autocthonous primary production and allocthonous terrestrial production (Smith & Prairie, 2004). Partially decomposed organic carbon, known as dissolved organic carbon (DOC) is used by heterotrophic bacteria for

growth. The quality, molecular weight, and abundance of DOC can select for different bacterial taxa (Cottrell & Kirchman, 2000; Fierer et al., 2007). A study on organic aggregate (OA) lake bacterial communities found that abundances of Phylum Bacteroidetes and Classes Alpha- and Betaproteobacteria were related to the physiochemical properties of OA (Tang et al., 2008). Bacteroidetes specifically was related to the availability of algal-derived substrates (Tang et al., 2008). Carbon availability can also influence bacterial metabolic activity. For example, a study of an arctic sub-catchment found that shifts in supply of dissolved organic matter resulted in changes in bacterial production, community composition, and rates of carbon processing (Judd et al. 2006). Phylum Bacteroidetes in freshwater consists mostly of carbon-degrading heterotrophs, with genus *Flavobacterium* being a notable example.

Dissolved oxygen

Dissolved oxygen is consumed by heterotrophic bacteria for respiration and is released as a byproduct in autotrophic photosynthesis. Dissolved oxygen concentration is sometimes used to estimate whether heterotrophic or autotrophic activity is dominant in a system (Doherty et al., 2017). Dissolved oxygen has often been associated with changes in bacterial community composition and structure, but is rarely identified as a dominant driver (Doherty et al., 2017; Liu et al., 2019; T. Liu et al., 2018). Dissolved oxygen can be influenced by pH, temperature, nutrient load, and physical processes such as turbulence (Yang et al., 2018), meaning these factors may explain more variation in bacterial communities than, or confound clear responses from, dissolved oxygen alone.

Unsurprisingly, members of autotrophic Cyanobacteria such as the common *Microcystis* genus are associated with increased oxygen levels (Casamatta & Hasler, 2016).

SPATIOTEMPORAL VARIATION IN BACTERIAL COMMUNITY DIVERSITY

Bacterial communities are sensitive to variation in salinity, pH, temperature, nutrients, dissolved oxygen, and other environmental factors. Temporal changes in these environmental factors caused by climatic changes in temperature, precipitation, terrestrial input of carbon and nutrients, and snowmelt can result in temporal patterns in bacterial communities (Crump et al., 2007; Portillo et al., 2012; Doherty et al., 2017). A study on the bacterioplankton communities of two temperate, non-intersecting rivers found the communities of both rivers were nearly identical due to the climatic influences of temperature and flow rate (Crump and Hobbie, 2005). Another study on bacterioplankton in a subtropical climate found community composition to differ between the wet and dry seasons in the Yangtze River (Liu et al., 2018). Spatial factors such as land-use and geomorphology can also influence environmental factors that cause bacterial community change. Liu et al. (2018) also observed that landform types (e.g., mountains and plains) influenced bacterial communities as geomorphology can affect drainage and erosion patterns which can introduce new growth substrates, nutrients, and bacteria into the system. Human land-use practices such as agriculture, animal grazing, and wastewater treatment can also be powerful spatial factors as they can introduce large amounts of nutrients, organic wastes, and pharmaceuticals into the environment (Kolpin et al., 2002; Li et al., 2016; Ma et al., 2016; Newton & Mclellan, 2015; Novo et al., 2013; Simonin et al., 2019).

TYPICAL FRESHWATER BACTERIA

Meta-analyses of studies investigating drivers of microbial community assembly have found that freshwater communities from dozens of rivers and lakes share remarkable similarities despite being geographically distant (Nold & Zwart, 1998; Lozupone & Knight, 2007; Fortunato et al., 2012). Ultimately, individuals from phyla Proteobacteria, Bacteroidetes, Actinobacteria, Cyanobacteria, and Verrucomicrobia have emerged as the dominant taxa in freshwater systems (Zwart et al. 2002). Most of what we know about these important phyla in freshwater systems has come from lakes, but the body of research on river bacterial communities has increased in recent years (Zinger et al., 2012; Zeglin, 2015).

PHYLUM PROTEOBACTERIA

Proteobacteria is a Gram-negative, diverse taxon that is typically the most abundant phylum in freshwater systems (Barberan & Casamayor 2010; Liu et al. 2019). Proteobacteria is currently divided into six classes: Alphaproteobacteria, Betaproteobacteria, Deltaproteobacteria, Epsilonproteobacteria, Gammaproteobacteria, and Zetaproteobacteria (Newton et al. 2011). Of these classes, Alphaproteobacteria, Betaproteobacteria, and Gammaproteobacteria are most commonly observed in freshwater (Newton et al. 2011). Progress has been made in understanding the ecology and phylogeny of Alphaproteobacteria Betaproteobacteria freshwater lineages, but little is still known about Gammaproteobacteria native to freshwater.

Class Alphaproteobacteria

Alphaproteobacteria is a persistent presence in freshwater systems, although its abundance is much lower than that of Betaproteobacteria (Newton et al. 2011). Freshwater members are generally small and have slow growth rates (Salcher et al., 2011). They are slow but efficient users of nutrients, which may explain why they can outcompete other groups like Gammaproteobacteria in abundance when nutrient concentrations are low (Salcher et al., 2011). Freshwater Alphaproteobacteria are currently divided into eight lineages labeled *alfI* through *alfVIII*. The most widely-distributed and well-studied of these lineages is LD12 (*alfV*) (Newton et al. 2011).

Lineage LD12

Lineage LD12 is a freshwater cluster within the abundant and common marine group SAR11 (Pelagibacterales). Members are small, obligately aerobic, chemoorganotorphic, and have streamlined genomes (Eiler et al., 2016; Henson et al., 2018). This group was previously thought to have low abundances in freshwater, but more recent studies have revealed that under the right conditions that LD12 abundances can predominate in lakes (Heinrich et al., 2013; Salcher et al., 2011). Lineage LD12 appears to be oligotrophic as it has been negatively associated with nutrient concentrations (Salcher et al., 2011) and areas of high productivity (Heinrich et al., 2013). However, Heinrich et al. (2013) observed a positive relationship between LD12 and nitrate in a eutrophic lake, potentially suggesting ecological diversification. This diversification may be driven by temperature, but little is still known about the various LD12 ecotypes (Henson et al., 2018). This group degrades low molecular weight compounds such as amino acids but can also degrade organic carbon such as glucose,

fructose, and acetate (Salcher et al., 2011). This group is difficult to culture, and so many aspects of its physiology and relationships with environmental factors remain unknown. There is currently only one member that has been successfully isolated (Henson et al., 2018).

Class Betaproteobacteria

Betaproteobacteria appears specially adapted for freshwater environments, as its abundance decreases with salinity (Nold & Zwart 1998, Wu et al 2006). This group is generally fast-growing, nutrient-loving, and heterotrophic (Newton et al. 2011). Many members of Betaproteobacteria such as *Simplicispira* in freshwater are nitrogen fixers, serving a key role in the cycling of nutrients (Liu et al. 2012). Betaproteobacteria appear to prefer higher temperatures and pH (Figueiredo et al. 2012, Jordaan& Bezuidenhout 2016) while other studies have found them positively associated with dissolved oxygen, nitrate, ammonia, and sulfate (Liu et al. 2012; Jordaan& Bezuidenhout 2016).

Freshwater Betaproteobacteria can be divided into seven lineages: *BetI*, *BetII*, *BetIII*, *BetIV*, *BetV*, *BetVI*, and *BetVII* (Newton et al. 2011). Family *Comamonadaceae* contains many common and abundant freshwater groups, including the wide-spread Genera *Limnohabitans* and *Hydrogenophaga.* Genus *Polynucleobacter* (*BetII, Burkholderiaceae*) is another cosmopolitan and highly abundant taxon and also one of the most well-studied.

Family Comamonadaceae

Family *Comamonadacae* (Order Burkholderiales) was first proposed as a taxon in 1991 (Williams, et al., 1991) and to date includes 29 genera and 104 species (Willems,

2013). Members of this group have been found in freshwater, groundwater, sediment, activated sludge, and industrial wastewater (Willems, 2013). Most members are aerobic heterotrophs (Willems, 2013) but other genera such as *Hydrogenophaga* and *Simplicispira* are also denitrifies (Willems & Gillis, 2015; Zubair et al., 2019). *Comamonadaceae* is an abundant and common group in freshwater systems (Jani et al., 2018; Simonin et al., 2019). Many members of this group are copiotrophic and have been found to increase in abundance in response to nutrient enrichment from urbanization (Balmonte et al., 2016; Simonin et al., 2019; Yang et al., 2018). As such, *Comamonadaceae* has been considered a potential indicator taxon for urbanization and stream modification (Simonin et al., 2019). Genus *Limnohabitans* is perhaps the most notable member of *Comamonadaceae* in freshwater due to its widespread abundance. Another common genus is *Hydrogenophaga*.

Genus *Limnohabitans*. *Limnohabitans*(Family *Comamonadaceae*) was first described in 2010 (Hahn et al. 2010). There are currently four described species within Genus *Limnohabitans*: *L. parvus, L. planktonicus, L. australis, and L. curvus* (Hahn et al. 2010a; Hahn et al. 2010b, Kasalicky et al. 2011). All described species are chemoorganotrophic, rod-shaped, non-motile, and aerobic or facultative anaerobic, with variation in the compounds they metabolize (Hahn et al. 2010a; Hahn et al. 2010b, Kasalicky et al. 2011). *Limnohabitans planktonicus* and *L. parvus* appear to be of special ecological importance as members of the R-BT lineage, a group of Betaproteobacteria which grow rapidly on algal-derived substrates and subject to high rates of bacterivory, making them an important part of the carbon cycle in freshwater systems (Simek et al. 2010, Kasalicky et al. 2011). This group is copiotrophic and increases in abundance in

urbanized systems where nutrient enrichment is high (Ma et al., 2016).

  Genus *Hydrogenophaga*. Genus *Hydrogenophaga* (Family Comamonadaceae) consists of five validated species, all of which were previously classified in the genus *Pseudomonas* (Willems & Gillis, 2015). These species are *H. flava*, *H.intermedia, H. palleronii, H. pseudoflava,* and *H. taeniospiralis* (Willems & Gillis, 2015). All validated species are motile, rod-shaped, facultative chemolithotrophs capable of oxidizing H_2 and carbohydrates for energy (Willems & Gillis, 2015). Two members, *H. pseudoflava* and *H. taeniospiralis,* are capable of denitrification (Jenni et al., 2008; Lalucat et al., 1982). *Hydrogenophaga* is a typical and often abundant member of freshwater environments (Ma et al., 2016). This group is copiotrophic and has been documented to increase in abundance in urbanized streams where nutrient enrichment is high, similar to *Limnohabitans* (Ma et al., 2016; Yang et al., 2018). Denitrifying strains have been discovered in the sand filters of a wastewater treatment plant (Lemmer et al., 1997).

Genus *Polynucleobacter*

  Genus *Polynucleobacter (*Family *Burkholderiaceae*)was first described in 1987 with the co-description of *P. necessarius spp.*, an obligate endosymbiont of ciliate *Euplotes aediculatus* (Heckman & Schmidt 1987). *Polynucleobacter* has since been observed in lakes, rivers, and ponds, and can be the most abundant taxon in a bacterioplanktonic community (Hahn et al. 2012; Liu et al. 2012; Ma et al. 2016). Members of *Polynucleobacter* are typically small, rod-shaped, non-motile, and are found in aerobic zones permeated by light (Watanabe et al. 2008, Wu & Hahn 2006). Members of *Polynucleobacter* have a low salinity tolerance and have not been found in marine environments (Wu & Hahn 2006, Hahn et al. 2012). *Polynucleobacter* generally prefer

lower pH environments with high nutrient loads, although this genus is tolerant of most conditions (Lindstrom et al., 2005; Newton & Mclellan, 2015; Ma et al., 2016). *Polynucleobacter* is classified into four lineages, PnecC containing *P. rarus*, PnecB containing *P. acidiphobus*, PnecC containing *P. necessarius*, and PnecD containing *P. cosmopolitans*. *P. necessarius* and its subspecies are one of the best-studied freshwater groups (Newton et al. 2010). Species *P. necessarius* was once thought to be solely an obligate endosymbiont, but free-living strains have been discovered (Vannini et al. 2007). PnecC is ubiquitous and found across a variety of environments, but seems to prefer low pH like other members of its genus (Lindstrom et al. 2005). This group is chemoorganotrophic and utilizes low-molecular substrates such as the products derived from photooxidation of humic substances, which may explain why it can make up to 60% of bacterioplankton in humic ponds (Hahn et al. 2005, Hahn et al. 2012).

PHYLUM BACTEROIDETES (FORMERLY CYTOPHAGA-FLEXIBACTER-BACTEROIDES (CFB) GROUP)

Bacteroidetes has been found in freshwater, salt water, and sediment. Along with Phylum Firmicutes it is dominant in the vertebrate gut microbiome (Thomas et al. 2011). Members of Bacteroidetes are Gram-negative, rod-shaped, heterotrophic, and can be aerobic or anaerobic (Thomas et al. 2011). This group is often the second most abundant group in stream systems after Proteobacteria in freshwater (Zwart et al. 2002). A study on bacterial communities in the Mooi River in South Africa found 16-60% of sequences to consist of Bacteroidetes depending on sampling site (Jordaan& Bezuidenhout, 2015).

Bacteroidetes can be an important group in the degradation of organic matter, namely high molecular weight (HMW) carbohydrates and proteins. One study found Bacteroidetes to be overrepresented in the consumption of chitin, protein, and N-acetylglucosamine for its relative abundance in a marine bacterial community, indicating its efficiency at processing organic material (Cottrell & Kirchman, 2000). Bacteroidetes members also metabolize allochthonous material such as plant matter that enters river systems (Kisand et al., 2002).

Members of Bacteroidetes are typically nutrient-loving and prefer environments rich in organic material (Sullivan et al., 2006; Figueiredo et al., 2012; Doherty et al., 2017). It is often associated with phytoplankton blooms that generate carbon (Doherty et al. 2007). Bacteroidetes abundance appears to be strongly influenced by anthropogenic activity near waterways; two studies demonstrated that sediment-associated Bacteroidetes members had dominant relative abundance in urban rivers with nearby wastewater discharge due to increase amount of complex organic compounds (Wu et al., 2012; Drury et al., 2013). Bacteroidetes can be found in saline and freshwater environments, but its abundance decreases with increasing salinity (Doherty et al. 2007; Wu et al. 2012).

Four classes comprise Phylum Bacteroidetes: Bacteroidia, Cytophagia, Flavobacteria, and Sphingobacteria. Flavobacteria is the largest class (Thomas et al. 2011) and contains family Flavobacteriaceae, which appears to be one of the most abundant taxa in freshwater systems (Jordaan& Bezuidenhout 2006; O'Sullivan et al. 2006; Figueiredo et al. 2011). Genus *Flavobacterium*, an aerobic, carbon-degrading group that often comprises the majority of Flavobacteriaceae found in freshwater (Jordaan& Bezuidenhout 2006; O'Sullivan et al. 2006; Figueiredo et al. 2011).

Genus *Flavobacterium.* Genus *Flavobacterium* is a group of impressive

physiological diversity, with 40 validated species (Bernardet & Bowman, 2015). Most

members are obligate anaerobes with chemoorganotrophic metabolisms (Bernardet &

Bowman, 2015). The physiological diversity of *Flavobacterium* has led to representatives

being discovered in soils, freshwater, and marine environments (Bernardet & Bowman,

2015). Salinity tolerance varies among freshwater species, with some strains being able to

subsist in brackish conditions at lower abundances (Kisand et al., 2005). *Flavobacterium*

is psychrotolerant and has been found to be a predominant part of bacterioplankton

communities in Antarctic lakes (Michaud et al., 2012). *Flavobacterium* appears to be an

ecologically-important group in the degradation of organic carbon (Kisand et al., 2002).

Eiler & Bertilsson (2007) found *Flavobacterium* abundances to increase with available

carbon from Cyanobacteria blooms. *Flavobacterium* can be one of the most, if not most

abundant bacterial genera in freshwater systems (Jordaan& Bezuidenhout 2006;

O'Sullivan et al. 2006; Figueiredo et al. 2011).

