Assessing landscape influences on the base of the aquatic food web across the North

Saskatchewan River basin

By

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ABSTRACT

Anthropogenic activities are forcing a shift in landscape types and climate regimes with concomitant effects on runoff draining into regional rivers and downstream water quality. Stream microbial communities at the base of aquatic food webs play an important role in overall ecosystem health, yet there has been limited work integrating the effects of landscape type and water quality on their community structure. Here we investigate these relationships across the North Saskatchewan River basin, an expansive watershed in Alberta (Canada), spanning forested, industrial, urban, and agricultural landscapes, and the primary drinking water source for a major city and 65 other communities. Over summer 2020 and 2021, samples for 48 water chemistry parameters (e.g., nutrients, ions, metals, oxygen isotopes), organic carbon character, and microbial community composition were collected from 78 tributaries across the North Saskatchewan River basin land use and land cover gradient. We found significant differences in water chemistry with lower nutrient and ion concentrations in alpine and forested regions compared to agricultural lands and city centers. Organic matter character across the basin was largely driven by differences in allochthonous input, where composition across the varying landscapes aligned with the changing soil type across the basin. Microbial community structure was also distinct across the basin, with the alpine sites containing the highest number of unique species (10,521) and the urban and agricultural sites being the most similar. Additionally, a universal core community was present across all sites (6-98%), composing the lowest relative abundance in the alpine sites, and the highest in the foothills which we propose to be a product of soil inoculation, as foothills sites have both the presence of well-developed soils, rolling topography, and high precipitation that would facilitate these inoculation events. Our findings highlight the importance of landscape influences in determining water chemistry, organic matter,

and microbial community composition across the North Saskatchewan River basin. These results imply a shift in stream microbial communities as landscape types and climate regimes shift, with a possible decrease in microbial diversity as landscapes become more connected to the terrestrial environment.

PREFACE

This thesis represents original research as part of a collaborative project between the University of Alberta and the Government of Alberta designed and planned by principal investigators Craig Emmerton, Cristina Buendia-Fores, Faye Wyatt, Rolf Vinebrooke, Mark Poesch, Stephanie Green, and Maya Bhatia. Field sampling, lab analysis, and data analysis were primarily done by me with the help of: research assistants Hannah Holzer and Lakoda Thomas who assisted with field preparation, sampling, and lab analysis; graduate students Charvanaa Dhoonmoon and Hayley Drapeau who assisted with field sampling; and lab technician Maria Cavaco who assisted with field preparation, sampling, lab analysis, and notably completed 16S rRNA gene sequencing and bioinformatics for the microbial community composition data. Chlorophyll *a* sampling and lab analysis were completed by Shelby Stenerson. Compilation of geospatial data in ArcGIS was done by Mina Nasr. Watershed delineation in ArcGIS and the flow assessment was done by Craig Emmerton. Edits to this manuscript were completed by Maya Bhatia, Craig Emmerton, and Maria Cavaco. No components of this thesis have been previously published.

The second chapter of this thesis will be formatted for submission to the journal Science of the Total Environment (STOTEN). This publication will be coauthored by: Craig Emmerton, Maria Cavaco, Mina Nasr, Hannah Holzer, Cristina Buendia-Fores, Suzanne Tank, Faye Wyatt, and Maya Bhatia.

DEDICATION

To Maya, who I owe my entire academic career to. If it wasn't for you, I never would have seen myself as a scientist. You meant so much to me.

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Chapter 1. General Introduction

1.1   The North Saskatchewan River basin and the watershed integrity project

The North Saskatchewan River (NSR) basin is a highly relied upon watershed for recreation, agriculture, and namely a drinking water source for the city of Edmonton and over 65 other communities (Alliance (NSWA), 2005). Beginning on the eastern slopes of the Rocky Mountains, and flowing east across the province, the watershed traverses a diverse range of landscape types including alpine, foothills, forested, urban, and agricultural land. These landscapes influence the streams and rivers of the basin through runoff, where precipitation flows across the landscape and enters the traversing streams. It is expected that landscape influences are being altered as land use and land cover change shifts to support a growing population (Martellozzo et al., 2015), landscape altering climate events become more frequent (IPCC, 2019), and precipitation regimes shift, altering runoff and thus stream-landscape connectivity (Newton et al., 2021). With potential for shifting climate across a large range of landscape and land uses across Alberta, it is of vital importance to understand the relationship between landscape type and stream ecosystem health in this greatly depended on river basin.

The watershed integrity project, partnering the University of Alberta and the Government of Alberta, aimed to create a geospatial model that could predict stream aquatic health based on the surrounding landscape types in the NSR basin. Where the relationship between aquatic health and surrounding landscapes is often inferred for these types of models, this project aimed to first assess the aquatic health of the basin and to understand how this was influenced by different landscapes by characterizing the aquatic ecosystem health of the NSR basin through spatially exhaustive sampling of its tributaries. Sampling included a whole ecosystem approach investigating fish, invertebrates, algae, microorganisms, and water chemistry. Sampling sites were selected using a multivariate statistical approach and geospatial data across the basin to ensure tributaries from a full range of landscape types and land uses would be sampled. Briefly, this was done through delineating sub watersheds across the NSR basin to the hydrologic unit code 10 (HUC-10), resulting in 231 individual subwatersheds basin-wide. Each HUC-10 was classified by natural region (i.e., mixed alpine, foothills, dry mixedwood, central parkland, and central mixedwood) and landscape conditions and land uses within each subwatershed were characterized using natural, climate, and stressor geospatial data. Using hierarchical cluster

analysis, three distinct landscape groupings within each natural region were identified, and streams were selected to span all groupings. The data collection, analysis, and write-up for this thesis was done as a part of the watershed integrity project, and will be focused on the water chemistry, organic matter (OM), and microbial community components.

1.2   Influences of landscapes on stream water chemistry

It has long been understood that stream water chemistry is largely influenced by its surrounding landscapes (Allan, 2004; Carpenter et al., 1998; Johnson et al., 1997). As runoff traverses the watershed it can accumulate and transport sediment, nutrients, metals, and ions to river networks. Consequently, rivers integrate a mosaic of the many different characteristics of the watershed they flow through. Such landscape characteristics include natural factors such as geology. The geology of a stream watershed can be an important determinant of stream water quality as runoff can weather and erode parent material and soils and deliver these products downstream (Nelson et al., 2011). Other natural variables influencing stream conditions include vegetation, which can improve stream water quality through stabilizing stream banks and limiting soil erosion (Wynn et al., 2004) and intercepting nutrients from runoff for its own biological uptake (Peterjohn & Correll, 1984). Additionally, these landscape controls on river water quality are mediated by the climate of the area, which will determine the amount of precipitation and thus runoff that traverses the landscape. The amount of time this runoff interacts with the landscape is determined by slope, where watersheds with higher slopes are often host to dilute streams due to limited interaction between the landscape and runoff (Clow & Sueker, 2000). Anthropogenic land use also greatly impacts water chemistry and is often associated with degrading water quality (Allan, 2004; Giri & Qiu, 2016). This can include increased nutrient loads from fertilizers (Carpenter et al., 1998), greater erosion and sediment delivery to streams due to increased impervious surfaces and unimpeded runoff in watersheds (Russell et al., 2017), and increased metals from industrial effluent and atmospheric deposition (Yu et al., 2014). Therefore, it is this complex interaction of many natural and anthropogenic landscape variables that shape a stream's water chemistry.