PHYLUM ACTINOBACTERIA

Actinobacteria is one of the most diverse bacteria phyla and is wide-spread across

terrestrial, freshwater, and marine habitat (Warnecke et al. 2004). Members are Gram-

positive, filamentous, typically small, and generally have high guanine and cytosine

nucleotide content (Zothanpuia et al., 2018). They were once considered to be an

intermediate form between bacteria and fungi as many members produce a mycelium and

reproduce through sporulation (Zothanpuia et al., 2018).

Phylum Actinobacteria along with Phylum Bacteroidetes and Class

Betaproteobacteria is one of the top three abundant groups of bacteria in rivers (Crump & Hobbie, 2005; Jordaan& Bezuidenhout, 2015; Li et al., 2016; Wang et al., 2015). One study found that Actinobacteria made up 63% of cell biomass in an Austrian lake (Glöckner et al., 2000). As a phylum, Actinobacteria can be abundant in a range of environmental conditions. For example, Actinobacteria made up one quarter of all bacteria in all sites sampled down an estuary gradient which varied in salinity and dissolved organic carbon (Holmfeldt et al., 2009). Another study found Actinobacteria alpha diversity remained constant across an urban river with varying degrees of anthropogenic activity (Wang et al. 2016).

Members of Actinobacteria are difficult to culture and so the ecology and physiology of freshwater Actinobacteria remains largely uncharacterized (Lipko, 2020). To date, most knowledge of freshwater Actinobacteria ecology has come from culture-free methods. The small cell size of this group makes it resistant to grazing, which may in turn contribute to its abundance in freshwater (Hahn et al., 2003). Abundance at the phylum level may be positively correlated with temperature (Holmfeldt et al. 2009) and zones where primary production is high (Figueiredo et al. 2009). Actinobacteria appears to play an important role in the cycling of carbohydrates and extracellular polymeric substances (Elifantz et al., 2005).

There are currently nine described freshwater lineages within Actinobacteria: *acI*, *acTH1*, *acSTL*, *Luna1*, *acIII*, *Luna3*, *acTH2*, *acIV*, and *acV* (Newton et al. 2011, Lipko 2020). Of these groups *acI* and *acII* appear nearly exclusively to freshwater, while *acI* and *acIV* are the dominant Actinobacteria lineages in freshwater systems.

Lineage *acI*

The *acI* lineage is the best-studied Actinobacteria lineage in freshwater systems and the most resolved (Lipko 2020). The *acI* lineage lies within order Actinomycetales and consists of 13 tribes across four clades labeled *acI-A* through *acI-C* (Newton et al. 2011). Members of *acI* have low G+C content despite Actinobacteria being characterized as G+C rich (Ghai et al. 2012). Members of *acI* are generally free-living (Warnecke et al. 2004). The *acI* lineage often comprises the majority of Actinobacteria in a freshwater system, with one study finding *acI* to make up to 80% of sequenced Actinobacteria (Allgaier & Grossart, 2006). This group is generally tolerant of a wide range of salinity conditions, although salinity tolerance does vary at the clade level (Holmfeldt et al. 2009).Water acidity can select for different tribes, with *acI-AI*, *-BII*, and *-BIII* preferring more acid conditions (pH <6) and *acI-AII*, *-AVI*, and *-BI* preferring more alkaline conditions (Newton et al. 2007).

Lineage *acIV*

Lineage *acIV* is associated with order Acidomicrobiales and is further divided into clades *acIV-A* through *acIV-D* (Newton et al. 2011). This group can be found in freshwater, sediment, and marine environments and is most closely related to clades of marine-associated Actionobacteria (Warnecke et al. 2004). Lineage *acIV* shares uncharacteristically low G+C for Actinobacteria with *acI* (Ghai et al. 2012). This group prefers more saline conditions and can be outcompeted in abundance by other lineages such as *acI* and *acII* in freshwater conditions (Holmfeldt et al. 2009) However, *acIV* is still a persistent and abundant member of most freshwater systems (Newton et al. 2011).

Lineage *acIV* abundance has been found to be positively correlated with chlorophyll-a and DOC, suggesting a preference for productive environments (Holmfeldt et al. 2009).

PHYLUM CYANOBACTERIA

The ecological significance of Cyanobacteria has resulted in it being one of the most comprehensively-studied bacterial phyla (Huisman et al., 2005;Whitton & Potts, 2002;Whitton, 2012; Stevenson, 2014). This group uses sunlight to grow and releases oxygen as a metabolic byproduct (Rasmussen et al., 2008). Primitive ancestors of Cyanobacteria are responsible for changing the Earth's atmosphere from anoxic to oxic~2.5 billion years ago (Rasmussen et al. 2008). Today, Cyanobacteria produce 20% to 30% of the planet's oxygen (Pisciotta et al., 2010). Cyanobacteria are a critical part of the aquatic food web as they provide energy-rich organic compounds to grazers (Pisciotta et al. 2010). Many members are nitrogen fixers and contribute greatly to the global nitrogen budget (Karl et al., 2002). Other members can store phosphorus and sequester iron and other trace minerals (Paerl & Otten, 2013).

Cyanobacteria are hugely diverse and occupy nearly every type of environment on the planet (Casamatta & Hasler, 2016.). Cyanobacteria tend to be less abundant than Actinobacteria and Bacteroidetes in freshwater systems but are still a persistent presence in these habitats (Zwart et al., 2002; Portillo et al., 2012; Doherty et al., 2017), with representatives of this group occupying all compartments of freshwater from the epilithon to surface waters (Casamatta & Hasler, 2016).

This phylum is often considered a nuisance taxa despite its general ecological importance as certain members under the right conditions can proliferate and form large

blooms (Newton et al. 2011). These blooms can greatly inhibit ecosystem function by depleting oxygen, blocking out sunlight, and releasing toxins (Paerl & Otten, 2016). Generally, high temperatures, slow-moving waters, and inputs of phosphorus and nitrogen select for taxa responsible for Cyanobacteria blooms, pointing to anthropogenic influence on waterways as a cause of this issue (Paerl & Otten, 2016).

Taxonomic description of Cyanobacteria can often be difficult as members are small and often lack morphologically distinct features (Casamatta& Hasler, 2016). Some common freshwater lake genera include *Microcystis, Anabaena, Aphanizomenon, Oscillatoria, Planktothrix, Synechococcus*, and *Cyanothece*(Newton et al. 2011). Genera associated with cyanobacterial blooms include N_2-fixers *Anabaena, Aphanizomenon, Cylindrospermopsis, Lyngbya, Nodularia, Oscillatoria*, and *Trichodesmium;* and non-fixers *Microcystis and Planktothrix*(Paerl & Otten 2016). The ecology and systematics of Cyanobacteria has been more thoroughly reviewed elsewhere (Huisman et al., 2005; Stevenson, 2014; Whitton, 2012; Whitton & Potts, 2002).

PHYLUM VERRUCOMICROBIA

Phylum Verrucomicrobia was first described in 1997 by Hedlund et al. and is closely related to sister phyla Plactomycetes and Chlamydiae (He et al., 2017). This phylum is wide-spread in freshwater systems but is generally rare, with abundances typically at 1-6% of total bacterial communities (Newton et al. 2011). Verrucomicrobia appears to be diverse in ecophysiology and members have appeared in mesotrophic (Parveen et al., 2012), eutrophic (Haukka et al., 2006; Kolmonen et al., 2004), and dystrophic (He et al., 2004) lakes. Members are generally heterotrophic and can be

aerobic, facultative aerobic, and anaerobic (He et al. 2004). Verrucomicrobia appear to use carbohydrates as carbon sources (Hedlund et al., 1997), specifically polysaccharides such as exudates from algal activity (He et al., 2004). Unsurprisingly, their abundance is strongly associated with the diversity and biomass of phytoplankton (Parveen et al., 2013). Verrucomicrobia relative abundance tends to increase in humic lakes, likely due to increased amounts of organic carbon substrates (Kolmonen et al., 2004; Haukka et al., 2006; Arnds et al., 2010). Arnds et al. (2010) reported Verrucomicrobia to be the dominant group in a humic lake, accounting for 19% of the total bacterial community. This group's relationship to nutrients is not fully understood as Arnds et al. (2010) reported no relationship between Verrucomicrobia abundance and nutrient loads while Lindstrom et al. (2004) found the relative abundance of one member to increase with phosphorus concentration. Freshwater Verrucomicrobia is understudied relative to other taxa and most of its ecophysiology and phylogeny remains unknown (Newton et al. 2011; He et al. 2017)

STUDY NEED

Rivers and streams are often complex environments. Hydrology and terrestrial interactions change not only spatially from headwater to confluence, but also temporally from seasonal changes in climate (Vannote et al., 1980; Crump & Hobbie, 2005; Naiman et al., 2008). This heterogeneity leads to higher bacterial diversity in lotic systems relative to marine waters (Barberán & Casamayor, 2010). The number of studies investigating the complex dynamics between lotic microbes and their environment have increased in recent years, but uncertainty remains as to which environmental factors are most important in community dynamics (Zeglin, 2015). For example, nutrients do not

have a consistent relationship with microbial diversity across studies (Zeglin, 2015). This uncertainty may in part stem from low spatial and or temporal resolution of existing studies on lotic microbial communities. Sampling a large area over multiple time points is necessary to investigate the complex dynamics of these systems (Portillo et al., 2012). However, most studies on lotic bacteria have focused on a single river (Zeglin, 2015) and many have infrequent or one-time sampling strategies (Crump et al., 2007; Fierer et al., 2007; Wang et al., 2015; Balmonte et al., 2016; Ma et al., 2016). These studies have been valuable in identifying typical lotic taxa and local relationships between bacterial communities and their environment. However, investigating lotic bacterial communities at larger spatiotemporal scales allows for consideration of factors such as climate and geomorphology that shape the environmental factors that drive bacterial diversity.

A study over a river catchment scale with multiple time points is required to fully characterize microbial communities and their dynamics in response to environmental change. The Platte Basin in Nebraska is an opportunity to investigate these relationships. Although many studies on microbial community dynamics are conducted in temperate systems, to our knowledge no such studies have been conducted in a Great Plains system. As a Great Plains system, the Platte Basin is prone to drying and flooding, especially in its upper reaches (Dodds, 2005). Canopy cover is often low which results in lower allochthonous input relative to other temperate systems (Dodds, 2005). The western portion of the basin consists of grass-covered sand dunes and is mostly used for animal grazing. The eastern portion of the basin is grassland that has mostly been developed for row-crop agriculture. These climatic, geomorphic, and land-use factors make the Platte

Basin an interesting opportunity to study spatiotemporal patterns in bacterial diversity.

STUDY OBJECTIVES

The goal of this study was to characterize the bacterial communities of the Platte River Basin, observe how these communities changed over space and time, and to identify relationships between these communities and environmental factors. Objectives were:

1) characterize composition and structure of bacterial communities in the Platte River Basin,

2) observe any differences in bacterial composition and structure across space and time, and

3) identify relationships between bacterial taxa and their environment.

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CHAPTER 2: SPATIOTEMPORAL TRENDS IN BACTERIAL DIVERSITY ACROSS THREE WATERSHEDS IN THE PLATTE RIVER BASIN, NEBRASKA

ABSTRACT

River bacteria are understudied despite being critical components of river ecosystems. There are limited studies investigating bacterial communities at large spatiotemporal scales, which may provide insight into drivers of community assembly. We investigated differences in bacterial diversity across environmental gradients within three sub-basins in the Platte River Basin in Nebraska, USA. Surface water samples were collected weekly at 36 sites from May to September by the Nebraska Department of Environment and Energy (NDEE) in 2019. Bacterial communities were characterized by sequencing the V4 region of the 16S rRNA gene using the Illumina MiSeq platform and subsequent sequence data were used to identify amplicon sequence variants (ASVs). Sub-basins had similar counts of unique ASVs but significantly different community structures. These bacterial community differences were partially driven by environmental factors influenced by climate, land-use, and geomorphology. The Upper Loup and Central Platte sub-basins exhibited some seasonal trends in bacterial community structure on the monthly scale, but the Lower Loup sub-basin exhibited no such trends. Relative abundances of typical freshwater genera such as *Flavobacterium* contributed the most to bacterial community structural differences between sub-basins. Copiotrophic bacteria were among the most abundant across all sub-basins, suggesting that our study areas were nutrient-enriched. These results provide evidence that observing bacterial community diversity at large spatiotemporal scales can provide useful insights into the relationships between bacteria and their environment.

INTRODUCTION

Bacteria are an essential component of river ecosystems as they decompose organic material, form the base of the aquatic food web, and cycle nutrients essential to life (Mcclain et al., 2002; Peterson et al., 2001; Tank et al., 2010; Withers & Jarvie, 2008). Despite the ecological significance of river bacteria, we have a poor understanding of which environmental factors drive bacterial community diversity and function in lotic systems (Zinger et al., 2012). However, with the development of cost-effective and sensitive molecular techniques that allow for accurate descriptions of bacterial communities (Ghanbari et al., 2015), the number of studies investigating the relationship between freshwater bacterial diversity and environmental factors have increased (Givens et al., 2015). Numerous environmental factors have been implicated in driving freshwater bacterial community diversity, including temperature (Crump & Hobbie, 2005; Hullar et al., 2006), organic carbon (Doherty et al., 2017), nutrients (Crump & Hobbie, 2005; Portillo et al., 2012), hydrological factors such as flow rate (Doherty et al., 2017), and pH (Fierer et al., 2007; Figueiredo et al., 2012; Jordaan & Bezuidenhout, 2015). Temporal variation in these and other environmental factors can result in predictable patterns in bacterial community composition and structure. For example, Crump and Hobbie (2005) found the composition of bacterial communities in two similar rivers to change synchronously according to seasonal changes in temperature, flow rate, oxygen, and nitrogen. Similarly, Doherty et al. (2017) found bacterial community composition followed seasonal changes in river discharge in the Amazon River.

Factors such as land-use and geomorpholology can also influence environmental factors, which leads to differences in microbial communities across space (Ma et al., 2016; Wang et al., 2015). A study on the Mooi River in South Africa found that bacterial community richness and evenness increased downstream of an urban settlement where nutrient pollution was high(Jordaan& Bezuidenhout, 2015).

Many studies have observed relationships between bacterial communities and environmental factors, but it is still uncertain as to which factors exert significant influence (Wang et al., 2015). Zeglin (2015) found that the relationship between environmental factors (e.g., nitrogen) and lotic bacterial community diversity was not consistent across studies. This inconsistency may be attributed to the often low spatial and/or temporal resolution of studies on lotic bacterial communities. Rivers and streams, depending on their size, local climate, and terrestrial interface, can be highly variable in terms of hydrology and chemistry (Portillo et al., 2012). This degree of variability can make predicting general patterns in lotic bacterial communities difficult at low spatiotemporal resolution(Portillo et al., 2012). Sampling a river or stream over multiple time points, therefore, is critical to understanding how lotic bacteria respond and interact with their environment. However, temporal resolution of lotic microbial communities is often low, with most studies collecting one sample for analysis (Fierer et al., 2007; Ma et al., 2016) or having long time lags between sampling collection (Balmonte et al., 2016; Crump et al., 2007; Wang et al., 2015). Such investigations may provide general descriptions of bacterial communities but are less effective at describing more complex temporal dynamics. In addition to being temporally-limited, lotic bacterial community studies are often spatially limited as well and largely focus on a single river (Zeglin

2015). Assessing bacterial communities at the basin scale may assist with identifying universal drivers of bacterial community change (Zeglin 2015) and allow us to observe patterns in bacterial diversity at different levels of the river hierarchy.

Bacterial studies focusing on lotic communities with large spatiotemporal scales are necessary to better understand relationships between lotic bacterial diversity and environmental factors. Here we used a basin-wide river monitoring effort in the Platte River basin to investigate broad spatiotemporal trends in diversity within and between surface water bacterial communities. Our objectives were to 1) characterize bacterioplankton community composition and structure of three sub-basins of the Platte River Basin, 2) identify differences in bacterioplankton community diversity over space in time within these regions, and 3) identify relationships between bacterioplankton diversity and environmental factors.

METHODS

Study area

The Platte River is a braided, shallow river that forms at the confluence of the North Platte and South Platte rivers in western Nebraska and flows east across the state, where it empties into the Missouri River. Most of Nebraska has been developed for grazing or row-crop agriculture, with major urban centers clustering around the Platte River. Three sub-basins nested within the Platte River watershed were selected for investigating bacterial community composition and structure: Upper Loup sub-basin, Lower Loup sub-basin, and Central Platte sub-basin (Figure 2-1, Figure 2-2).

Sample Collection

Water samples were collected weekly at 36 sites across the three sub-basins from May to September, 2019 through a collaboration with the Nebraska Department of Environment and Energy (NDEE) (Figure 2-1). The NDEE conducts intensive annual sampling in designated basins each year to assess water quality and community health of fish and macroinvertebrates. Water samples were provided by the NDEE at each site-week combination. Only surface water was sampled. A 500ml autoclaved Nalgene bottle was filled with water from just under the water surface and its contents frozen until processing. In addition to water collection, water quality parameters were also measured at each site. Measured parameters that we used in our analyses include temperature, dissolved oxygen, Kjeldahl nitrogen, phosphate-phosphorus, total suspended solids, pH, and chloride. Information on NDEE sampling methodology and measured water quality parameters can be found in the 2018-2019 Nebraska Monitoring Programs Report published by NDEE (2020). Quantum Geographic Information System (QGIS) was used to assign a land use type to each sampling site, with land use type being either row-crop agriculture or grasslands.

Concentrating Bacteria and DNA Extraction

Samples were thawed and divided into two 250ml autoclaved bottles once in the laboratory. Each bottle was centrifuged at 24500xg for 20 minutes to pellet all bacteria present in the water sample. The water was then decanted and the resulting bacterial pellet was re-suspended in 450ul of Tris (10mM, pH 8). The resulting bacterial suspension was aliquoted into two,1.5ml autoclaved centrifuge tubes containing 0.2g of

acid-washed 500um garnet beads and stored at -80°C until processed for DNA extraction.

A Mag-Bind Stool DNA 96 Kit (Omega Bio-tek, Norcross, GA, USA) was used for the DNA extraction according to the instructions provided by the manufacturer. The cell lysis step was modified by using aTissueLyser (Qiagen Inc., Valencia, CA, USA) to physically disrupt the cell wall by bead beating the samples for 10 minutes at 30hz in addition to the lysis solution. Samples were then incubated at 90°C for 10 minutes in a water bath to further increase cell lysis.

The lysate collected after centrifugation was used for nucleic acid precipitation by adding 0.2x volume of 10M ammonium acetate and one volume of 100% isopropanol before being vortexed and incubated overnight at -80°C. The next day, tubes were centrifuged again at 16,000xg for 15 minutes at 4°C and their supernatant discarded. The nucleic acid pellets were washed with 70% ethanol chilled at -80°Cto remove residual salt and centrifuged at 13,000xg for 15 minutes at room temperature. The ethanol wash was discarded and pellets were air dried for 3 minutes before resuspending the pellet in 300μL of Tris (10mM, pH 8). A Kingfisher automated DNA purification system (Thermo Fisher Scientific, Waltham, MA) was used for further purification of the DNA according to the manufacturer's instructions.

Library Preparation and sequencing of the bacterial community

The V4 region of the 16S rDNA gene was amplified using polymerase chain reaction (PCR) and barcoded universal primers as described previously by Kozich et al. (2013). The PCR reaction volume was 25ul and contained 0.75 units of Terra PCR Direct Polymerase Mix (Takara Bio USA Inc., Mountain View, CA, USA), 1X Terra PCR Direct Buffer (Takara Bio USA Inc., Mountain View, CA, USA), 0.4uM indexed

primers, and20-50ng of Dante thermal regime was an initial denaturation step at 98.0°C

for 3 minutes followed by 30 cycles of 98°C for 30s, 55°C for 30s, 68°C for 45 seconds,

with a final extension at 68°C for 4 minutes. The quality of the PCR product was

assessed using gel electrophoresis.

The resulting PCR amplicons were normalized using a NGS Normalization 96-

Well Kit (NorgenBiotek Corp., Thorold, ON, Canada). The normalized libraries were

pooled using equal volume and were sequenced using the Illumina MiSeq platform using

a 250 bp paired-end sequencing strategy. A V2 500 Cycle sequencing kit was used for

sequencing according to the protocol provided by the manufacturer (Illumina, San Diego,

CA, USA).

16S rDNA community analysis

Sequences were processed using the DADA2 pipeline (Callahan et al., 2016)in R

(R Core Team 2019). Forward and reverse reads were trimmed where the quality of the

nucleotides dropped below a Q-score of 30. A maximum error rate for forward and

reverse reads was set to "2" to filter reads. Filtered and trimmed reads were used to

calculate error rates, which were used to infer true sequence variants. Denoised forward

and reverse reads were then merged and used to identify amplicon sequence variants

(ASVs). The resulting ASVs were further checked for chimeric sequences and such

sequences were removed. Taxonomy was assigned using the SILVA reference alignment

database (Yilmaz et al., 2014, v. 132) and a phylogenetic tree was generated from the

ASV sequences using MOTHUR (Schloss et al., 2009, v. 1.43). The resulting ASV

abundance table, ASV taxonomy table, metadata, and phylogenetic tree were combined

to create a phyloseq object using the "phyloseq" package (McMurdie & Holmes,

2013)which was used for subsequent analyses. The ASV contaminants identified in our negative controls from reagents used in extraction and amplification were removed from the phyloseq object using the"decontam" package (Davis et al., 2017). Amplicon sequence variants with a prevalence of less than 10% of all samples and a read abundance of <30 reads were removed. Rarefaction curves were generated from the samples. Finally, samples with fewer than 5000 reads were removed from the dataset using the rarefaction curve as a guideline to ensure all samples used in analyses had a robust read depth.

Analysis

Relative abundance for taxa at the phylum and genus level were calculated to characterize composition and structure of bacterioplankton communities in the Platte River Basin and the three sub-basins. Similarity Percentage (SIMPER) was used to identify ASVs most likely to contribute to differences in community structure between sub-basins and land-use factors.

Observed species richness, or number of unique ASVs, was determined for each sub-basin. A Wilcoxon Rank Sum test was used to determine differences in species richness between sub-basins. As a cursory look into longitudinal differences in species richness with increasing stream order, species richness was also calculated for each river in the study and their similarity tested using a Wilcoxon Rank Sum test.

Bray-Curtis dissimilarity was used to generate dissimilarity matrices for each sub-basin bacterial community. These matrices were used in a PERMANOVA to test if bacterial communities were different between sub-basins. These tables were also used for a pairwise PERMANOVA with Bonferroni correction to test if bacterial community

structure was different between months. These structural differences were visualized using Non-metric Multidimensional Scaling (NMDS) using the same Bray-Curtis dissimilarity matrices. Bray-Curtis matrices were also calculated for the communities of grasslands and row crop agriculture land-use types. Structural differences between bacterial communities of land-use types were also tested using PERMANOVA.

Box and whisker plots were generated using environmental parameters PO_4-P, Kjeldahl nitrogen, dissolved oxygen, total suspended solids (TSS), pH, and chloride to summarize the environmental conditions of each sub-basin. Significant difference in mean environmental parameters between sub-basins was tested for using ANOVA. Relationships between environmental variables were tested using Pearson's correlation to identify collinearity between variables that may have affected results of Canonical Correspondence Analysis (CCA).

Canonical Correspondence Analysis was used to examine the correlative relationship between bacterial communities and environmental factors. The set of environmental factors with the strongest correlation to bacterial community dissimilarity was identified for each sub-basin using the "bioenv" function in the R package "vegan" (Oksanen et al., 2020). These sets of factors along with community dissimilarity matrices were used to run the CCAs. Percent of possible variance explained by each CCA was calculated by dividing the sum of the first two eigenvalues by the sum of total eigenvalues. Spearman's rank correlation was used to observe relationships between environmental factors and differential ASVs.

RESULTS

Characterization of composition and structure of bacterial communities

Taxonomic summary of sub-basin communities

Five phyla made up 92% of ASVs across all samples: Proteobacteria (44%), Bacteroidetes (18%), Actinobacteria (14%), Cyanobacteria (13%), and Verrucomicrobia (4%) (Table 2-1). Class Betaproteobacteria was most abundant group in Proteobacteria (56%), followed by Alpha- (28%) and Gamma-proteobacteria (13%). The most abundant genus across all samples was *Flavobacterium* (Phylum Bacteroidetes) which accounted for 14% of all reads (Table 2-1). The next most abundant genera were *Polynucleobacter*(7%, Betaproteobacteria), *hgcI* clade (6%, Actinobacteria), *Limnohabitans*(5%, Betaproteobacteria), and *Simplicispira*(3%, Betaproteobacteria). Each of these genera are common chemoorganotrophs that are often native to freshwater.

The five most abundant phyla were also consistently the most abundant in each sub-basin, but sub-basins varied in the ranking of relative abundances of these groups (Table A-2, A-3, A-4). Central Platte sub-basin had highest relative abundances of autotrophic Cyanobacteria (19%) and Upper Loup had the lowest (8%). The majority of Cyanobacteria ASVs corresponded to chloroplasts and not bacteria (Upper Loup = 59%, Lower Loup = 81%, Central Platte = 79%). *Comamonadaceae* was the most abundant group at the family level across each sub-basin (Table A-2, A-3, A-4). Members of *Comamonadaceae* are often copiotrophic and like other Betaproteobacteria have been associated with areas where urbanization or nutrient input is high. The most abundant genera (>5% total ASVs) in each sub-basin were all cosmopolitan, chemoorganotrophic

groups (Table A-2, A-3, A-4). *Flavobacterium* was the most abundant group in the Upper Loup (15%) and Lower Loup (8%) sub-basins, whereas the *hgcI_*clade was the most common in Central Platte (6%). The only autotrophic genus with >1% relative abundance was *Synchococcus* (3%*),* a common member of Cyanobacteria, in Central Platte sub-basin. *Simplicispira*, a chemoorganotrophic and sometimes denitrifying group which is commonly associated with wastewater treatment and sewage, was more abundant in Upper Loup sub-basin relative to other sub-basins (5%).

<u>Taxa contributing to differences between land-use types and sub-basins</u>

We also investigated the top ASVs which together contributed 20% of the difference between grassland and row crop agriculture bacterial communities (Figure 2-4). Genus *Flavobacterium* contributed the most to differentiating land-use types (6%). Classes Flavobacteriia (Phylum Bacteroidetes) and Betaproteobacteria (Phylum Proteobacteria) contributed most overall (14%) and chloroplasts and Phylum Actinobacteria represented the rest (6%). *Flavobacterium* was the most abundant taxa identified by SIMPER in grasslands and was less abundant in row crop agriculture. Chloroplasts were the most abundant SIMPER ASVs in row crop agriculture, but their presence was negligible in grasslands. Abundances of *Comamonadaceae* (Betaproteobacteria) were similar at the family level between land-use types, but at the genus level abundances of *Alicycliphilus*, *Limnohabitans*, *Simplicispira*, and unnamed genera varied.

The top 4 ASVs differentiating Upper Loup and Lower Loup sub-basins (10%) corresponded to Genera *Flavobacterium*, *Polynucleobacter*, *Simplicispira*, and another member of *Flavobacterium* (Figure 2-5). These four ASVs were also the top ASVs

differentiating Upper Loup and Central Platte sub-basins. Upper Loup sub-basin, which consists of all grassland samples, had higher mean relative abundances of these ASVs compared to Central Platte sub-basin (row crop), but lower abundances relative to Lower Loup sub-basin (mixed land use). The top four ASVs differentiating Lower Loup and Central Platte sub-basins corresponded to two chloroplasts, a member of *Comamonadaceae*, and *Polynucleobacter*. Central Platte sub-basin had higher mean relative abundance in chloroplast ASVs relative to Lower Loup sub-basin with one exception (ASV 6) but overall lower abundance of other ASVs identified by SIMPER.

There were 8 ASVs that appeared in each sub-basin comparison: ASV-1, 2,4,5,6,9,11, and 13, which corresponded to a member of *Comamonadaceae*, *Polynucleobacter*, *Simplicispira*, *Limnohabitans*, a chloroplast, *Flavobacterium*, *Flavobacterium*, and *Flavobacterium*(Table A-6, A-7, A-8). Overall, Lower Loup sub-basin had the highest average abundance of SIMPER-identified ASVs and Central Platte sub-basin had the lowest (Figure 2-5).

Spatiotemporal differences in community richness and structure

Species richness across sub-basins

Observed species richness was similar between sub-basins. Upper Loup sub-basin had 4253 unique ASVs, Lower Loup sub-basin had 4303, and Central Platte sub-basin had 4186. Central Platte sub-basin had lower richness than Lower Loup sub-basin, but no other basin was different in terms of richness (Wilcoxon Rank Sum; $p < 0.05$). Our cursory look into longitudinal change in alpha diversity from tributaries to mainstem did not reveal any patterns as most rivers were not different in terms of species richness

according to the Wilcoxon Rank Sum test. The only two rivers that were different from each other in species richness were the Platte River and its direct tributary the Middle Loup River (p <0.05).

<u>Spatiotemporal differences in community structure</u>

Bacterial community structure was different between grassland and row crop agriculture land-use types (PERMANOVA, DF = 1, F = 15.5, P = 0.001). Bacterial community structure was also different between sub-basins (Table 2-2). Bacterial community structure changed from month to month within each sub-basin, with some exceptions observed in Upper Loup and Central Platte sub-basins (Table 2-3, 2-4, Pairwise PERMANOVA, p < 0.05). Bacterial communities were not structurally different between May and June within Upper Loup sub-basin, although all other months were different (Table 2-3). This suggests that early summer communities in the Upper Loup were distinct from later months. In Central Platte sub-basin, bacterial communities across May, June, and July were not structurally different and communities across August and September were not structurally different (Table 2-4). This suggests that in Central Platte sub-basin bacterial communities experienced a structural shift between early and late summer. No such seasonal patterns were observed in Lower Loup sub-basin as community structures differed between each month (Table 2-5). The NMDS plot for Upper Loup sub-basin visualizes the differences in May-June community structure from other months (Figure 2-6a). The Central Platte NMDS visualizes May and June community structures together but contrary to PERMANOVA results clusters July communities more closely with August and September communities (Figure 2-6b). The

NMDS for Lower Loup, similarly to PERMANOVA results, show no clear temporal trends in bacterial community structure (Figure 2-6c).

Relationships Between Bacterial Communities and Environmental Factors

Environmental characterization of sub-basins

Sub-basins differed in mean PO4-P, temperature, dissolved oxygen, pH, and total suspended solids (TSS) (One-way ANOVA, $p < 0.05$; Figure 2-3, Table 2-X). Only mean Kjeldahl nitrogen was not different between sub-basins. Upper Loup sub-basin had the lowest mean pH (7.7), TSS (79 mg/L), and PO4-P (0.27 mg/L) of the three sub-basins (t-test, $p < 0.05$). Central Platte had the highest mean temperature (22.8 °C), pH (8.2), and dissolved oxygen (8.5 mg/L) (t-test, $p < 0.05$). The Lower Loup sub-basin had a larger number of outliers across each environmental parameter relative to the other sub-basins.

Relationships between communities and environment using CCA

Dissolved oxygen, PO_4-P, Kjeldahl nitrogen, pH, chloride, and TSS were the set of variables with the strongest correlation to community dissimilarity in the Upper Loup sub-basin ($\rho = 0.37$, Table A-15). The first two axes (eigenvalues) of the Upper Loup sub-basin CCA using these environmental factors accounted for 54% of possible variation that could be explained by the model (Table 2-6, Figure 2-7a). Three main clusters of species data can be seen on the Upper Loup sub-basin CCA plot: one associated with increasing TSS and pH, one associated with increasing dissolved oxygen, and one with increasing chloride (Figure 2-7a).

The CCA for Lower Loup sub-basin used Kjeldahl nitrogen, PO$_4$-P, and pH (ρ = 0.37, Table A-15)and the first 2 axes accounted for 84% of explainable variation (Table 2-6). The largest cluster of species data was associated with increasing PO$_4$-P and Kjeldahl nitrogen (Figure 2-7b). The Central Platte sub-basin CCA used temperature, pH, and PO$_4$-P (ρ = 0.53, Table A-15) and the first 2 axes accounted for 81% of explainable variation (Table 2-6). The largest cluster was associated with decreasing temperature and the second-largest cluster was associated with increasing PO$_4$-P (Figure 2-7c).