Several studies have assessed the relationship between landscape conditions and river water quality at a watershed scale, with many focusing on land use impacts as demand for urban and agricultural land increase with population rise. In these large scale studies, it is commonly

found that urban and agricultural landscapes are attributed to degrading water quality when compared to undisturbed (often forested) regions as seen across the world in the Patagonia region (Miserendino et al., 2011), New York City (Mehaffey et al., 2005), England (Rothwell et al., 2010), and China (S. Li et al., 2009). Other common findings include the upland regions of a watershed, often less suitable for human development, to have more pristine, dilute water quality as seen in the highlands of watersheds in north western England (Rothwell et al., 2010) and the Colorado Rocky Mountains (Clow & Sueker, 2000). These studies that encompass several different landscape types have further illustrated the landscape controls on water chemistry, which when quantified, as was done in a study in Michigan, USA assessing 62 streams, were found to explain 56% variance in the water chemistry (Johnson et al., 1997). As such it is well documented that streams are highly influenced by the characteristics of the surrounding watershed.

1.3   Influences of landscapes on stream organic matter composition

OM in stream ecosystems can be generally separated into allochthonous OM, that which is produced outside of the stream, and autochthonous OM, that which is produced by microorganisms or plants within the stream. Allochthonous OM is inherently linked to the landscape as it is washed to the streams as precipitation flows through vegetation and soil of the watershed and is composed of complex, humic-like compounds (Fellman et al., 2008). Autochthonous OM, produced by in stream communities, is influenced by site specific conditions that can be influenced by landscapes through altering of stream nutrient concentrations (Wilson & Xenopoulos, 2009), light availability (Reche et al., 1998), and the lability of the organic matter sourced into the stream (Amon & Benner, 1996) and is composed of less complex compounds with more nitrogenous molecules (Yamashita et al., 2011). They are often differentiated by their lability, or the ease with which they can be decomposed, where allochthonous is often more complex, and thus less labile that autochthonous. In most streams the OM pool is largely of allochthonous origin (Brooks et al., 1999). As such, the surrounding landscapes are a large control on the stream OM pool. Despite this importance, the relationship of varying landscapes with stream OM is not well characterized. Studies integrating differing landscape conditions and land uses at the watershed scale have found various controls of landscape type on both allochthonous and autochthonous OM in streams.

In terms of OM quantity, agricultural landscapes have been associated with both higher (Graeber et al., 2012; Mattsson et al., 2009) and lower (Wilson & Xenopoulos, 2009) allochthonous OM transport to streams, when compared to wetland or forested areas. Explanations for a higher export include agricultural soils being more compact from heavy equipment as well as livestock, which results in flow paths for runoff to be more shallow, thus traversing the more organic rich upper layer of soils (S. Chen et al., 2021), or that tillage mobilizes organic carbon from this upper layer increasing export to streams (Kelsey et al., 2020). In contrast, higher export from wetlands, when compared to agriculture, has been observed and is attributed to the organic rich soils that wetlands harbour (Wilson & Xenopoulos, 2009). In terms of composition, studies have found both an increase in the relative proportion of microbial-like (Lu et al., 2013; Williams et al., 2010; Wilson & Xenopoulos, 2009) and humic-like OM (Graeber et al., 2012; Shang et al., 2018) in streams of agriculture catchments when compared to wetland or forested catchments. Explanations for increased microbially-derived OM include that nutrients from agriculture runoff stimulate autochthonous production (Wilson & Xenopoulos, 2009), that agriculture soils are less humic so easier for stream microorganisms to decompose (Kalbitz et al., 2003), or alternatively that agriculture processes such as tillage increase soil microbial activity that are eventually transported to the stream (S. Chen et al., 2021). Explanations for increased relative abundance of humic-like OM similarly attribute the organic-rich shallow flow paths of agricultural land (Graeber et al., 2012). Confounding the relationship between landscape type and stream OM, is differences in soil type which are often shifting alongside types of land use or landcover. In a study attempting to differentiate soil and land use controls, organic soil composition was found to be the most significant predictor of OM quantity and composition (Autio et al., 2016). Thus, on a watershed scale, landscape influences on OM in streams have many interacting factors and can be spatially variable, thus making it difficult to understand the processes linking the two conditions.

1.4 Microbial community composition across landscapes

Processes that shape microbial communities across different spatial scales include species sorting, where environmental gradients of nutrients or temperature for example, allow certain taxa to thrive and dispersal, where the community is composed of taxa that have been transported there from other environments. Proponents for species sorting argue that microbial communities

are adjusted to the local conditions due to their short generation times (Van der Gucht et al., 2007). This is supported in studies that find unique microbial communities across various spatial scales (Fierer & Jackson, 2006; Van der Gucht et al., 2007). In contrast many studies have found that local communities are largely composed of taxa that are ubiquitous within the region, supporting the argument that communities are shaped by immigration from surrounding communities (Östman et al., 2010). This is similarly supported by studies that have found no difference in microbial community composition across environmentally distinct sampling sites (Nesbø et al., 2006; Papke et al., 2003). Additionally, a microcosm experiment attempting to determine the dominant mechanism found both dispersal and species sorting to be evident in the initial assembly of microbial communities (Langenheder & Székely, 2011).

Understanding the role of species sorting and dispersal in shaping microbial communities in stream environments, where distinct communities have been observed across different landscape types (Jones et al., 2020; Staley et al., 2014; Zeglin, 2015), is an ongoing area of research. When considering the influence of different landscape types on stream microbial communities, it is difficult to disentangle species sorting and dispersal effects because changing landscapes often result in both new soil microbial communities that could inoculate the stream and new environmental conditions, such as the aforementioned water chemistry and organic matter, for the communities to respond to. Species sorting has been attributed as the dominant mechanism, as seen in the Mississippi watershed, where the majority of variation in the microbial community (88%) was attributed to environmental parameters (Staley et al., 2015), boreal Quebec, where there was no evidence of dispersal limitation across major lakes and rivers (Niño-García et al., 2016), and the Hubbard Brook experimental forest, where geographic distance had no impact on benthic microbial community composition (Fierer & Jackson, 2006). Other studies emphasize the importance of terrestrial microbial communities in initial assembly of stream microbial community composition, arguing that the community is first shaped by dispersal from soils, then species sorting follows downstream (Besemer et al., 2013; Crump et al., 2012). As such, further research is needed to clarify the mechanisms shaping stream microbial communities across different landscapes.

1.5   Research objectives

Our study aims to further investigate the influence of different landscapes on water quality and the base of the aquatic food webs in stream ecosystems. To do so I will use field data collected in the summers of 2020 and 2021 across 78 tributaries in the NSR basin, for water chemistry, OM, and microbial community composition. Additionally, data to characterize the watersheds of each tributary with land cover, land use, geology, and climatological data were acquired using GIS. Using these data, the primary objectives were to: 1) characterize the water chemistry, organic matter, and microbial community composition across the NSR basin; 2) investigate how each of these environmental components shift with differences in landscapes and 3) understand the mechanisms driving microbial community composition on a watershed scale.

1.6   Significance

Microbial communities play crucial roles in stream ecosystems such as performing biogeochemical functions, decomposing organic matter, and transporting nutrients to higher trophic levels. Although this is well documented, microorganisms are often excluded from ecosystem response studies (Allison & Martiny, 2008), and specifically microbial communities in streams are the least studied when compared to marine, terrestrial, and lake environments (K. Li et al., 2021). Globally, only 35 basins have assessed surface water microbial communities across more than 50 sites (K. Li et al., 2021). Studies have documented differences in stream microbial communities across differences in landscapes, but the mechanisms remain unclear. Our study aims to further investigate this gap in understanding through sampling of stream microbial communities, and the stream environment of water chemistry and OM, across a landscape gradient in the NSR basin. Our study provides unique insight as we assess these variables across highly variable climate and landscape conditions. This study will be the first, of our knowledge, to spatially characterize stream microbial communities in Alberta. Additionally, because the NSR basin is utilized heavily by the province, understanding the aquatic health and how that is influenced by the surrounding watershed is of vital importance. Therefore, this study will enhance our understanding of landscape controls on the base of the aquatic food web while also characterizing the state of a highly relied upon river basin.