Relationship of differential ASVs to environmental variables

We further investigated the relationships between differential ASVs and environmental factors to identify drivers of these notable taxonomic groups (Figure 2-8). No relationship between SIMPER-identified ASVs and environmental variables had a rho value >0.50 (Table A-14). The ASVs corresponding to the two most abundant genera in our study, *Flavobacterium* and *Polynucleobacter*, had a negative or no relationship to Kjeldahl nitrogen or PO$_4$-P. *Limnohabitans* had a positive relationship with PO$_4$-P but a negative relationship with Kjeldahl nitrogen. This relationship with nutrients is unusual as *Flavobacterium, Polynucleobacter, and Limnohabitans* are typically copiotrophic and have been used as indicator taxa for nutrient-enriched environments. Chloroplast ASVs had positive relationships with dissolved oxygen, pH, and temperature, which is typical for algae. All differential ASVs except for chloroplasts and *hgcI*_clade had a negative relationship with temperature and TSS. Despite this negative relationship with temperature, temperatures across all study cites rarely exceeded 30°C, which is within the upper thermal tolerance limit for common psychrophiles such as *Flavobacterium* and *Polynucleobacter*.

DISCUSSION

Composition and structure of sub-basin communities

Proteobacteria, Bacteroidetes, Actinobacteria, Cyanobacteria, and Verrucomicrobia were the most dominant phyla in each sub-basin, with some variation in relative abundance between sub-basins (Table 2-1). Other studies have found these phyla to be typical members of freshwater systems (Newton et al. 2011), with Actinobacteria, Bacteroidetes, or Proteobacteria being the most abundant depending on the system (Jordaan & Bezuidenhout, 2015; P. Wang et al., 2016; Yang et al., 2018). In our study Proteobacteria, specifically Betaproteobacteria, was the most abundant group (22% of all reads). Betaproteobacteria includes nutrient-loving, heterotrophic groups that have been found to increase in abundance in response to amendments of carbon, nitrogen, and phosphorus (Newton & Mcmahon, 2011). Family *Comamonadaceae* is one such group (Simonin et al., 2019) and the most abundant family across each sub-basin, suggesting high concentrations of nutrients such as phosphorus and nitrogen have selected for the proliferation of Betaproteobacteria in our study system.

The most abundant genera in our study were *Flavobacterium*, *Polynucleobacter*, *hgcI*, *Limnohabitans*, and *Simplicispira*(Table 2-1). The relative abundances of ASVs from these genera contributed the most to differentiating community structures of land-use types and sub-basins (Figures 2-4, 2-5). *Flavobacterium, Polynucleobacter,* and *Simplicispira* contributed the most to differentiating grassland and row crop samples and were more highly abundant in grasslands. They were also more abundant in Upper Loup

(grasslands) and Lower Loup (mixed land-use) sub-basins relative to Central Platte (row crop) sub-basin. *Flavobacterium*, *Polynucleobacter*, and *Simplicispira* are heterotrophic degraders of organic carbon and in this study had negative correlations to dissolved oxygen, temperature, Kjeldahl nitrogen, PO_4-P, pH and TSS (Figure 2-8). The negative association with pH and dissolved oxygen may indicate high heterotrophic activity where these organisms are abundant. Heterotrophic respiration consumes oxygen and releases CO_2, which reacts with water to form carbonic acid that reduces pH. The high abundance of *Simplicispira* in grasslands is notable as most members of this group are nitrogen reducers that were first isolated from sewage sludge (Cho et al., 2018; Grabovich et al., 2006; Lu et al., 2007; Zubair et al., 2019). This suggests that grassland samples have large amounts of nitrogenous waste, possibly due to cattle grazing.

Chloroplast ASVs, which may have been derived from autotrophic Cyanobacteria or algae, were also important in differentiating land-use types and sub-basins (Figures 2-4, 2-5). Their abundance was higher in row crop agriculture samples than grassland samples and higher in Central Platte and Lower Loup sub-basins than the Upper Loup sub-basin. Chloroplasts identified had positive correlations to temperature, dissolved oxygen, and pH but no relationship with nutrients or TSS (Figure 2-8). The positive association of chloroplasts with dissolved oxygen, pH, and temperature suggest that they were isolated from areas of high primary productivity as warmer temperatures can select for higher abundances of certain photosynthetic taxa. These organisms take up CO2 as part of photosynthesis, thereby increasing water pH, and release oxygen as a byproduct.

Spatiotemporal differences in bacterial richness and structure

Bacterial communities can experience temporal shifts in composition and structure across an annual cycle (Crump et al., 2007; Doherty et al, 2017; Fortunato et al., 2012; Hullar et al., 2006; Ma et al., 2016; Wang et al., 2016). These shifts can follow seasonal changes in temperature (Crump &Hobbie, 2005; Ma et al. 2016), flow rate (Crump &Hobbie, 2005; Doherty et al., 2017; Fortunato et al., 2012), dissolved or particulate organic matter (Hullar et al., 2006; Doherty et al., 2017), and nutrient levels (Crump &Hobbie, 2005). We observed some seasonality affecting bacterial communities in the Upper Loup and Central Platte sub-basins (Tables 2-3, 2-4). In the Upper Loup sub-basin, bacterial communities in samples collected in the months of May through June had similar structures (Table 2-3) and in Central Platte sub-basin relatively persistent communities formed May-July and August-September (Table 2-4). These results are similar to those in a study on the Columbia River which found bacterial community compositional change corresponded to maximum discharge rates in the spring and lower discharge rates in the summer and fall (Fortunato et al., 2013). Similarly, Doherty et al. (2017) reported river discharge as a master variable in governing bacterial change in the Amazon River where discharge influenced temperature, fluxes in nutrients and organic matter, turbidity, and residence time. Unfortunately, discharge rates for our sample sites are unavailable. However, water velocity averaged across all sub-basins was similar in May and June, which may correspond to the structural similarities of early summer communities for Upper Loup and Central Platte sub-basins (Figure A-1). It should be noted that in March 2019 the Platte Basin experienced flooding that resulted in

persistently high discharge rates into the late summer (Figure A-2). Thus, temporal

dynamics in bacterial communities for 2019 may be different from those of other years

due to increased erosion, lower temperatures, and shorter river residence times from

increased discharge (Liu et al., 2018; Wang et al., 2016).

Seasonal patterns observed in the community structures of Central Platte and

Upper Loup sub-basins were not seen in Lower Loup sub-basin as all months were

different from each other (Table 2-5). This temporal variability may be partially

explained by the sizes of rivers sampled in the Lower Loup sub-basin, as the Lower Loup

has the greatest number of low-order streams. Streams with lower water volumes relative

to larger streams and rivers are more vulnerable to environmental change (Resh et al.,

1988). Bacterial communities in small streams may then undergo frequent structural

shifts but not follow clear seasonal patterns due to the stochasticity of their habitat. This

variability was observed by Portillo et al. (2012) who found no seasonal patterns in

microbial communities of several small streams. Conversely, Central Platte sub-basin had

the largest river in the study (the Platte River) and most obvious seasonality (Table 2-5).

Inputs like sediment, groundwater, and organic matter become less significant as water

volume increases (Savio et al., 2015; Vannote et al., 1980). Instead, larger rivers receive

most organic material and bacteria from upstream and tributaries (Savio et al., 2015).

Reduced terrestrial interactions and the buffering effect of a large water volume in larger

rivers may result in more gradual seasonal shifts in bacterial community diversity relative

to that of small streams, which are more susceptible to stochastic events such as flooding

and drying.

While species richness was similar between sub-basins, each sub-basin had a

unique community structure (Table 2-2). Other studies on lotic environments have found differences between bacterial community diversity in space, mostly at the within-river scale. Geographic variation in factors such as land-use (Jordaan & Bezuidenhout, 2015; Wang et al., 2018), land geomorphology (Liu et al., 2018), stream-order (Portillo et al., 2012), and river-groundwater interfaces (Crump et al., 2012; Savio et al., 2015) can influence factors that cause bacterial community variation. Our study further supports the existence of relationships between geographic factors and bacterial communities, as bacterial community structure was different between row crop and grassland land-use types (Table A-9). A major potential difference between sampled land-use types may be nutrient concentration as Central Platte sub-basin (row-crop) had the highest mean value of PO_4-P and Upper Loup sub-basin (grassland) had the lowest mean value of PO_4-P (Figure 2-3, Table A-12). Other geomorphologic differences besides land-use type may also contribute to structural differences observed between sub-basins. For example, Upper Loup sub-basin rivers are mostly ground-fed, which may result in more allocthonous sources of bacteria.

Upper Loup, Lower Loup, and Central Platte sub-basins are not independent systems despite their differences in bacterial community diversity and geography. Upper Loup sub-basin drains into the Lower Loup sub-basin. Subsequently, the Lower Loup sub-basin ultimately drains into the Platte River of Central Platte sub-basin, although most samples were taken upstream of this confluence. The connection between sub-basins interested us in seeing if longitudinal patterns in bacterial diversity existed from tributaries to the Platte River mainstem. There appeared to be no longitudinal pattern in species richness in our study as most sampled rivers did not differ in species richness

regardless of sub-basin. The only significantly different comparison was that the Platte River had slightly lower richness than Middle Loup River. A possible explanation for this lower diversity is that the buffering effect of the Platte River's water volume on environmental factors results more homogenized environmental conditions that select for taxa.

Relationships between bacterial communities and environmental factors

Increases in nutrients have been tied to increased bacterial abundance and diversity in other systems (Zeglin, 2015). Jordaan& Bezuidenhout (2015) found that bacterial richness and evenness increased downstream of an urban settlement where nutrient inputs were high. Our results suggest that not all bacteria responded positively to increasing nutrient load (PO_4-P and Kjeldahl nitrogen). For example, numbers of bacteria in Lower Loup and Central Platte sub-basins were associated with decreasing nutrient concentrations (Figure 2-7b, 2-7c). Furthermore, all differential heterotrophic bacteria identified by SIMPER had negative or no relationship with nutrients despite several of these genera being characterized as copiotrophic (*Flavobacterium, Polynucleobacter,* and *Limnohabitans*) (Figure 2-8). This antagonistic relationship with nutrients has also been observed by Eiler et al. (2012), who found that closely related groups within Betaproteobacteria had negative relationships with nutrients due to competition between species for a similar niche. Nutrients may have positively affected the proliferation of these copiotrophic taxa which then led to competition between or within these groups, resulting in the observed negative association with nutrients. Other differential ASVs such as those corresponding to chloroplasts from algae had no relationship to nutrients

(Figure 2-8). An explanation for the lack of relationship between certain ASVs and nutrients is that nutrients in the Platte River Basin are likely not limiting. Therefore, minor fluctuations in nutrient concentrations may not result in a response from certain taxa (Johnson et al., 2009; Reisinger et al., 2016). This effect has been observed in lotic biofilms with nearby urban or agricultural development (Johnson et al., 2009). We propose that nutrient input from widespread agricultural development in the Platte River Basin may be resulting in atypical responses from common, nutrient-loving groups. However, nutrients still had a positive relationship with a large number of other ASVs in Lower Loup and Central Platte basins.

Nutrients did not have as much influence on bacterial communities in the Upper Loup sub-basin compared to the other two sub-basins (Figure 2-7a). The two key drivers in this system appear to be TSS and chloride. Total suspended solids can influence bacterial community composition as they can include organic substrates required for heterotrophic growth (Tang et al., 2009). Tang et al., (2009) found that bacterial abundance was correlated to TSS and that the organic matter within the TSS was dominated by heterotrophs. Bacteria in our study associated with increasing TSS were also associated with decreasing chloride, suggesting that these taxa are heterotrophic with lower salinity tolerance such as Betaproteobacteria (Nold& Zwart, 1996). High loads of TSS may also increase the amount and variety of particle habitat for particle-associated bacteria to grow on. The composition and structure of particle-associated bacterial communities has been found to be distinct from that of free-living bacterial communities (Rieck et al., 2015). Therefore, some of the ASVs which increase in abundance with TSS load may be particle-associated bacteria. Communities associated with increasing

chloride were also associated with increasing PO4-P and pH, two variables associated with autotrophic growth (Eiler et al., 2012). Chloride-favoring communities may then be autotrophs or associated with autotrophic activity.

Temperature, along with nutrients, is another factor associated with increased bacterial abundance in other studies (Liu et al. 2018). While some Central Platte and Lower Loup sub-basin communities responded positively to temperature, others did not (Figure 2-7b, 2-7c). Temperatures within the sub-basins never exceeded the optimal growth range for common psychrophillic taxa such as *Limnohabitans* (~30°C , Figure 2-6). As such, we propose that the negative relationship observed between temperature and these taxa may be related to increased growth rates from warm temperatures that increases competition within similar niches (Mayo & Noike, 1996).

CONCLUSIONS

If we are to understand river bacterial diversity and its drivers, it is necessary to consider bacterial diversity as a function of its total environment. Rivers are dynamic systems which experience shifts in hydrology, terrestrial interactions, and biological processes down the river continuum. Investigating how river bacteria communities respond to such environmental gradients can provide insights into river bacterial ecology as well as overall river ecosystem health and function. We found large scale spatial and temporal differences in bacterial community structure between three sub-basins within the Platte River Basin, Nebraska. Structural differences between sub-basins appeared influenced by environmental factors which in turn may be affected by climate, land-use, and geomorphology. The relative abundances of common freshwater bacterial taxa such as *Flavobacterium* and *Polynucleobacter* contributed the most to structural differences in

bacterial communities between sub-basins and land-use types. High abundances of copiotrophic bacteria suggest that our study areas are nutrient enriched. Overall, the trends in bacterial diversity observed in this study help establish the ecological relevance of investigating bacterial diversity at large scales.

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Table 2-1. Left: Phyla contributing 99% of total bacteria across all three sub-basins.

Right: Genera contributing >1% of total bacteria across all three sub-basins.

Phylum	Count	% All	Genus	Count	% All
Proteobacteria	4845859	44	Flavobacterium	1012967	14
Bacteroidetes	2021614	18	Polynucleobacter	482732	6
Actinobacteria	1554861	14	hgcI_clade	458795	6
Cyanobacteria	1410007	13	Limnohabitans	351041	5
Verrucomicrobia	455635	4	Simplicispira	254481	3
Acidobacteria	187600	2	Pseudarcicella	242002	3
Planctomycetes	153345	1	Candidatus_Rhodoluna	187262	3
Firmicutes	100179	1	Rhodobacter	163901	2
Chloroflexi	81039	1	Novosphingobium	145037	2
Gemmatimonadetes	40044	<1	Synechococcus	138648	2
Armatimonadetes	35677	<1	Sphingomonas	120970	2
			CL500-29_marine_group	118639	2
Euryarchaeota	35008	<1	Hydrogenophaga	113233	2
Nitrospirae	32308	<1	Fluviicola	103549	1
Parcubacteria	30413	<1	Alpinimonas	96547	<1
Thaumarchaeota	29124	<1	Luteolibacter	96506	<1
			Sediminibacterium	96491	<1
			Massilia	94291	<1
			Arenimonas	93282	<1
			Candidatus_Planktophila	88196	<1
			Pseudomonas	86766	<1
			Roseomonas	84711	<1
			Candidatus_Planktoluna	84207	<1
			12up	78315	<1
			Methylotenera	76312	<1

Table 2-2. PERMANOVA testing if bacterial communities are different between sub-basins, between rivers, and between sampling sites. Bray-Curtis distance was used to calculate community dissimilarities. PERMANOVA was nested to account for non-independence between factors. Difference is considered significant if Pr<0.05.

Spatial Scale	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
Sub-basin	**2**	**7.801**	**3.900**	**17.058**	**0.062**	**0.001**
River	18	23.751	1.319	5.770	0.188	0.001
Sampling Site	15	7.472	0.498	2.179	0.059	0.001
Residuals	382	87.349	0.229	NA	0.691	
Total	417	126.373	NA	NA	1.000	

Table 2-3. Pairwise PERMANOVA comparing bacterial communities between months in the Upper Loup sub-basin. Bray-Curtis distance was used to create community dissimilarity matrices. The community compositions between two months were considered different if $p < 0.05$. Bonferroni corrections are accounted for in the adjusted p-value.

Pairs	Df	SumsOfSqs	F.Model	R2	p.value	p.adjusted
May vs. June	1	0.472	2.97	0.114	0.012	**0.12**
May vs. July	1	1.392	6.102	0.135	0.001	**0.01**
May vs. August	1	1.358	5.722	0.156	0.001	**0.01**
May vs. September	1	1.437	7.486	0.333	0.001	**0.01**
June vs. July	1	1.669	7.8	0.157	0.001	**0.01**
June vs. August	1	1.568	7.157	0.174	0.001	**0.01**
June vs. September	1	1.721	10.417	0.367	0.001	**0.01**
July vs. August	1	0.59	2.323	0.044	0.01	**0.1**
July vs. September	1	1.539	6.373	0.158	0.001	**0.01**
August vs. September	1	1.111	4.327	0.143	0.001	**0.01**

Table 2-4. Pairwise PERMANOVA comparing bacterial communities between months in the Central Platte sub-basin. Bray-Curtis distance was used to create community dissimilarity matrices. The community compositions between two months were considered different if $p < 0.05$. Bonferroni corrections are accounted for in the adjusted p-value.

Pairs	Df	SumsOfSqs	F.Model	R2	p.value	p.adjusted
May vs. June	1	0.598	2.239	0.096	0.018	**0.270**
May vs. July	1	0.751	2.432	0.104	0.009	**0.135**
May vs. September	1	0.945	3.403	0.120	0.001	**0.015**
May vs. August	1	0.849	2.946	0.148	0.001	**0.015**
June vs. July	1	0.760	2.813	0.086	0.005	**0.075**
June vs. September	1	1.087	4.319	0.113	0.001	**0.015**
June vs. August	1	0.965	3.847	0.129	0.001	**0.015**
July vs. September	1	0.777	2.803	0.076	0.005	**0.075**
July vs. August	1	0.423	1.489	0.054	0.106	**1.000**
September vs. August	1	0.508	1.941	0.061	0.051	**0.765**

Table 2-5. Pairwise PERMANOVA comparing bacterial communities between months in the Lower Loup sub-basin. Bray-Curtis distance was used to create community dissimilarity matrices. The community compositions between two months were considered different if $p < 0.05$. Bonferroni corrections are accounted for in the adjusted p-value.

Pairs	Df	SumsOfSqs	F.Model	R2	p.value	p.adjusted
May vs. June	1	1.004	4.390	0.055	0.001	**0.015**
May vs. July	1	2.735	10.193	0.088	0.001	**0.015**
May vs. August	1	2.615	10.311	0.118	0.001	**0.015**
May vs. September	1	2.816	10.638	0.103	0.001	**0.015**
June vs. July	1	1.837	7.232	0.056	0.001	**0.015**
June vs. August	1	2.407	10.137	0.098	0.001	**0.015**
June vs. September	1	2.156	8.649	0.074	0.001	**0.015**
July vs. August	1	1.279	4.748	0.037	0.001	**0.015**
July vs. September	1	1.597	5.805	0.040	0.001	**0.015**
August vs. September	1	1.707	6.402	0.055	0.001	**0.015**

Table 2-6. Summary statistics for CCAs run on each sub-basin. Environmental variables used in CCA were determined using the "bioenv" function in R package "vegan". Eigenvalues > 0.3 were considered indicative of a strong gradient (terBraak&Verdonschot, 1995).