Chapter 2.   Assessing landscape influences on water chemistry, organic matter, and microbial community composition across the North Saskatchewan River Basin

2.1   Introduction

Rivers are a key fresh water source, heavily relied upon for recreation, agriculture, and drinking water on a global scale. The ecosystem health and water quality of rivers are significantly impacted by the watersheds they drain, where unique chemical (Allan, 2004; Carpenter et al., 1998) and biological (Jones et al., 2020; Zeglin, 2015) conditions have been attributed to differences in watershed landscape type. In a fluvial network, connection to the landscape is primarily driven by runoff, where precipitation that flows through the watershed delivers sediment, nutrients, and ions to streams. Thus, changes in landscapes or landscape connectivity (e.g., runoff frequency) may have important consequences for traversing streams. As human populations increase, land use and land cover (LULC) change is expected to occur as demand for urban areas and agriculture grow (IPCC, 2019), thus possibly altering runoff composition to streams. Additionally, precipitation regimes are expected to shift with climate change (Newton et al., 2021), thereby altering connectivity between land and stream ecosystems. To understand the full implications of these future shifts on watershed processes, it is of critical importance to characterize the role that landscapes play in impacting the aquatic state of stream ecosystems.

Landscape influences on stream ecosystems have been heavily assessed from a water quality perspective where agriculture and urban land use is often associated with degrading water quality (e.g., higher nutrients and suspended sediment) (Carpenter et al., 1998; Walsh et al., 2005) and watersheds with less disturbed environments, such as forested and alpine watersheds, are associated with more pristine water chemistry (Clow & Sueker, 2000). It is this well-developed link between watershed characteristics and water quality that has long motivated expansive monitoring efforts (Puckett, 1995). Similarly, organic matter (OM) transport to streams is also largely influenced by surrounding landscapes, where unique OM signatures are delivered to streams from landscapes varying in soil and vegetation character (Fellman et al., 2008). Tightly connected to water chemistry and organic matter, are microbial communities, which play important roles in stream ecosystems given their position at the base of the food web and the functions they perform in carbon cycling, organic matter decomposition, and nutrient transport to higher trophic levels. Yet, despite their importance in biogeochemical processes, it is

common for microbial communities to be excluded in studies assessing ecosystem response (Allison & Martiny, 2008). This tendency, and ensuing gap in our knowledge, is particularly relevant for rivers, where stream microbial communities are least studied when compared to ocean, soil, and lake ecosystems with only 35 river basins worldwide having been assessed across more than 50 sites (K. Li et al., 2021).

Of the studies in stream environments, it has been well established that microbial communities appear to shift in response to landscape types (Jones et al., 2020; Staley et al., 2014; Zeglin, 2015). These differences are characterized by both variations in the relative abundance of ubiquitous taxa as well as the presence/absence of specific species. Stream microbial community structure has historically been thought to be driven by two processes: species sorting as a result of environmental selection, and passive dispersal (Crump et al., 2012; Staley et al., 2015). Species sorting occurs as environments shift downstream in networks, and the in situ stream microbial communities in turn, also shift in response to this environmental change, resulting in differences in microbial taxa found along the stream gradient (Niño-García et al., 2016). In contrast, passive dispersal results from communities upstream and in the surrounding terrestrial environment being transported into the stream, resulting in a more homogenous microbial community throughout the stream network (Crump et al., 2012). The importance of each of these mechanisms seems to be highly dependent on the local watershed conditions, and thus region-specific studies are necessary to fully understand how microorganisms will be affected by changing land use and landscape connectivity.

This study aims to decipher landscape controls on microbial community structure in stream ecosystems by assessing changing water chemistry, organic matter, and microbial community composition across diverse landscapes of the North Saskatchewan River (NSR) basin. The NSR basin spans the province of Alberta in western Canada beginning in pristine alpine environments on the eastern slopes of the Rocky Mountains, and flowing eastward through forests, wetlands, urban centers (e.g., City of Edmonton), and farmland. The NSR serves as the drinking water source to over 65 communities (Alliance (NSWA), 2005). The importance of this river to the surrounding communities, as well as the range of landscape types present across the watershed, makes it an ideal environment to assess landscape-driven differences at the base of the food web. Here, we sampled 78 tributary streams across the NSR basin in the summers of 2020 and 2021 for 48 parameters of water chemistry, organic matter composition,

and microbial community composition. Tributaries were selected to encompass the complete range of landscape types across the basin, thus spanning alpine, forested, urban, and agriculture landscapes. Geospatial data were used to characterize LULC data including geology, land cover, and land use across the basin. Our primary objectives are to: 1) characterize water chemistry, organic matter composition, and microbial community composition across the NSR basin; 2) investigate how all three of these environmental components vary with differences in landscape; and 3) investigate controls on microbial community composition across this large spatial gradient. To our knowledge, a study characterizing stream microbial communities over this spatial extent has never been done in Alberta. Our primary findings illustrate the significant influence of landscapes on stream water chemistry, organic matter, and microbial community composition across a diverse watershed. We find species sorting and dispersal controls to both be shaping the microbial community, with potential importance of soil inoculation during precipitation events. Collectively, the results from this work provide important insight into landscape influences at the base of the food web as well as locally characterize the state of a highly relied upon river basin.

2.2   Methods

2.2.1   Study site description

The NSR headwaters begin at the Saskatchewan Glacier on the eastern slopes of the Rocky Mountains in Banff National Park, Alberta, Canada. The NSR flows eastward from the mountains across the province of Alberta where it eventually merges with the South Saskatchewan River, and later flows into Lake Winnipeg, Manitoba. Notably, the NSR is a critical provider of ecosystem resources in Alberta (i.e., drinking water) upon which over 65 communities, including the provincial capital city of Edmonton (population of ~ 1 million), depend. In the NSR basin, mean summer temperature ranges between 10-14°C and precipitation ranges from 290-340 mm (Alliance (NSWA), 2005). The basin spans 57,000 km^2 and drains 12.5% of Alberta's land mass, spanning diverse natural land cover, including Rocky Mountains, Foothills, Boreal Forest, Parkland, and Grassland (Downing & Pettapiece, 2006). Intensive land use across the NSR basin has occurred since the mid to late 1800s, when the province was first settled, and includes agriculture, resource extraction for oil and gas, forestry, and major urban areas. Tributary streams across the river basin, ranging in order from 1-7, drain these diverse

natural and anthropogenically modified landscapes, spanning varying types of runoff and degrees of landscape connectivity. Landscape connectivity is greater in the western Alberta tributaries, where there is higher precipitation and a greater slope, compared to the eastern Alberta tributaries, where smaller streams flow more slowly across the flatter, dry Prairie region and with increased urban and agricultural influence (Jencso et al., 2009).

To assess the impact of varying landscapes on the base of the food web in NSR tributary stream ecosystems, 78 tributary streams of stream order 2-6, encompassing the range of LULC present across the basin, were sampled for water chemistry, organic matter composition, and microbial community composition. Sampling took place in July-August of 2020 and 2021 across the diversity of landscapes found in the NSR basin (Table 2.1). Precipitation between summers was highly variable, as 2020 was wetter than average and 2021 was much drier than average, with 411 mm and 194 mm total precipitation recorded, respectively, in the city of Edmonton each year (ACIS, 2023).