	Upper Loup	Lower Loup	Central Platte
# Samples	79	268	71
# Environmental variables	6	3	3
Species x Environment Correlations	0.372	0.38	0.526
Total Inertia	5.57	8.74	6.83
Constrained Inertia	1.29	0.366	0.936
Eigenvalues - CCA1 (CCA2)	0.377(0.317)	0.202 (0.128)	0.435 (0.325)
Sum All Eigenvalues	1.28	0.366	0.936
% Variance			
Total Inertia	12.5	3.51	11.1
Species x Environment	54.2	83.9	81.2
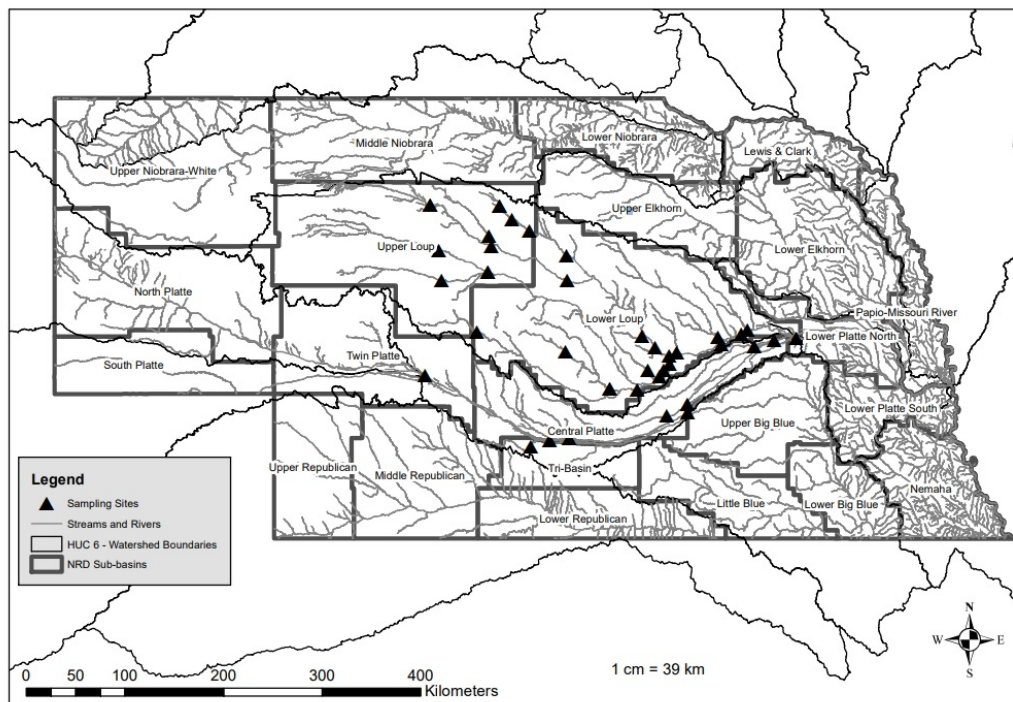
Figure 2-1. Map depicting sampling locations across three sub-basins in the Platte River Basin, Nebraska. Surface samples were obtained weekly from May to September,2019. Thick gray lines delineate sub-basins. Upper Loup, Lower Loup, and Central Platte were the sub-basins measured in this study.
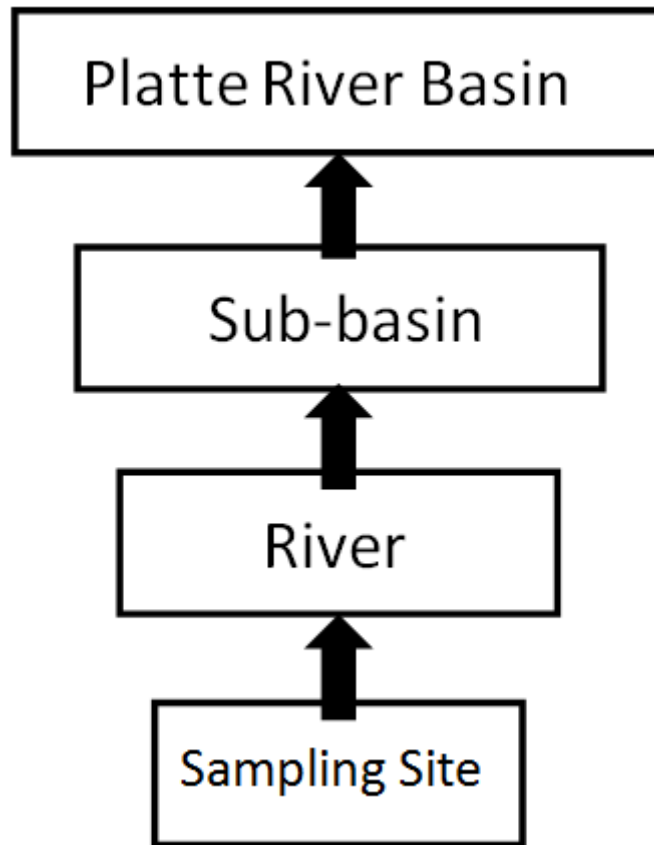
Figure 2-2. Flow chart showing structure of spatial scales. Sampling sites were nested within rivers, rivers nested within sub-basins, and sub-basins nested within the Platte River Basin.
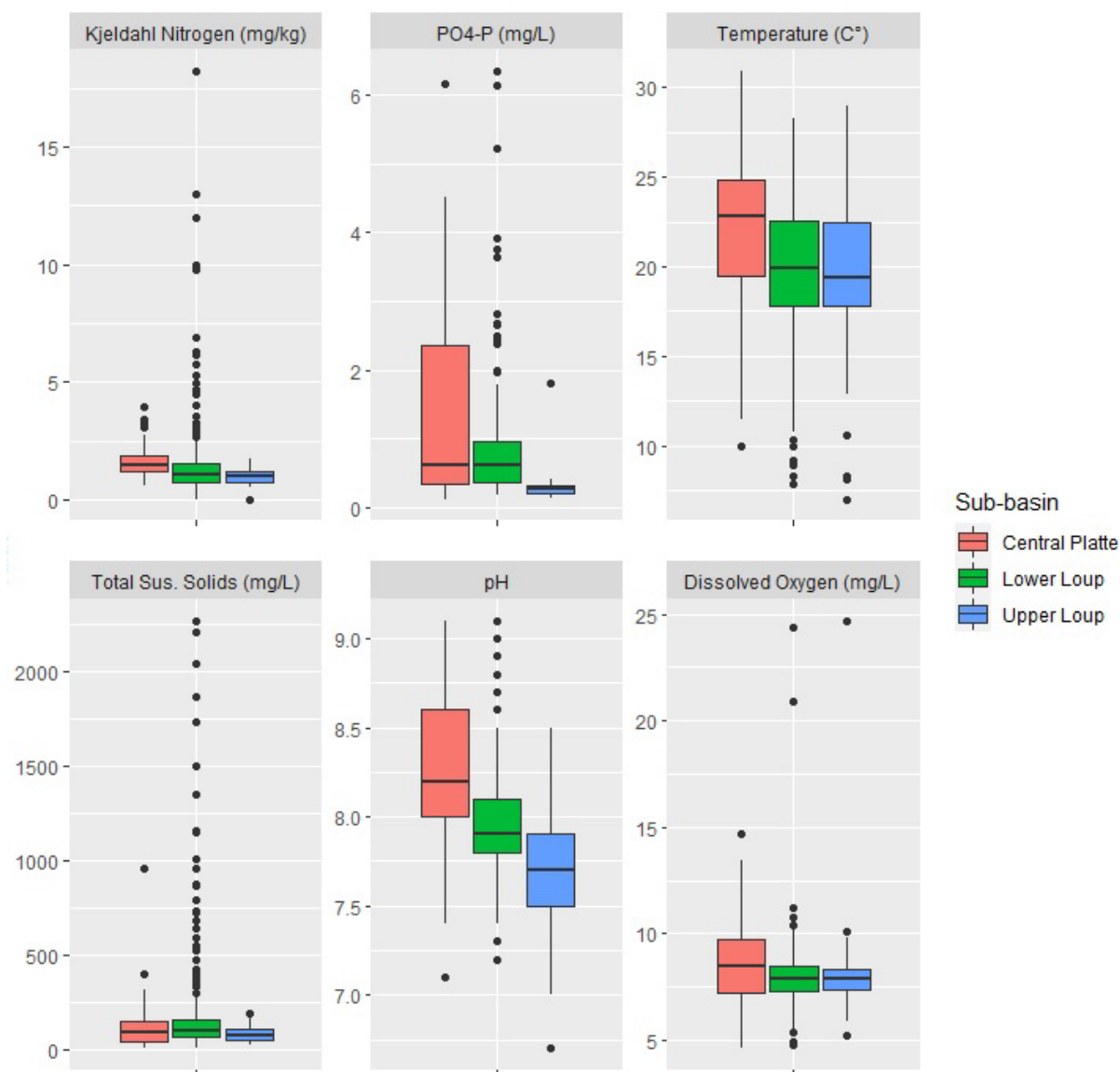
Figure 2-3.Box and whisker plots summarizing environmental parameters within each sub-basin. Environmental data were collected from May 2019 to September 2019. Sampled parameters were Kjeldahl nitrogen, phosphate-phosphorus, temperature, total suspended solids, pH, and dissolved oxygen.
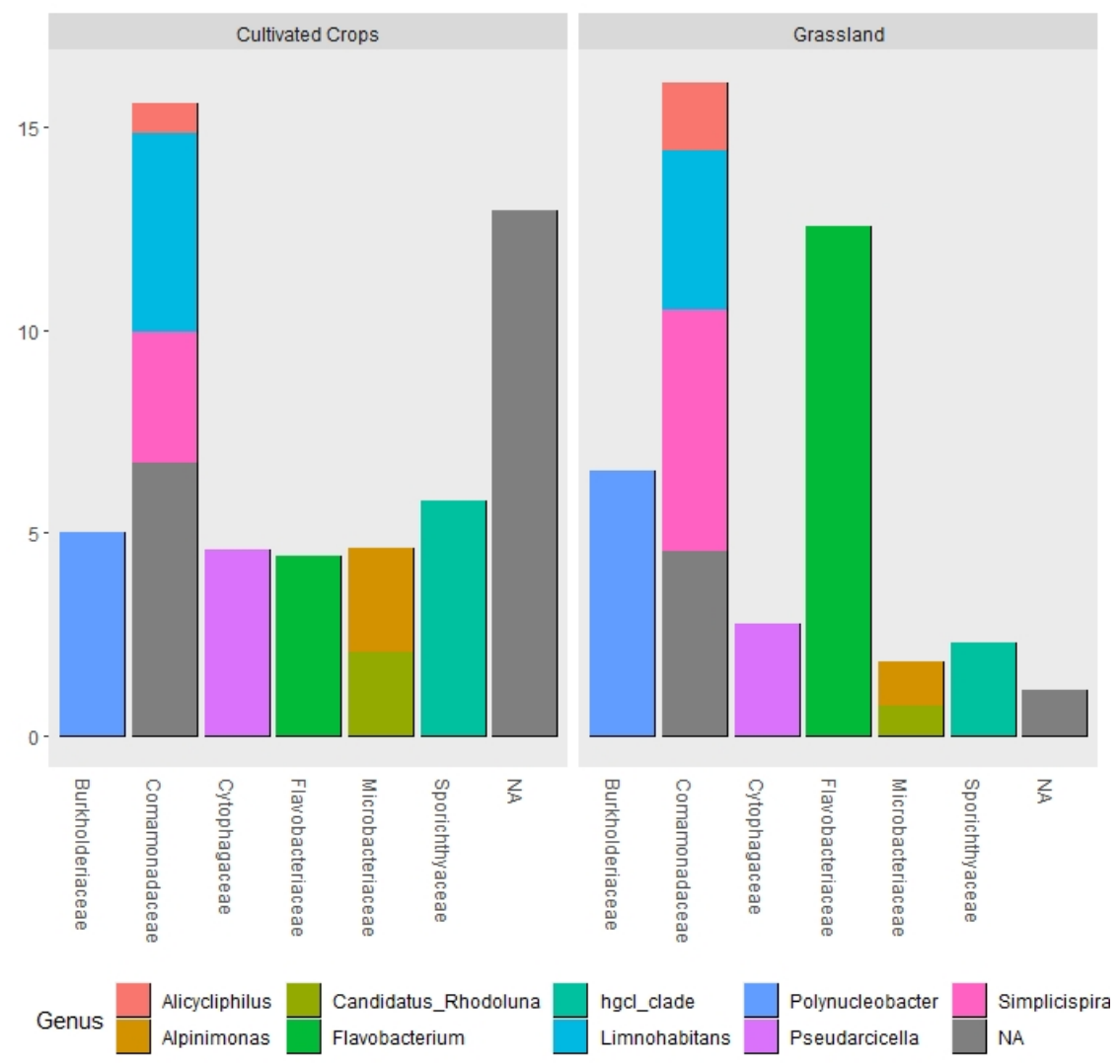
Figure 2-4. Normalized abundances of ASVs identified by SIMPER as important in differentiating land-use types and sub-basins, broken down by land-use type(i.e., row agriculture and grassland). Bars are families faceted by genus. Group labeled as "NA" corresponds to chloroplasts ASVs.
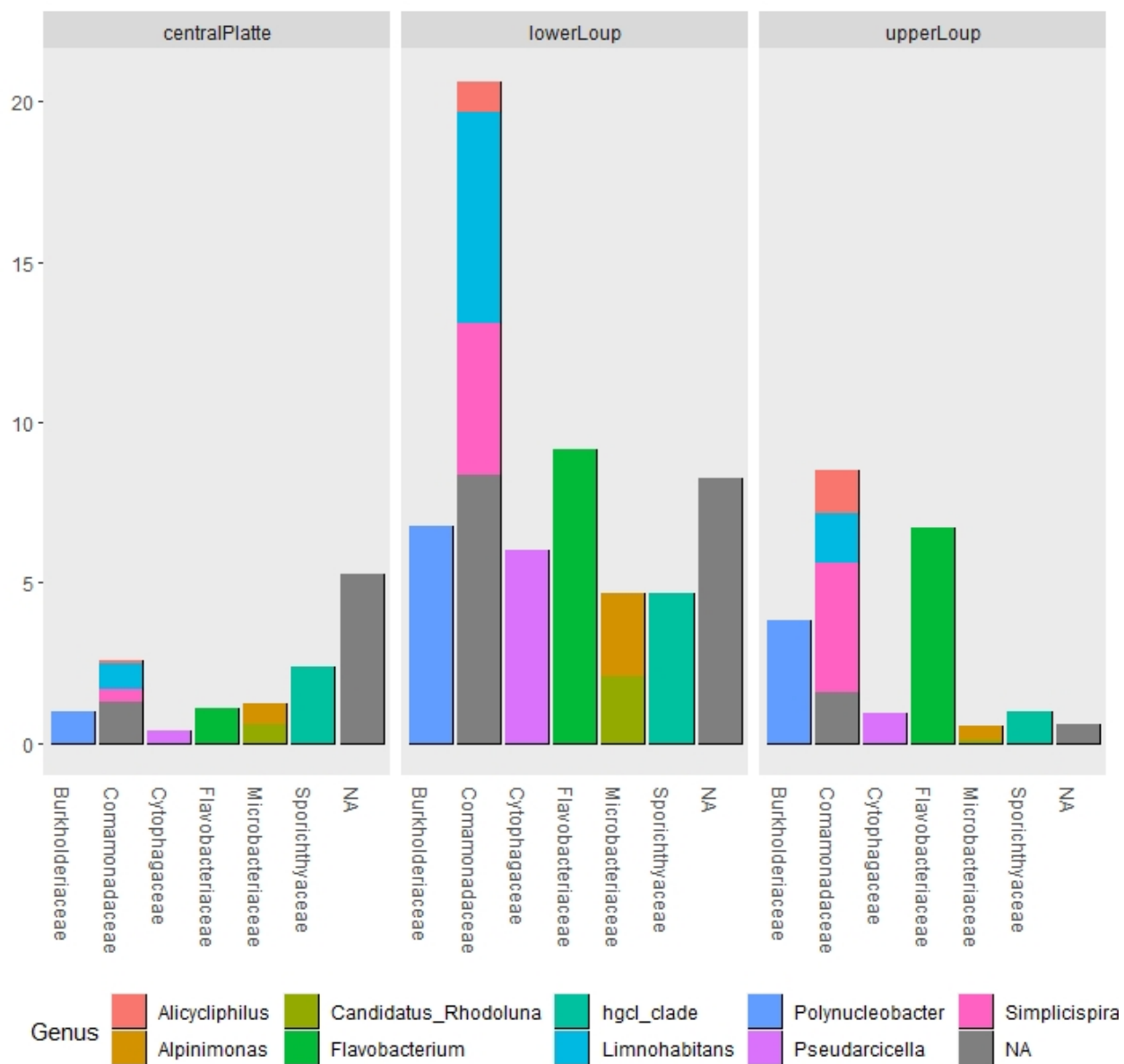
Figure 2-5. Bar plot showing normalized abundances of ASVs identified by SIMPER as important in differentiating sub-basins and land-use types, broken down by sub-basin. Bars are families faceted by genus. Group labeled as "NA" corresponds to chloroplast ASVs.
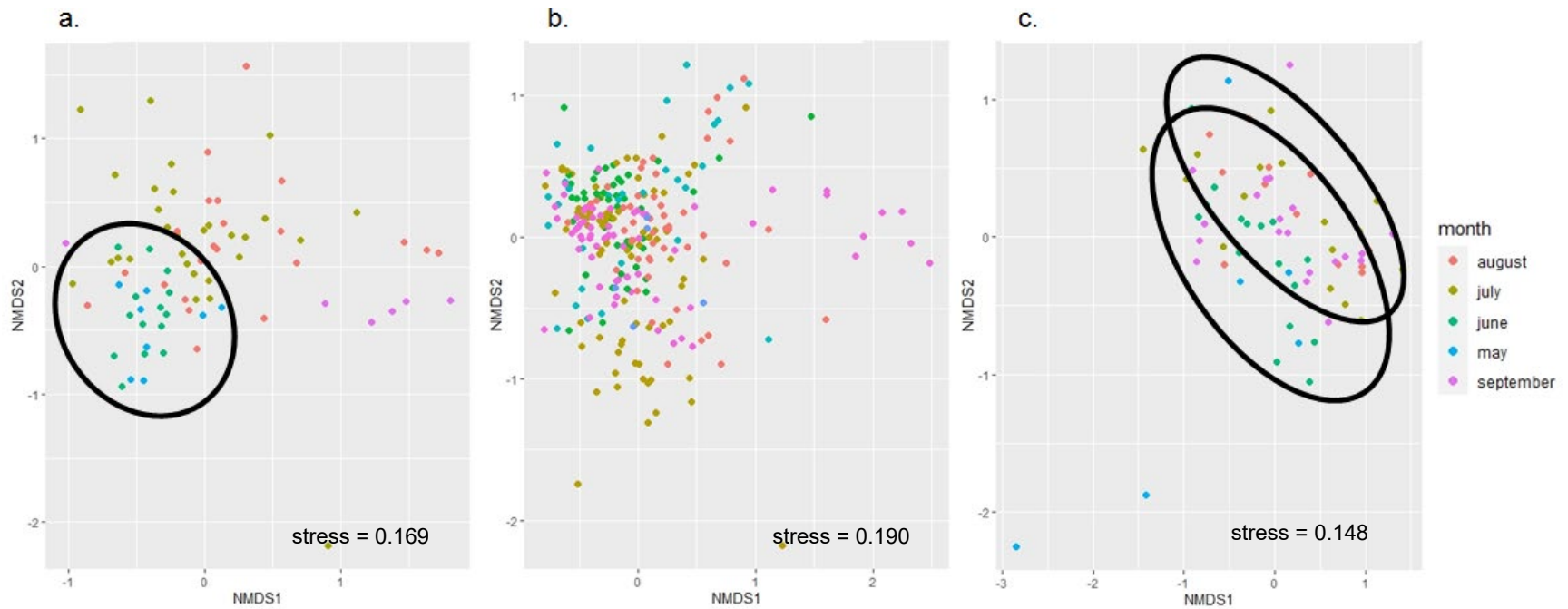
Figure 2-6. Visualization of community structure for a) Upper Loup sub-basin, b) Lower Loup sub-basin, and c) Central Platte sub-basin using non-metric multidimensional scaling (NMDS). Plots were generated using sub-basin Bray-Curtis dissimilarity matrices. The ASVs are colored by month. Circles indicate periods where bacterial communities were similar in structure according to PERMANOVA. Upper Loup sub-basin communities were similar in structure during the months of May and June. Central Platte sub-basin communities were similar May through July and August through September. Communities varied month to month in Lower Loup sub-basin.
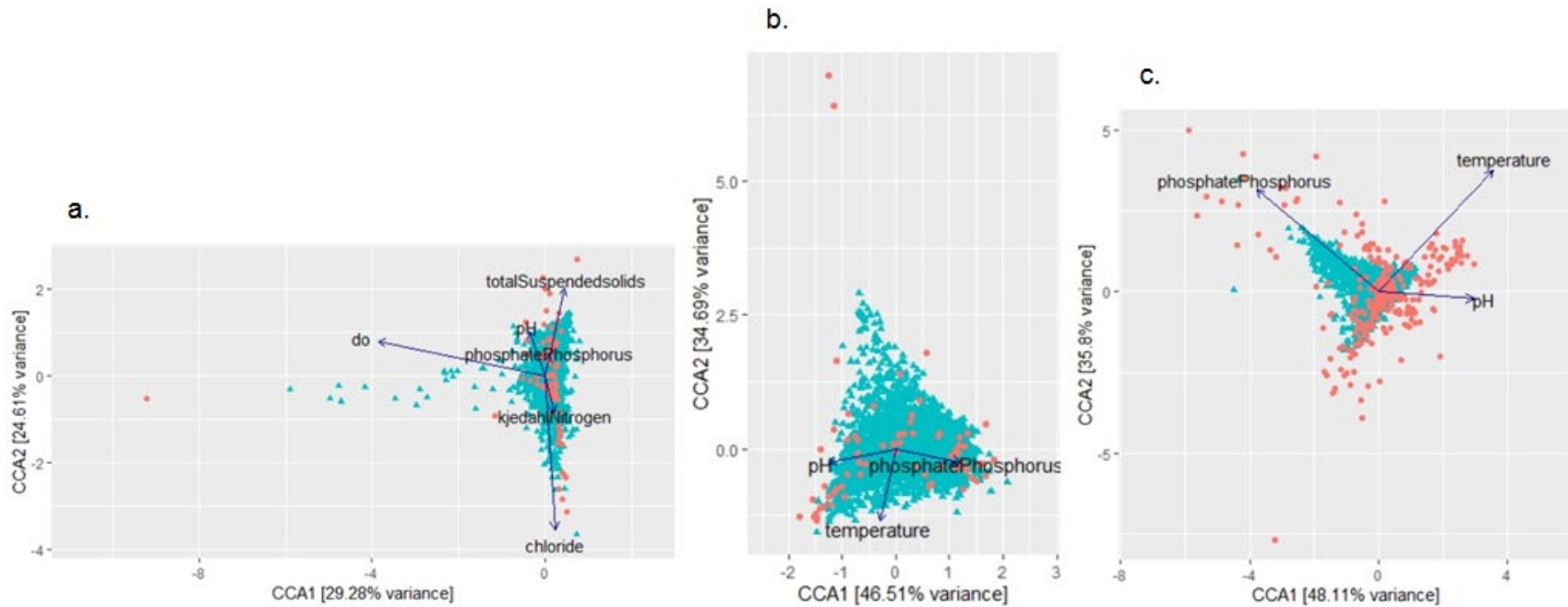
Figure 2-7. Relationships between bacterial community structures and environmental factors for a) Upper Loup sub-basin, b) Lower Loup sub-basin, and c) Central Platte sub-basin using canonical correspondence analysis (CCA). Environmental factors used in each CCA were selected using the "bioenv" package for "vegan" in R. Blue triangles correspond to ASV abundances (species) and red circles correspond to environmental scores (sites). Biplot arrows represent environmental factors with arrowheads indicating the direction of increase of value. A perpendicular projection of an ASV species score onto an environmental biplot arrow allows us to infer the relationship between that ASV's abundance and selected environmental factor
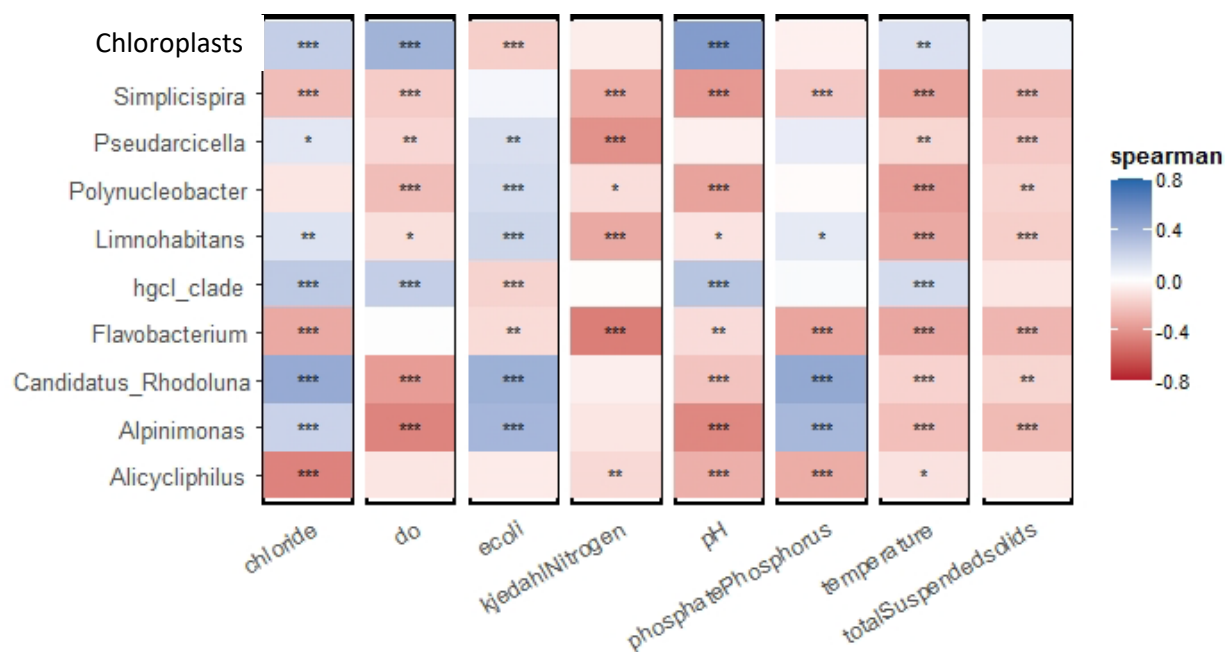
Figure 2-8. Heat map showing Spearman rank correlation values between ASVs identified by a SIMPER test as important in differentiating 1) land-use types and 2) sub-basins. Red color indicates a negative relationship, blue color indicates positive relationship, and white indicates no relationship. Asterisk(s) indicate where a relationship is significant (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$).

APPENDIX
Supplemental tables and figures referring to Chapter 2

Table A-1.Latitude, longitude, and IDs for all stations sampled weekly by the NDEE from May
to September 2019.

Station ID	Latitude	Longitude	Station ID	Latitude	Longitude
SLO4MUDCR259	41.29223	-99.39479	SLO4SLOUP405	41.42505	-100.20263
SLO2NLOUP225	41.77686	-99.3797	SMP2WHORS109	41.13331	-100.67674
SLO2CALAM108	41.94686	-99.38639	SMP2PLUMC033	40.64155	-99.71069
SLO3DSMLR225	41.77876	-100.5253	SLO3OAKCR209	41.16478	-98.64315
SMP2BUFCR102	40.69724	-99.35985	SLO3TRKEY122	41.16884	-98.49993
SLO4SLOUP135	41.03213	-98.74043	SLO2CALAM301	42.18723	-99.88641
SLO4MUDCR133	41.03795	-98.99283	SMP1CLEAR116	41.38004	-97.29289
SLO3MLOUP128	41.20346	-98.44603	SMP2PLATT245	40.68248	-99.54048
SLO2MUNSN104	41.32262	-98.57862	SMP2WOODR187	40.93462	-98.28264
SLO2DAVSC120	41.40012	-98.69837	SMP2PLATT133	40.87397	-98.28215
SLO2CALAM210	42.11249	-99.7224	SLO3OAKCR116	41.1216	-98.53872
SLO2GOOSE129	42.07554	-100.09154	SMP2WOODR225	40.85446	-98.47417
SLO2NLOUP304	42.0089	-100.07301	SLO1SPRNG112	41.28557	-98.37895
SLO2NLOUP401	42.28642	-100.62581	SLO2NLOUP105	41.26415	-98.44966
SLO3MLOUP707	41.97972	-100.55007	SLO2CALAM401	42.27708	-99.99639
SLO1CEDAR109	41.39466	-98.00395	SLO3MLOUP105	41.83101	-100.10082
SLO1LOUPR330	41.34496	-97.97536	SLO2CALAM401	42.28642	-100.62581
SLO1LOUPC150	41.40932	-97.79404	SLO2NLOUP105	41.28557	-98.37895
SLO1BEAVR114	41.4422	-97.73648	SLO2NLOUP401	42.27708	-99.99639
SMP1PRAIR116	41.32907	-97.67539	SLO2NLOUP105	41.83101	-100.10082
SMP1PLATT225	41.36793	-97.49489	SLO4MUDCR259	41.03795	-98.99283

Table A-2: Phyla, families and genera that made up >1% of total bacteria in the Upper Loup sub-basin.

Phyla	Count	% Sub-basin	Family	Count	% Sub-basin	Genus	Count	% Sub-basin
Proteobacteria	961989	45	Comamonadaceae	354071	17	Flavobacterium	323444	21
Bacteroidetes	457136	22	Flavobacteriaceae	325069	15	Polynucleobacter	146108	10
Actinobacteria	263433	12	Burkholderiaceae	147304	7	Simplicispira	114765	7
Cyanobacteria	164499	8	Microbacteriaceae	85073	4	hgcI_clade	64026	4
Verrucomicrobia	90628	4	Sporichthyaceae	80969	4	Limnohabitans	56711	4
Acidobacteria	40980	2	FamilyI	66445	3	12up	44234	3
Planctomycetes	37182	2	Rhodocyclaceae	52667	2	Alicycliphilus	36603	2
Chloroflexi	21309	1	Sphingomonadaceae	46130	2	Pseudarcicella	27912	2
			Cytophagaceae	36661	2	Roseomonas	26935	2
			Planctomycetaceae	35087	2	Novosphingobium	22332	1
			Verrucomicrobiaceae	34878	2	Pseudomonas	21861	1
			Rhodobacteraceae	34040	2	Candidatus_Rhodoluna	21356	1
			Chitinophagaceae	33679	2	Massilia	20868	1
			Acetobacteraceae	28933	1	CL500-29_marine_group	18492	1
			MNG7	28453	1	Fluviicola	18405	1
			Oxalobacteraceae	25547	1	Synechococcus	17108	1
			Acidimicrobiaceae	23435	1	Rhodobacter	16837	1
			LD29	22966	1	Sediminibacterium	16108	1
			Pseudomonadaceae	21882	1	Alpinimonas	15735	1
						Cyanobium	15657	1

Table A-3: Phyla, families and genera that made up >1% total bacteria in Lower Loup sub-basin.

Phyla	Count	%Sub-basin
Proteobacteria	3107388	44
Bacteroidetes	1307594	19
Actinobacteria	975930	14
Cyanobacteria	900064	13
Verrucomicrobia	287007	4
Acidobacteria	115415	2
Planctomycetes	77090	1

Family	Count	%Sub-basin
Comamonadaceae	1111386	16
Flavobacteriaceae	608385	9
Microbacteriaceae	399020	6
Sporichthyaceae	338121	5
Burkholderiaceae	289752	4
Cytophagaceae	259395	4
Sphingomonadaceae	255893	4
Rhodobacteraceae	205366	3
Verrucomicrobiaceae	192867	3
Chitinophagaceae	179716	3
FamilyI	169174	2
Methylophilaceae	104275	1
Xanthomonadaceae	100735	1
MNG7	87272	1
Oxalobacteraceae	83176	1
Cryomorphaceae	77702	1
Acidimicrobiaceae	74077	1
Planctomycetaceae	71164	1

Genus	Count	%Sub-basin
Flavobacterium	593310	8
Polynucleobacter	282177	4
hgcI_clade	279641	4
Limnohabitans	257765	4
Pseudarcicella	197153	3
Candidatus_Rhodoluna	142696	2
Simplicispira	125919	2
Rhodobacter	114410	2
Novosphingobium	101097	1
Sphingomonas	89643	1
Hydrogenophaga	82591	1
Fluviicola	73809	1
Sediminibacterium	71041	1

Table A-4: Phyla, families and genera that made up >1% or more of total bacteria in Central Platte sub-basin.

Phylum	Count	%Sub-basin
Proteobacteria	678039	39
Cyanobacteria	338179	19
Actinobacteria	288976	17
Bacteroidetes	232023	13
Verrucomicrobia	69208	4
Planctomycetes	35902	2
Firmicutes	25265	2
Acidobacteria	25047	1

Family	Count	%Sub-basin
Comamonadaceae	166574	10
Sporichthyaceae	130814	7
FamilyI	98339	6
Flavobacteriaceae	85996	5
Microbacteriaceae	77236	4
Rhodobacteraceae	63809	4
Sphingomonadaceae	53373	3
Xanthomonadaceae	46704	3
Verrucomicrobiaceae	44354	3
Acidimicrobiaceae	39701	2
Burkholderiaceae	38037	2
Chitinophagaceae	37175	2
Planctomycetaceae	33014	2
MNG7	32945	2
Cytophagaceae	29588	2
Saprospiraceae	28456	2
Xanthomonadales	28091	2
Erythrobacteraceae	21826	1
Methylophilaceae	20149	1
Alcaligenaceae	17704	1

Genus	Count	%Sub-basin
hgcI_clade	109529	6
Flavobacterium	82472	5
Synechococcus	59000	3
Polynucleobacter	34759	2
CL500-29_marine_group	34395	2
Limnohabitans	31509	2
Rhodobacter	30553	2
Arenimonas	24229	1
Candidatus_Rhodoluna	20764	1
Sphingomonas	19607	1
Novosphingobium	19556	1
Candidatus_Planktophila	19284	1
Dinghuibacter	18041	1

Table A-5. SIMPER-identified ASVs contributing to 20% of difference between land-use types grasslands and row crops. Each sampling site was assigned a land-use type using QGIS. "Percent contributed" is the percent amount that an ASV contributed to the difference between land-use types. "Mean abundance" is mean abundance of an ASV in a given land-use type. Each ASV is identified down to the genus level.

ASV	Percent Contributed	sd	Mean abundance grasslands	Mean abundance row crops	Phylum	Class	Order	Family	Genus
ASV_8	2.69	0.056	1075.0	237.8	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium
ASV_2	2.68	0.037	1126.0	502.0	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	Polynucleobacter
ASV_11	2.44	0.054	1131.0	62.2	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium
ASV_4	2.07	0.023	933.1	363.5	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Simplicispira
ASV_1	1.92	0.016	666.7	705.7	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	NA
ASV_9	1.66	0.018	409.8	540.8	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Pseudarcicella
ASV_5	1.63	0.014	574.6	552.1	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Limnohabitans
ASV_6	1.36	0.029	87.5	529.0	Cyanobacteria	Chloroplast	NA	NA	NA
ASV_10	1.30	0.033	19.8	548.4	Cyanobacteria	Chloroplast	NA	NA	NA
ASV_7	1.01	0.008	309.4	417.8	Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	hgcI_clade
ASV_13	0.93	0.013	297.8	260.3	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium
ASV_16	0.72	0.009	185.2	252.2	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Alpinimonas

Table A-6. SIMPER-identified ASVs contributing to 20% of difference between Lower Loup and Upper Loup sub-basins. "Percent contributed" is the percent amount that an ASV contributed to the difference between sub-basins. "Mean abundance" is mean abundance of an ASV in a given sub-basin. Each ASV is assigned taxonomy to the genus level.

ASV	Percent Contributed	sd	Mean abundance Lower Loup	Mean abundance Upper Loup	Phylum	Class	Order	Family	Genus
2	3.56	0.047	694.8	1539.0	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	Polynucleobacter
11	3.47	0.060	443.6	1211.0	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium
4	2.90	0.029	467.3	1417.0	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Simplicispira
8	2.74	0.050	547.6	803.8	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium
1	1.92	0.017	824.1	472.7	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	NA
5	1.67	0.015	651.8	448.2	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Limnohabitans
9	1.66	0.019	589.9	324.5	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Pseudarcicella
6	1.15	0.028	444.2	64.3	Cyanobacteria	Chloroplast	NA	NA	NA
13	1.00	0.014	249.2	381.4	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium

Table A-7. SIMPER-identified ASVs contributing to 20% of difference between Upper Loup and Central Platte sub-basins. "Percent contributed" is the percent amount that an ASV contributed to the difference between sub-basins. "Mean abundance" is mean abundance of an ASV in a sub-basin. Each ASV is assigned taxonomy to the genus level.

ASV	Percent contributed	SD	Mean abundance Upper Loup	Mean abundance Central Platte	Phylum	Class	Order	Family	Genus
2	3.04	0.047	1539.0	224.0	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	Polynucleobacter
4	2.76	0.030	1417.0	124.9	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Simplicispira
11	2.73	0.053	1211.0	18.3	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium
8	2.11	0.042	803.8	176.5	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium
10	1.49	0.029	27.2	570.3	Cyanobacteria	Chloroplast	NA	NA	NA
26	1.30	0.033	0.8	606.5	Cyanobacteria	Chloroplast	NA	NA	NA
1	1.28	0.012	472.7	477.3	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	NA
7	1.25	0.010	304.7	564.2	Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	hgcI_clade
5	1.09	0.012	448.2	286.1	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Limnohabitans
6	1.00	0.019	64.3	434.0	Cyanobacteria	Chloroplast	NA	NA	NA

Table A-8. SIMPER table listing ASVs contributing to 20% of difference between Lower Loup and Central Platte sub-basis. "Percent contributed" is the percent amount that an ASV contributed to the difference between sub-basins. "Mean abundance" is mean abundance of an ASV in a given sub-basin. Each ASV is assigned taxonomy to the genus level.

ASV	Percent contributed	SD	Mean abundance Lower Loup	Mean abundance Central Platte	Phylum	Class	Order	Family	Genus
10	2.10	0.038	403.8	570.3	Cyanobacteria	Chloroplast	NA	NA	NA
1	1.85	0.016	824.1	477.3	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	NA
6	1.75	0.030	444.2	434.0	Cyanobacteria	Chloroplast	NA	NA	NA
2	1.71	0.022	694.8	224.0	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	Polynucleobacter
8	1.55	0.044	547.6	176.5	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium
5	1.47	0.014	651.8	286.1	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Limnohabitans
9	1.37	0.016	589.9	144.8	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Pseudarcicella
26	1.31	0.031	1.28	606.5	Cyanobacteria	Chloroplast	NA	NA	NA
7	1.20	0.009	361.0	564.2	Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	hgcI_clade
4	1.05	0.012	467.3	124.9	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Simplicispira
23	0.98	0.023	15.09	385.7	Cyanobacteria	Chloroplast	NA	NA	NA
11	0.87	0.035	443.6	18.4	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium
16	0.74	0.010	247.7	167.4	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Alpinimonas
39	0.74	0.012	72.01	248.7	Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	hgcI_clade
13	0.69	0.012	249.2	132.5	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium
21	0.65	0.007	213.2	157.6	Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Candidatus_Rhodoluna

Table A-9. PERMANOVA testing if bacterial communities between land-use types grassland and row crops are different from each other. Bray-Curtis distance was used to create community dissimilarity matrices. P-values < 0.05 indicate significant difference.

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
Land-Use Type	1	4.54	4.534	15.49	0.0325	0.001
Residuals	461	135.02	0.293	NA	0.967	NA
Total	462	139.56	NA	NA	1	NA

Table A-10. One-way ANOVA results for testing if means for Kjeldahl nitrogen, phosphate-phosphorus, temperature, dissolved oxygen, pH, and total suspended solids were different between sub-basins.

Kjeldahl Nitrogen ~ Sub-basin					
	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Sub-basin	2	45	22.37	1.644	0.195	
Residuals	415	5648	13.61			

Phosphate-Phosphorus ~ Sub-basin						
	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Sub-basin	2	45.8	22.876	10.06	5.39E-05	***
Residuals	415	943.3	2.273			

Temperature ~ Sub-basin						
	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Sub-basin	2	405	202.42	12.71	4.39E-06	***
Residuals	415	6610	15.93			

Dissolved Oxygen ~ Sub-basin						
	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Sub-basin	2	26.8	13.412	4.034	0.0184	*
Residuals	415	1379.7	3.325			

pH ~ Sub-basin						
	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Sub-basin	2	9.08	4.542	15.85	2.32E-07	***
Residuals	415	118.91	0.287			

Total Suspended Solids (mg/L) ~ Sub-basin						
	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Sub-basin	2	1994777	997389	4.461	0.0121	*
Residuals	415	9.3E+07	223578			

Table A-11. Post hoc pair-wise t-test for identifying which sub-basins were different from each other in terms of mean environmental parameters. A p-value < 0.05 indicates significance.

Kjehldahl Nitrogen		
	centralPlatte	lowerLoup
lowerLoup	1	-
upperLoup	0.74	0.21

Phosphate Phosphorus		
	centralPlatte	lowerLoup
lowerLoup	0.0613	-
upperLoup	3.60E-05	0.0039

Temperature		
	centralPlatte	lowerLoup
lowerLoup	7.40E-06	-
upperLoup	6.00E-05	1

Dissolved Oxygen		
	centralPlatte	lowerLoup
lowerLoup	0.015	-
upperLoup	0.138	1

Total Suspended Solids		
	centralPlatte	lowerLoup
lowerLoup	0.159	-
upperLoup	1	0.026

pH		
	centralPlatte	lowerLoup
lowerLoup	0.00044	-
upperLoup	1.00E-07	0.00464

Table A-12. Five number summary plus standard deviations for the environmental

parameters measured in each sub-basin.

Upper Loup	Q1	Q2	Q3	Min	Max	SD
Kjeldahl Nitrogen	0.741	0.986	1.18	0	1.74	0.45
Phosphate Phosphorus	0.21	0.269	0.323	0.142	1.81	0.19
Temperature	17.8	19.4	22.6	7	29	4.24
Total Suspended Solids	54	79	113	23.5	193	40.8
pH	7.5	7.7	7.9	6.7	8.5	0.33
Dissolved oxygen	7.3	7.9	8.4	5.2	24.7	2.11

Lower Loup	Q1	Q2	Q3	Min	Max	SD
Kjeldahl Nitrogen	0.74	1.06	1.52	0	18.2	1.89
Phosphate Phosphorus	0.366	0.617	0.971	0.172	6.34	0.79
Temperature	17.8	19.9	22.55	7.9	28.3	3.74
Total Suspended Solids	69	102	162	7.7	2270	339.8
pH	7.8	7.9	8.1	7.2	9.1	0.34
Dissolved oxygen	7.3	7.9	8.5	4.8	24.4	1.65

Central Platte	Q1	Q2	Q3	Min	Max	SD
Kjeldahl Nitrogen	1.16	1.44	1.89	0.561	3.96	0.71
Phosphate Phosphorus	0.33	0.616	2.5	0.117	6.17	1.37
Temperature	19.4	22.8	25.1	10	30.9	4.60
Total Suspended Solids	44	196	154	6.5	956	134.0
pH	8	8.2	8.6	7.1	9.1	0.47
Dissolved oxygen	7.2	8.5	9.8	4.6	14.7	1.88

Table A-13. PERMANOVA testing for significant relationship between environmental variables and bacterial community dissimilarity for each sub-basin. Community dissimilarity was calculated using Bray-Curtis distance. P-values < 0.05 indicate a significant relationship.