2.2.2   Sample collection

Prior to sampling, all plasticware and glassware used in this study were soaked in a dilute acid bath (1.2 mol/L HCl) overnight, and triple rinsed with MilliQ (18.2 MΩ). Glassware was subsequently combusted at 560°C for at least 4 hours and sample bottles for microbial analysis were autoclaved. Glass fiber filters for CHN analyses were combusted at a lower temperature of 460°C for at least 4 hours. At each of our tributary stream sampling sites using nitrile gloves, all sample bottles and syringes were triple rinsed with stream water and sampled from below the surface of the running stream (~30 cm), avoiding still/stagnant pools of water. Stream water was collected from the centre of the channel for lower flow tributaries and from the bank at higher flow tributaries. All samples were stored in coolers in the dark for transport back to the laboratory that same day. Field blanks were taken during each sampling season (three in 2020, and two in 2021) consisting of MilliQ water from the laboratory that was brought into the field and processed in the same manner as stream water samples.

2.2.2.1   Water chemistry measurements

A full suite of water chemistry was sampled at each tributary stream including multi-probe sensor measurements, and sub-surface water collections for nutrients, ions, carbon, isotopes of O and H in water, and trace metals analyses (Table A2.2). In situ measurements of temperature (°C), pH, specific conductance (µS/cm), dissolved oxygen (mg/L and %), turbidity (NTU), and oxidative reduction potential (mV) were taken using a Hydrolab DS5X Water Quality Multiprobe sonde suspended in the middle of the water column. Whole water (unfiltered) samples were collected for total nitrogen (TN), total Kjeldahl nitrogen (TKN), total phosphorus (TP) analyses, and for particulate carbon and nitrogen (CHN), particulate phosphorus (PP), and total suspended solids (TSS) analyses. All whole water samples were collected in polypropylene bottles with the exception of CHN, which was collected in polytetrafluoroethylene bottles. Samples for dissolved nutrients (total dissolved nitrogen (TDN), ammonium (NH_4^+), nitrite (NO_2^-), nitrate/nitrite (NO_3^-/NO_2^-), total dissolved phosphorus (TDP), soluble reactive phosphorus (SRP), silica), major ions (Al^{3+}, Ca^{2+}, K^{2+}, Mg^{2+}, Na^+, Cl^-, SO_4^{2-}), alkalinity, water isotopes ($\delta^{18}O$ and δ^2H), UV-Vis spectroscopy (absorbance and fluorescence), dissolved organic carbon (DOC), and dissolved inorganic carbon (DIC) had whole water passed through a 0.22 µm polyethersulfone (PES) syringe filter (Fisher Scientific) on site. Samples for nutrients were collected in 15-mL centrifuge vials, major ions and alkalinity in 50-mL centrifuge vials, and isotopes in 20-mL HDPE scintillation vials with no headspace, UV-Vis and DOC in amber EPA vials, and DIC in glass exetainers with no headspace. Samples for trace metals (Ag, As, Ba, Be, Cd, Co, Cu, Fe, Mg, Mn, Ni, Pb, Sb, Se, Sn, Sr, Ti, Tl, V, and Zn) had whole water passed through 0.02 µm Anotop filters (Millipore) into 15-mL centrifuge vials to access the bioavailable fraction. Finally, samples for planktonic chlorophyll *a* pigment concentrations were collected directly from the stream and filtered on site through a 47-mm glass fibre filter (Whatman GF/C; 1.0 µm pore size).

Back at the laboratory, whole water samples TN, TKN, and TP were stored at 4°C until analysis. Particulate samples CHN, PP and TSS were filtered using a plastic filter tower fitted for 25-mm glass fibre (Whatman GF/F; 0.7 µm pore size)filters for CHN, and 47-mm GF/F filters for PP and TSS. CHN and PP filters were stored at -20°C until analysis, and TSS was immediately analyzed. Samples for dissolved nutrients were stored at -20°C until analysis. Samples for DOC were acidified with 40 µL concentrated (37%) trace metal grade HCl and trace

metals were acidified with 25 µL of (40%) trace metal grade HNO_3. Samples for isotopes, major ions, UV-Vis, DIC, DOC, and trace metals were stored at 4°C, in the dark, until analysis. Chlorophyll *a* filters were freeze dried until analysis.

2.2.2.2 Microbial community composition

Samples for microbial community composition were collected as whole water samples directly from the stream in polypropylene bottles. Upon return to the laboratory, within 12 hours of collection, samples were filtered through a 0.22 µm Sterivex filter (Millipore) via peristaltic pump, set to a rate of ~50 mL/min to avoid cell breakage. Filtration continued until either the filter clogged or 2 L of water passed through, whichever occurred first. Excess air and water were expelled with a sterile 60 mL syringe and the filter cartridge was stored at -80°C until further analysis. Prior to sample collection on the Sterivex cartridge, the silicon pump tubing was rinsed with 200 mL of sample water and 200 mL of 10% hydrochloric acid, and 400 mL of MilliQ was used to rinse the tubing between samples.

2.2.3 Sample analysis

2.2.3.1 Water chemistry

Instrument analyses and detectable limits for all water chemistry is summarized in Table A2.2. Samples for nutrients, trace metals, and ions were analyzed at the Biogeochemical Analytical Services Laboratory (BASL) (Univ. of Alberta) following standard operating protocols (http://www.biology.ualberta.ca/basl/index.html). Briefly, nutrient samples were analyzed on a Flow Injection Analyser (Lachat QuikChem 8500 FIA automated ion analyzer). Trace metals were analyzed on an Inductively Coupled Plasma Mass Spectrometer (Agilent ICP-MS 7900). Cations Al^{3+}, Ca^{2+}, K^{2+}, Mg^{2+}, Na^+ were analyzed on an Inductively Coupled Plasma Optical Emission Spectrometer (Thermo Scientific ICAP6300, ICP-OES), anions, Cl^- and SO_4^{2-}, were analyzed on an ion chromatograph (Dionex DX600 and Dionex ICS 2500), and alkalinity was analyzed on an Autotitrator (Man-Tech PC-Titrate with conductivity probe). Chlorophyll *a* concentration was calculated using high performance liquid chromatography (HPLC) following Vinebrooke and Leavitt (1999) on an Agilent 1100 Series HPLC.

DIC samples were analyzed using the Apollo SciTech AS-C3 DIC Analyzer coupled to a LI-COR LI-7000 infrared CO_2 analyzer. Sample concentrations were calculated using a standard curve (ranging from either 401.9-3014.2 μM or 2391-17402 μM) created using a Certified Reference Material (CRM) standard (Scripps Institute for Oceanography). Two measurements within 0.10% were averaged to attain a final sample measurement. The CRM standard was run every 5 samples and also at the end of the run to assess instrument accuracy, and remained within 10% of its expected value.

DOC samples were analyzed using a Shimadzu TOC-L using a regular sensitivity catalyst (150 μL injection with 5 min sparge time). Sample concentrations were calculated using multiple 5-point (0-5 ppm, 0-20 ppm, 0-50 ppm, or 0-200 ppm) standard curves with R^2 values ranging 0.9994-0.9999. Standard curves and reference waters were created through dilution of 5 ppm, 100 ppm, and 1000 ppm KHP standards (SCP Science). Reference waters and MilliQ blanks were run every ten samples to monitor instrument drift, and were within 5% of accepted value. Samples were blank corrected by subtracting the nearest blank concentration of MilliQ to account for instrument drift.

Water isotopes were analyzed on the Picarro L2130 isotope and gas concentration analyzer. Standards from Ice Core Water (USGS46) and Puerto Rico precipitation (USGS48) were assessed at the beginning, middle, and end of the run to determine instrument accuracy (within +/- 0.2 standard deviations for $\delta^{18}O$ and +/- 6 for δ^2H). Raw data were calibrated using these standards with the Picarro Post-Process ChemCorrect software. Standard curves were manually checked to ensure accepted triplicate injections for the standards did not include outliers (outside of the accepted standard deviations), and this same manual check was applied to the sample injections.

Prior to analysis, CHN filters were dried at 50°C, then fumigated to drive off inorganic carbon. Fumigation involved placing filters in a desiccator for 48 hours containing concentrated HCl in a beaker and wetting the filters with MilliQ to enhance acid diffusion. The filters were then neutralized in a desiccator containing NaOH pellets and drierite for 24 hours. To ensure complete desiccation, the filters were then dried at 50°C for 2 hours, and then packed into tin capsules, and submitted to BASL (Univ. of Alberta) for analysis on a CE440 Elemental Analyzer.

TSS samples were analyzed using a gravimetric approach. Filters were weighed prior to sampling, dried overnight, and re-weighed after filtration to measure a net mass of particles captured on the filter. Measured particle masses were standardized to the filtered sample volume.

UV-Vis samples were analyzed within one week of collection on a Horiba Aqualog using a 1 cm quartz cuvette, to obtain both absorbance and fluorescence data. Absorbance samples were analyzed from 240-800 nm at 1 nm increments with an integration time of 0.1 s. For fluorescence samples, Excitation and Emission Matrices (EEMs) were obtained from 230-500 nm excitation wavelengths, in 5 nm increments, with an integration time of 0.5 s. Samples were normalized using the RSU correction factor for each run. Both absorbance and fluorescence samples were blank corrected for each run.

2.2.3.2 *Microbial DNA extraction, PCR amplification, and 16s rRNA gene sequencing*

The DNeasy PowerWater Sterivex kit (Qiagen) was used to extract environmental genomic DNA from microbial communities within collected water samples. The manufacturer's protocol was followed with the exceptions of placing the Sterivex in a rotisserie incubator at 72°C for 1 hour (instead of 5°C for 90 minutes) during cell lysis to maximize DNA yield, and omitting bead beating during the physical lysis step to avoid cell shearing. Following genomic DNA extraction, the V4-V5 region of the 16S rRNA gene was amplified from each extracted sample using universal prokaryotic primers 515-F (5'GTGYCAGCMGCCGCGGTAA'3) and 926-R (5'CCGYCAATTYMTTTRAGTTT'3) (Earth Microbiome Project). Each primer also contained a 6-base index sequence for sample multiplexing and flow cell binding. The polymerase chain reaction (PCR) amplification protocol included: denaturation at 98°C (3 min), 35 cycles of denaturation (30 s), primer annealing at 60°C (30 s) and extension at 72°C, and final extension at 72°C (10 min). Samples were subsequently visualized on a 1.5% agarose gel, and all amplicons were pooled and purified using AMPure XP beads (at a 9:10 ratio of sample vs beads). Using i5 and i7 adapters (Illumina), unique indices were added to construct amplicon libraries. The quality of each pool was determined on an Agilent 2100 Bioanalyzer at the Molecular Biology Service Unit (MBSU) (Univ. of Alberta) to ascertain the average size of amplified product and to ensure overall integrity of samples prior to 16S rRNA gene sequencing. Subsequently, a 4pM library containing 10% PhiX control v3 (Illumina) was sequenced on a MiSeq Illumina platform (Illumina), using a 2 x 250 cycle Miseq Reagent kit v3 (Illumina) at the

MBSU laboratory for 2020 samples, and at The Applied Genomics Core (TAGC, Univ. of Alberta) for 2021 samples.

The unassembled fastq files were demultiplexed using the MiSeq Reporter software (version 2.5.0.5) and Miseq Local Run Manager GenerateFastQ Analysis Module 3.0. The assembly results were written into the fasta format, and assembled reads were analyzed using the Quantitative Insights Into Microbial Ecology (QIIME2) pipeline. Sequences were paired, denoised, and had chimeras and singletons removed using DADA2. Sequences were then parsed into amplicon sequence variants (ASVs). All sequences were classified using the SILVA database (version 138) (Quast et al., 2013; Yilmaz et al., 2014) with a stringent confidence threshold of 0.8.

2.2.4 Data analysis

2.2.4.1 Land use and land cover

LULC data were obtained from Alberta Biodiversity Monitoring Institute (ABMI), Government of Alberta (GOA), Alberta Geological Survey and Alberta Census of Agriculture. A total of 41 parameters were obtained from these datasets, encompassing geology, natural land cover, anthropogenic land use, climate, and topography (Table A2.3). These parameters were used to characterize the tributaries based on watershed LULC. To do so, watersheds for each tributary stream were delineated using ArcGIS (version 10.7.1) hydro toolbox and an Alberta-based digital elevation model (GOA, 2017). The LULC parameters were then overlaid onto delineated watersheds and percentage of each LULC parameter was calculated for each watershed. LULC percentages and climatological variables (Table A2.3) were standard scaled (variable mean subtracted and divided by the standard deviation) and used to construct a Euclidean distance dissimilarity matrix. Using this distance matrix a hierarchical cluster analysis with Ward linkage was used to group tributaries based on similarities in watershed LULC (Figure 2.1). These clusters were then used to assess water chemistry, organic matter composition, and microbial community composition across the basin. To further visualize the prominent LULC types associated with the hierarchical cluster determined groupings, LULC percentage was plotted against longitude.
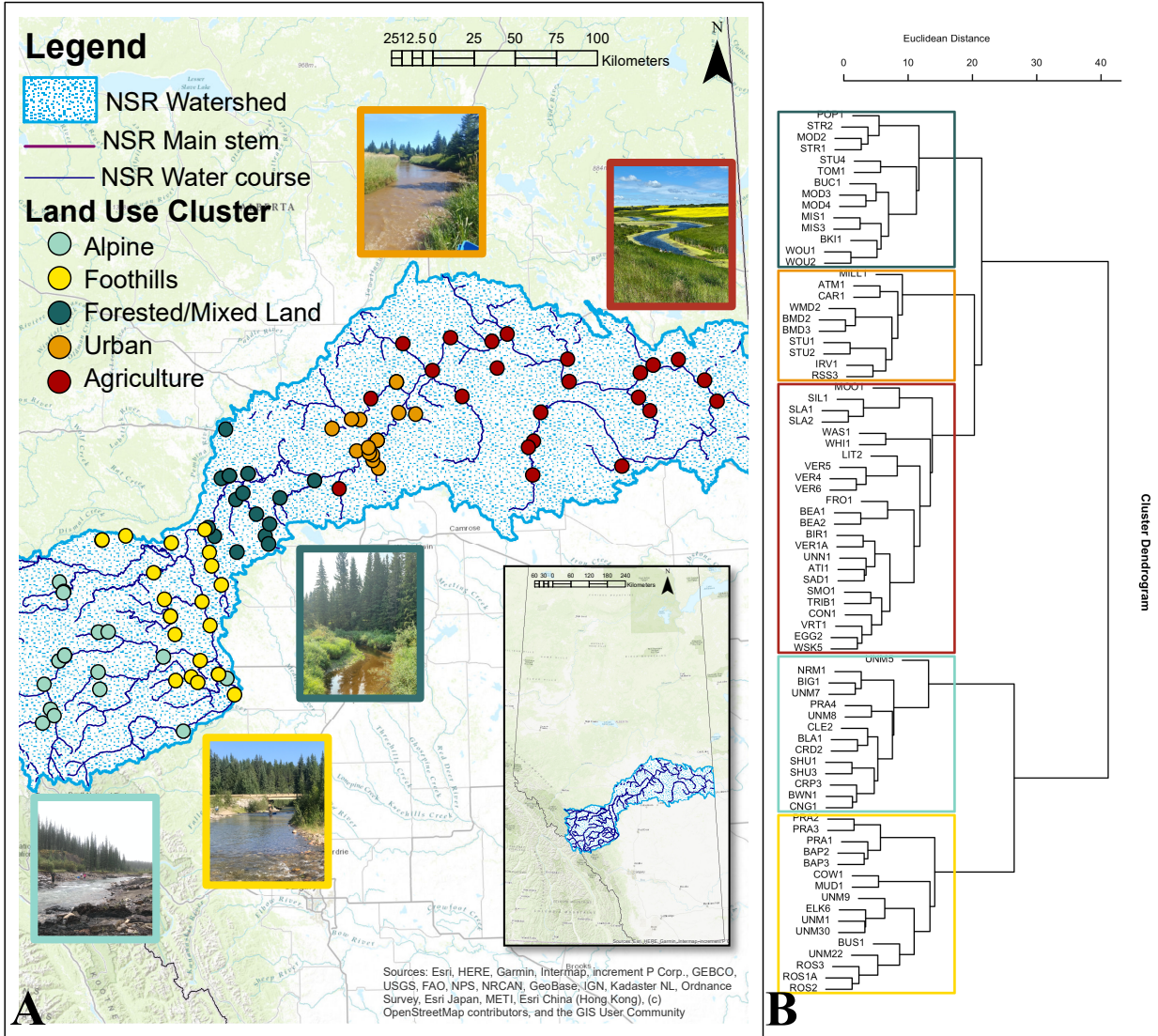
Figure 2.1. (A) Map of sampling sites spanning the North Saskatchewan River basin where each dot represents a stream sampled for water chemistry, organic matter, and microbial community composition. Colours are indicative of land use and cover (LULC) type (alpine, foothills, forested/mixed land, urban, and agriculture), as determined by a **(B)** hierarchical cluster analysis (Euclidean distance matrix, ward linkage) on LULC parameters for each tributary stream (See Table A2.3). Site coordinates are detailed in Table A2.1.