Upper Loup						
	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
Temperature	1	0.948	0.948	4.425	0.044	0.001
pH	1	0.540	0.540	2.518	0.025	0.010
Dissolved Oxygen	1	0.945	0.945	4.409	0.044	0.001
PO4-P	1	0.768	0.768	3.586	0.036	0.001
Kjeldahl Nitrogen	1	1.105	1.105	5.157	0.051	0.001
Total Suspended Solids	1	0.449	0.449	2.095	0.021	0.011
Chloride	1	1.517	1.517	7.078	0.071	0.001
Residuals	71	15.213	0.214	NA	0.708	NA
Total	78	21.484	NA	NA	1.000	NA

Lower Loup						
	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
Temperature	1	2.843	2.843	11.485	0.037	0.001
pH	1	1.086	1.086	4.386	0.014	0.001
Dissolved Oxygen	1	1.348	1.348	5.444	0.018	0.001
PO4-P	1	1.568	1.568	6.336	0.021	0.001
Kjeldahl Nitrogen	1	2.547	2.547	10.289	0.034	0.001
Total Suspended Solids	1	1.388	1.388	5.606	0.018	0.001
Chloride	1	0.852	0.852	3.442	0.011	0.001
Residuals	260	64.369	0.248	NA	0.847	NA
Total	267	76.001	NA	NA	1.000	NA

Central Platte						
	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
Temperature	1	1.204	1.204	5.104	0.057	0.001
pH	1	2.424	2.424	10.279	0.115	0.001
Dissolved Oxygen	1	0.306	0.306	1.296	0.014	0.160
PO4-P	1	1.061	1.061	4.501	0.050	0.001
Kjeldahl Nitrogen	1	0.516	0.516	2.190	0.024	0.010
Total Suspended Solids	1	0.292	0.292	1.236	0.014	0.198
Chloride	1	0.426	0.426	1.806	0.020	0.040
Residuals	63	14.858	0.236	NA	0.705	NA
Total	70	21.087	NA	NA	1.000	NA

Table A-14. Spearman correlation coefficients between ASVs and environmental variables. Each ASV in this table was identified by SIMPER as contributing to differentiating land-use types or differentiating sub-basins. The rho value indicates the strength of the relationship and the sign indicates the direction of the relationship.

Taxa	Variable	rho	p	Significance
Alicycliphilus	temperature	-0.106	0.031	*
Alicycliphilus	kjedahlNitrogen	-0.141	0.004	**
Alicycliphilus	phosphatePhosphorus	-0.308	0	***
Alicycliphilus	do	-0.092	0.06	
Alicycliphilus	pH	-0.298	0	***
Alicycliphilus	ecoli	-0.076	0.122	
Alicycliphilus	totalSuspendedsolids	-0.072	0.144	
Alicycliphilus	chloride	-0.467	0	***
Alpinimonas	temperature	-0.239	0	***
Alpinimonas	kjedahlNitrogen	-0.092	0.061	
Alpinimonas	phosphatePhosphorus	0.351	0	***
Alpinimonas	do	-0.459	0	***
Alpinimonas	pH	-0.444	0	***
Alpinimonas	ecoli	0.361	0	***
Alpinimonas	totalSuspendedsolids	-0.258	0	***
Alpinimonas	chloride	0.219	0	***
Candidatus_Rhodoluna	temperature	-0.166	0.001	***
Candidatus_Rhodoluna	kjedahlNitrogen	-0.064	0.191	
Candidatus_Rhodoluna	phosphatePhosphorus	0.432	0	***
Candidatus_Rhodoluna	do	-0.369	0	***
Candidatus_Rhodoluna	pH	-0.22	0	***
Candidatus_Rhodoluna	ecoli	0.396	0	***
Candidatus_Rhodoluna	totalSuspendedsolids	-0.156	0.001	**
Candidatus_Rhodoluna	chloride	0.424	0	***
Flavobacterium	temperature	-0.334	0	***
Flavobacterium	kjedahlNitrogen	-0.482	0	***

Table A-14. Continued.

Taxa	Variable	rho	p	Significance
Flavobacterium	phosphatePhosphorus	-0.338	0	***
Flavobacterium	do	0.008	0.873	
Flavobacterium	pH	-0.128	0.009	**
Flavobacterium	ecoli	-0.132	0.007	**
Flavobacterium	totalSuspendedsolids	-0.275	0	***
Flavobacterium	chloride	-0.32	0	***
Limnohabitans	temperature	-0.322	0	***
Limnohabitans	kjedahlNitrogen	-0.32	0	***
Limnohabitans	phosphatePhosphorus	0.102	0.037	*
Limnohabitans	do	-0.115	0.019	*
Limnohabitans	pH	-0.104	0.034	*
Limnohabitans	ecoli	0.2	0	***
Limnohabitans	totalSuspendedsolids	-0.18	0	***
Limnohabitans	chloride	0.137	0.005	**
Polynucleobacter	temperature	-0.363	0	***
Polynucleobacter	kjedahlNitrogen	-0.124	0.011	*
Polynucleobacter	phosphatePhosphorus	-0.013	0.79	
Polynucleobacter	do	-0.244	0	***
Polynucleobacter	pH	-0.343	0	***
Polynucleobacter	ecoli	0.169	0.001	***
Polynucleobacter	totalSuspendedsolids	-0.16	0.001	**
Polynucleobacter	chloride	-0.092	0.06	
Pseudarcicella	temperature	-0.153	0.002	**
Pseudarcicella	kjedahlNitrogen	-0.411	0	***
Pseudarcicella	phosphatePhosphorus	0.091	0.064	
Pseudarcicella	do	-0.15	0.002	**
Pseudarcicella	pH	-0.058	0.237	
Pseudarcicella	ecoli	0.156	0.001	**
Pseudarcicella	totalSuspendedsolids	-0.2	0	***
Pseudarcicella	chloride	0.115	0.018	*

Table A-14. Continued.

Taxa	Variable	rho	p	Significance
Simplicispira	temperature	-0.339	0	***
Simplicispira	kjedahlNitrogen	-0.302	0	***
Simplicispira	phosphatePhosphorus	-0.201	0	***
Simplicispira	do	-0.189	0	***
Simplicispira	pH	-0.383	0	***
Simplicispira	ecoli	0.042	0.389	
Simplicispira	totalSuspendedsolids	-0.246	0	***
Simplicispira	chloride	-0.245	0	***
Unclassified	temperature	0.145	0.003	**
Unclassified	kjedahlNitrogen	-0.071	0.148	
Unclassified	phosphatePhosphorus	-0.055	0.26	
Unclassified	do	0.377	0	***
Unclassified	pH	0.498	0	***
Unclassified	ecoli	-0.184	0	***
Unclassified	totalSuspendedsolids	0.069	0.158	
Unclassified	chloride	0.242	0	***
hgcI_clade	temperature	0.174	0	***
hgcI_clade	kjedahlNitrogen	-0.01	0.832	
hgcI_clade	phosphatePhosphorus	0.026	0.597	
hgcI_clade	do	0.242	0	***
hgcI_clade	pH	0.287	0	***
hgcI_clade	ecoli	-0.161	0.001	***
hgcI_clade	totalSuspendedsolids	-0.092	0.061	
hgcI_clade	chloride	0.262	0	***

Table A-15: BIOENV results showing correlation between sets of environmental factors and bacterial community dissimilarity for each sub-basin. Bolded lines indicate the set of environmental factors with the strongest correlation to community dissimilarity

Upper Loup	
Variable(s)	r
dissolved oxygen	0.198
dissolved oxygen, Kjeldahl nitrogen	0.277
phosphate-phosphorus, Dissolved oxygen, Kjeldahl nitrogen	0.323
phosphate-phosphorus, dissolved oxygen, chloride, Kjeldahl nitrogen	0.344
phosphate-phosphorus, pH, dissolved oxygen, chloride, Kjeldahl nitrogen	0.366
phosphate-phosphorus, pH, dissolved oxygen, TSS, chloride Kjeldahl nitrogen	**0.372**
phosphate-phosphorus, temperature, pH, dissolved oyxgen, TSS, chloride, Kjeldahl nitrogen	0.368

Lower Loup	
Variable(s)	r
pH	0.319
phosphate-phosphorus, pH	0.374
phosphate-phosphorus, pH, Kjeldahl nitrogen	**0.380**
phosphate-phosphorus, pH, dissolved oxygen, Kjeldahl nitrogen	0.367
phosphate-phosphorus, pH, dissolved oxygen, TSS, Kjeldahl nitrogen	0.358
phosphate-phosphorus, temperature, pH, dissolved oxygen, TSS, Kjeldahl nitrogen	0.335
phosphate-phosphorus, temperature, pH, dissolved oxygen, TSS, Kjedlahl nitrogen	0.310

| Central Platte	
Variable(s)	r
pH	0.352
phosphate-phosphorus, temperature	0.485
phosphate-phosphorus, temperature, pH	**0.552**
phosphate-phosphorus, temperature, pH, dissolved oxygen	0.526
phosphate-phosphorus, temperature, pH, dissolved oxygen, Kjeldahl nitrogen	0.487
phosphate-phosphorus, temperature, pH, dissolved oxygen, chloride, Kjeldahl nitrogen	0.445
phosphate-phosphorus, temperature, pH, dissolved oxygen, TSS, chloride, Kjeldahl nitrogen	0.407
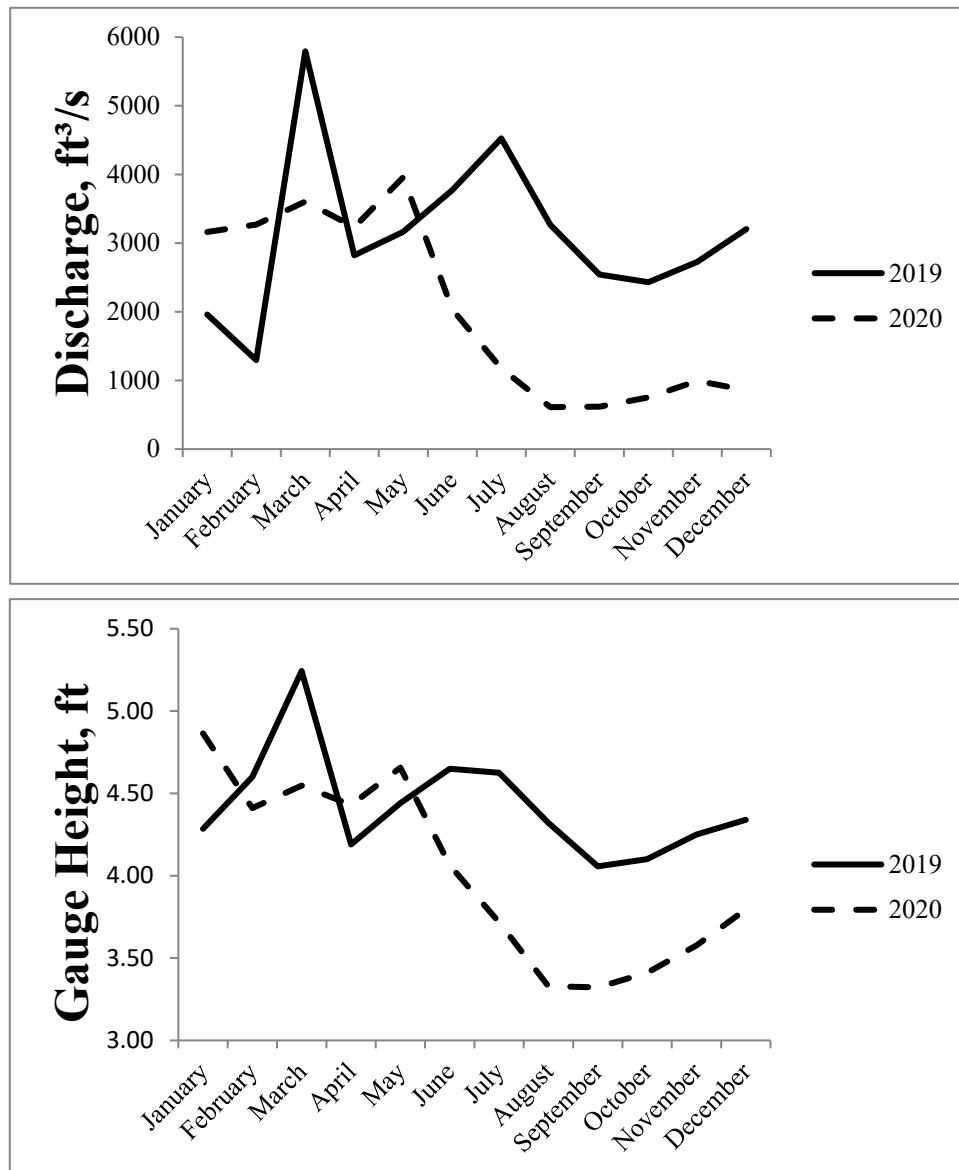
Figure A-1: Gauge height and velocity of discharge averaged across sites in the Platte River basin. Measurements obtained from the NDEE.
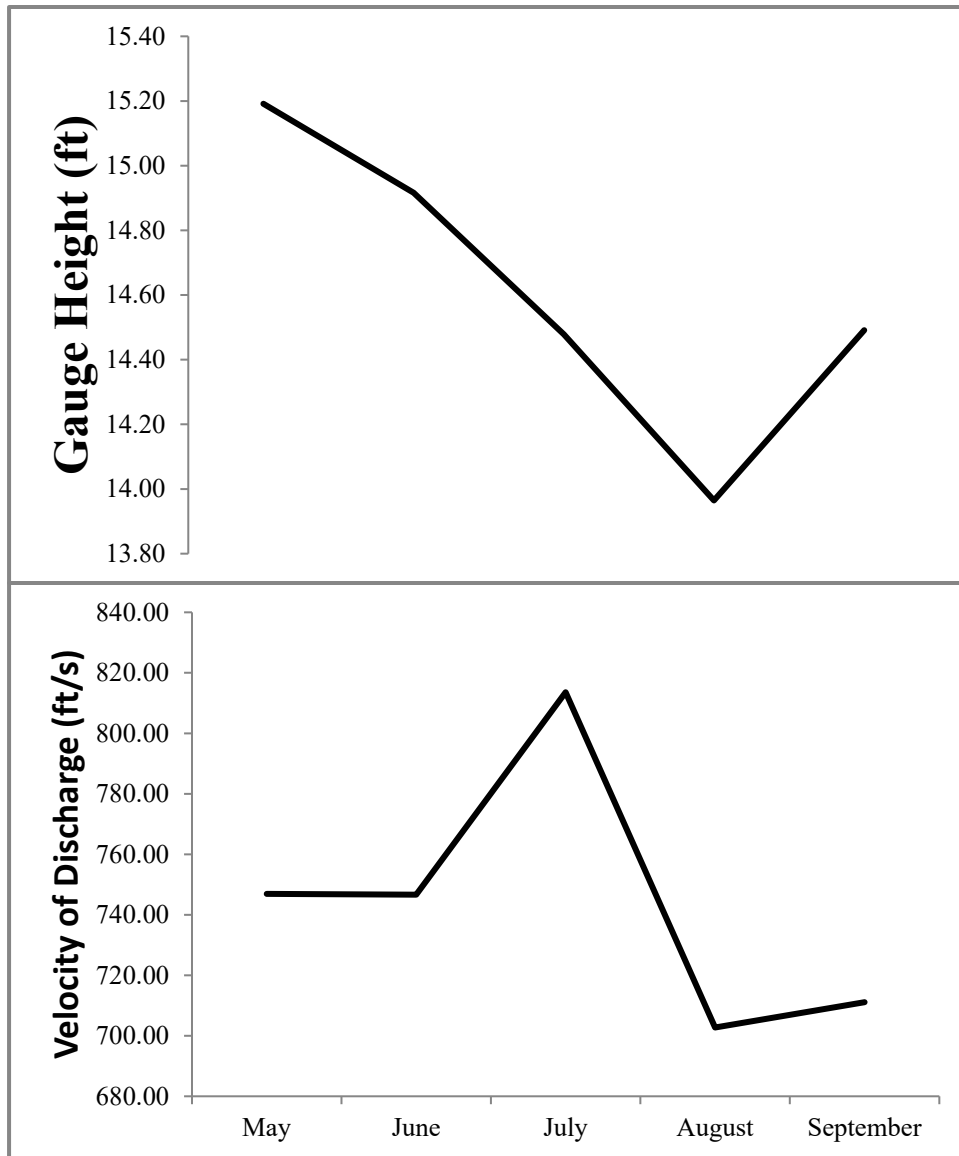
Figure A-2. Discharge rates and gauge height values for the Platte River in years 2019 and 2020. Values obtained from USGS river monitoring station in Grand Island, Nebraska.