2.2.4.2 Water chemistry

All water chemistry field blanks had negligible concentrations and were excluded from further analysis. To account for water chemistry values below the detectable limit (BDL), the percentage of BDL data for each parameter was determined (Table A2.4). Variables with BDL percentages over 40% for the entire dataset were excluded from further analysis (Table A2.4). For variables containing between 1-40% of measurements BDL we substituted the BDL samples with the instrument detectable limit multiplied by $\sqrt{2}/2$ (Antweiler, 2015). This substitution allows for the data to be represented at a very low, but non-zero, value. Water chemistry variables underwent the same standard scaling as the LULC data (variable mean subtracted and divided by the standard deviation) to account for varying units (e.g. °C vs ug/L), and were combined in a Principal Component Analysis (PCA), to visualize differences across the LULC categories. Pairwise PERMANOVA (permutations=999) tests were done to assess statistical differences between LULC categories water chemistry.

2.2.4.3 Organic matter composition

To describe the character of dissolved organic matter (DOM) fluorescence within the tributary streams, parallel factor analysis (PARAFAC) using the drEEM Matlab package (Murphy et al., 2013) was conducted. PARAFAC is a statistical tool that can be used to model the main DOM components which best describe the EEMs of a given sample set (Bro, 1997; Murphy et al., 2013). EEMs were corrected for inner filter effects and Raman and Rayleigh scattering prior to PARAFAC analysis. Models including up to seven components were considered, and ultimately, a three-component model was chosen and validated by split half analysis. These components were compared to published sources on the OpenFluor database, and components were relativized per sample to control for differences in DOM concentration. Additional analysis of absorbance and fluorescence spectra peaks was also used to assess DOM character. Fluorescence data were used to calculate the Biological Index (BIX) (Huguet et al., 2009), Humification Index (HIX) (Ohno, 2002), and Peaks A, M, C, T, and B – all of which are indicative of different sources of OM (Hansen et al., 2016). Absorbance at 254nm was used to calculate SUVA$_{254}$ to assess DOM aromaticity (Weishaar et al., 2003). Similar to water

chemistry, variables were standard scaled and combined in a PCA, and pairwise PERMANOVA (permutations=999) were used to assess the OM character across LULC clusters.

2.2.4.4 *Microbial community composition*

Microbial ASVs were assessed using rarefaction curves to ensure that our resolved species abundance was not a function of sample size. Samples with less than 7,500 reads (n = 5 out of 78 total) were excluded. Of our three field blanks analyzed, all contained less than 7,500 reads except for one (field blank Q1). The top 10 ASVs from this field blank were attributed to contamination due to excessive read count observed in this sample (~70,000 reads), and were removed from all samples. Once these ASVs were removed the read count of Q1 was below 7,500 and was excluded from further analysis. In total 73 samples were retained, and all samples were rarified to the lowest sample read count of 7,545 to control for differential total read counts (i.e., sample size) across our dataset (Sanders, 1968). A Bray Curtis distance matrix was constructed and Nonmetric Multi-Dimensional Scaling (NMDS) and pairwise PERMANOVA (permutations=999) were used to assess differences in microbial community composition across LULC clusters.

2.2.4.5 *Indicator species analysis*

To identify taxa associated with the LULC clusters, we performed an indicator species analysis (ISA) on the raw (non-rarified) microbial ASVs. ISA assigns an indicator value to taxa which is based on how specific that species is to the group (i.e., higher IV value if a species is exclusive to a group), and how often it is found in that group (i.e., higher IV value if a species is present at all sites within the group) (Dufrene & Legendre, 1977). We ran an ISA on the ASVs across our significantly different LULC clusters (alpine, foothills, forested/mixed land, and ag/urban combined) (Figure 2.2C) and defined alpine indicators as those with an IV > 0.85, and a p-value of < 0.05. We ran a second ISA excluding the alpine sites and identified foothills, forested/mixed land, and ag/urban indicators as those with an IV > 0.65, and a p-value of < 0.05. Spearman rank correlation was used to analyze trends between indicator species relative abundance and a subset of water chemistry and OM parameters.

2.2.5 Software

All data analysis was done in R Studio (version 2022.07.2+576 Build 576). Packages used were *vegan* (Oksanen et al., 2009), *RVAideMemoire* (Hervé, 2022*), dendextend* (Galili, 2015), *corrplot* (Wei and Simko, 2021) and *ggplot2* (Wickham, 2011). Matlab (version 9.12.0) was used for PARAFAC analysis.

2.3 Results

2.3.1 Land use and land cover across the North Saskatchewan River basin

Hierarchical cluster analysis distinguished five LULC clusters across the NSR basin from west to east: alpine, foothills, forested/mixed land, urban, and agriculture (Figure 2.1A, B). Generally, the natural land cover of our tributary stream watersheds shifts from coniferous trees in the alpine sites, to more mixed forest and swamps in the foothills and forested/mixed land, to a near absence of trees in the urban and agricultural areas, with an increased presence of lakes and marshes (Figure A2.1). The anthropogenic land use of these watersheds increases as you move east (Figure A2.1). Notably, watersheds are relatively untouched by human influence in the alpine sites, with increased forestry in the foothills and oil and gas well sites in the forested/mixed land sites, increased industrial and residential land use in the urban sites, and increased farmland in the forested/mixed land, urban and agriculture sites (Figure A2.1).

Table 2.1 Characteristics of the land use and land cover (LULC) clusters as defined by the hierarchical cluster analysis (Figure 2.1B), the number of sites in each cluster, and the years these sites were sampled.

LULC Cluster	Defining characteristics/conditions	# tributary watersheds	Years sampled (2020, 2021)
alpine	high slope, low riparian area, coniferous trees, bedrock	14	4, 10
foothills	swamps, coniferous/deciduous mixed forest	16	8, 8
forested/mixed land	deciduous trees, bogs, well sites	14	7, 7
urban	residential, industrial, agriculture	10	7, 3
agriculture	agriculture, lakes, ice thrust moraine deposits	24	14, 2

2.3.2 *Water chemistry properties of the land use and land cover clusters*

Our PCA of 41 water chemistry parameters showed that the first axis (PC1) explains 39.6% of the variation in our water chemistry dataset and positively associated with TN, temperature, Cl^-, K^{2+}, and negatively associated with DO% (Figure 2.2A). LULC clusters similarly followed this PC1 gradient with alpine and foothills sites loading negatively, forested/mixed land being around zero, and urban and agriculture sites loading more positively (Figure 2.2A). The second axis (PC2), accounted for 11.7% of the observed variation in the water chemistry dataset, and is positively associated with TSS, NO_3^-/NO_2^-, Al^{3+}, some metals, and negatively associated with alkalinity, conductivity, pH, silica, and TDP (Figure 2.2A). The ordination is based off of all water chemistry variables (shown in Figure A2.2) with the exception of POC, PON, and chlorophyll *a*, which were incomplete for the dataset and thus excluded from the ordinations, but are thought to still be represented by correlated variables (Figure A2.3). The alpine and foothills sites are tightly clustered around zero, whereas the forested/mixed land, urban, and agriculture load across both the positive and negative axes of PC2 (Figure 2.2A). All clusters were significantly different (pairwise PERMANOVA on Euclidean distances, $p < 0.05$).
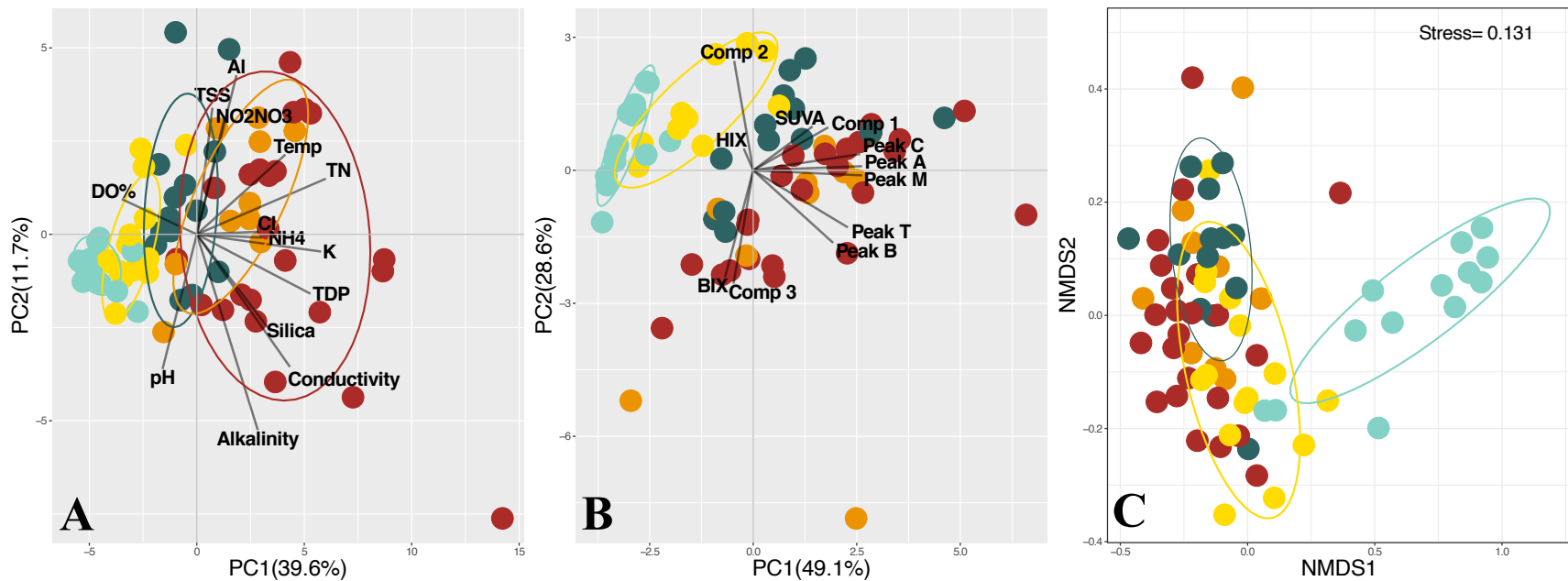
Figure 2.2 Principal components analysis of (A) water chemistry, (B) organic matter (OM) composition, and (C) nonmetric multi-dimensional scaling of microbial community composition. Select vectors are plotted, but ordinations are based on the entirety of each individual dataset (see Methods and Table A2.2 for details). Each dot represents the water chemistry, OM, or microbial community composition at one tributary stream, with the dot colouring indicative of land use and land cover (LULC) type as determined by the hierarchical cluster analysis shown in Figure 2.1B. Ellipses represent clusters that are significantly different from all other LULC clusters (pairwise PERMANOVA, $p < 0.05$).

2.3.3 *Organic matter composition*

PARAFAC analysis identified three modelled components of Organic Matter (OM) composition; two terrestrial, humic-like components (Comp 1 and Comp 2), and one microbial, protein-like component (Comp 3) (OpenFluor) (Table 2.2). A PCA of these components as well as peak data, SUVA, BIX, and HIX indices, revealed 77% of the DOM data set to be explained by the first two axes (Figure 2.2B). The alpine and foothills sites are tightly clustered, loading negatively on PC1. Forested/mixed land, urban, and agriculture are more variable, loading more positively on PC1 (49.1% variance explained) along with variables Peaks A, M, C, T, B, SUVA, and the humic-like Comp 1. Across the second axis, PC2, in which 28.6% variance in the DOM dataset is explained, alpine and foothills sites cluster positively. Again, the forested/mixed land, urban, and agriculture sites are more variable where some load positively, associated with humic-like Comp 2, and other sites loaded negatively, associated with protein-like Comp 3 and the BIX index. Alpine and foothills sites were significantly different from the rest of the sites (pairwise PERMANOVA, $p < 0.05$).

Table 2.2 Summary of three PARAFAC components resolved in North Saskatchewan River basin dissolved organic matter. Excitation and emission matrices are shown for each component, as well the peak excitation and emission wavelength and likely carbon source as indicated by top three matches from OpenFluor (Literature).
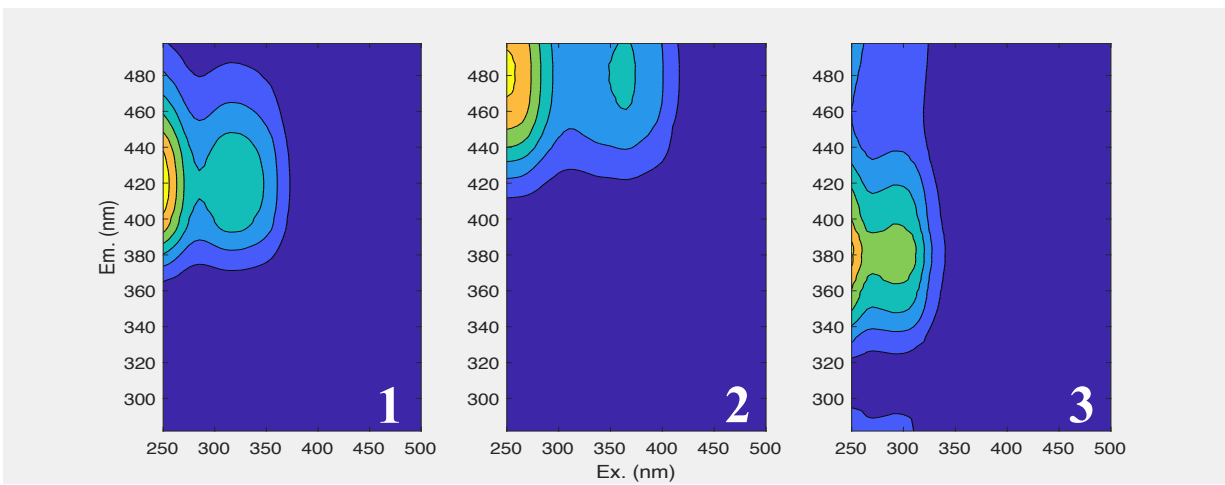
Component	Excitation: Emissions (nm)	Carbon Source	Literature
1	250: 417	Humic-Like	(Graeber et al., 2012) (Weigelhofer et al., 2020) (Guéguen et al., 2016)
2	250: 479	Humic-Like	(Williams et al., 2010) (Shutova et al., 2014) (DeFrancesco & Guéguen, 2021)
3	250: 375	Microbial-Like	(Borisover et al., 2011) (Andersson et al., 2018) (Yamashita et al., 2011)

2.3.4 Microbial community composition

After sequencing of the 16S rRNA gene, total read counts across the 73 sites for which we had sequencing data ranged from 7,545 to 180,780. In total 20,854 unique ASVs were identified across all sites. At the family taxonomic level (Figure A2.4), 42 microbial families had a relative abundance of >2% across our entire dataset, and illustrated notable differences between our five LULC clusters. NMDS analysis on the ASV microbial dataset revealed the alpine, foothills, and forested/mixed land clusters to be significantly different from the other two clusters (pairwise PERMANOVA, $p<0.05$) (Figure 2.2C). In particular, the NMDS showed a substantial separation in ordinal space of the alpine sites from the other clusters across NMDS1 (Figure 2.2C). Generally, the *Comamonadaceae, Flavobacteriacea*, and *Spirosomaceae* families were consistently present across a majority of the sites. *VadinHA49* was present across our alpine sites,and were generally absent from the rest of the sites except for one agriculture sample. Outside of the alpine cluster, the *Sporichthyaceae, Chitinophagaceae*, and *Burkholderiaceae* families were consistently present across the foothills, forested/mixed land, agriculture, and urban clusters. Reads assigned to chloroplasts, presumed to be associated with eukaryotic photosynthesizers, were more relatively abundant in the forested/mixed land, urban, and agriculture clusters, whereas the cyanobacteria families *Microcystaceae* and *Cyanobiaceae* exhibited the greatest relative abundance in the urban sites located around the city of Edmonton.

ASVs across the five LULC clusters were found to be both unique to the individual clusters as well as shared amongst all five (Figure 2.3A). Across our LULC, the alpine sites had the highest number of unique ASVs (10,521) with the other four clusters having comparatively fewer unique ASVs (foothills=1,320, forested/mixed land=1,520, urban=1,178, agriculture= 2,190). The alpine cluster sites showed greater variation in the relative abundance of unique species (ranging between 1% and 45%), whereas sites from the other four clusters showed lower relative abundance with only four sites having greater than 10% contribution of unique species (Figure 2.3C). The high number of unique ASVs present in the alpine cluster is reflected in the significantly higher alpha diversity (calculated using the Shannon Diversity Index) observed in these samples compared to our other LULC clusters ($p<0.05$, Pairwise Wilcox test). The urban and alpine clusters were not significantly different, but only slightly ($p= 0.0520$) (Figure 2.3B). Additionally, the foothills LULC cluster has the fewest number of unique species and a significantly lower alpha diversity than the other four clusters ($p<0.05$, Pairwise Wilcox test).

Across all of our LULC clusters, 338 ASVs were shared (referred to as 'core' herein) (Figure 2.3A). Sites in the foothills, forested/mixed land, urban and agriculture LULC clusters had a high relative abundance of the 338 core ASVs, accounting for between 30-98% of the relative community abundance in these samples (Figure 2.3C). In comparison, within the alpine cluster, individual samples exhibited a wider range of relative abundance of the core ASVs (ranging between 6% and 93%). Alpine sites SHU1, SHU3, and BWN1 showed greater similarity to the non-alpine sites with 86%, 92%, 93% core ASVs and 2%, 1%, and 6% unique ASVs.
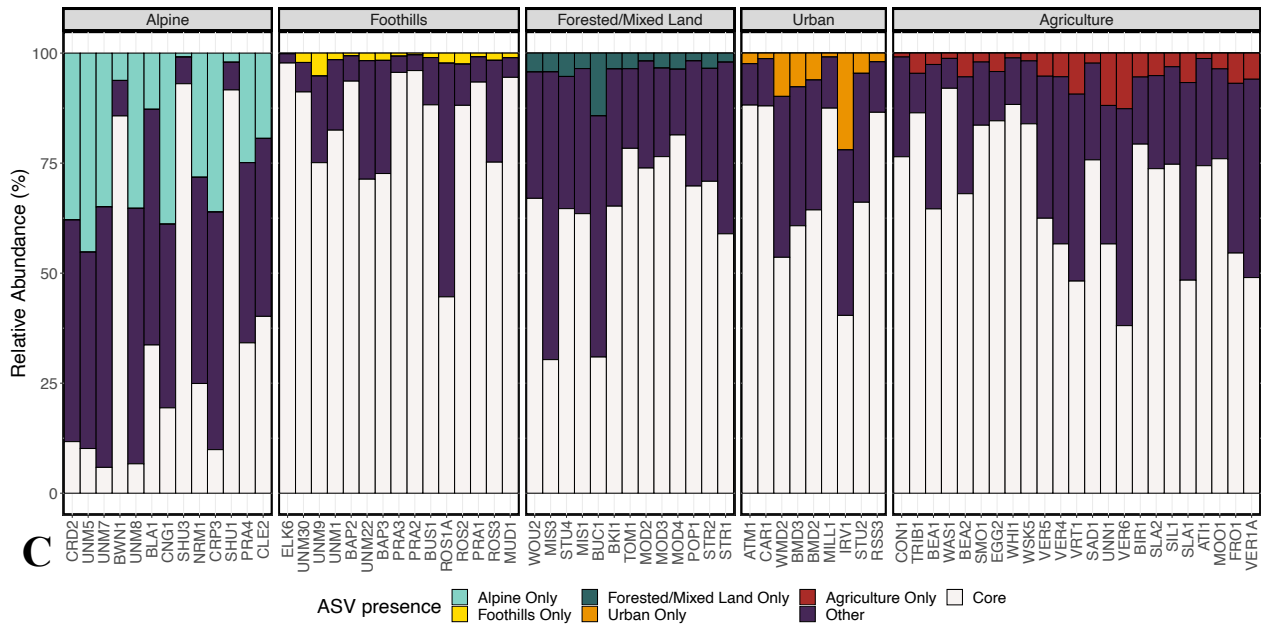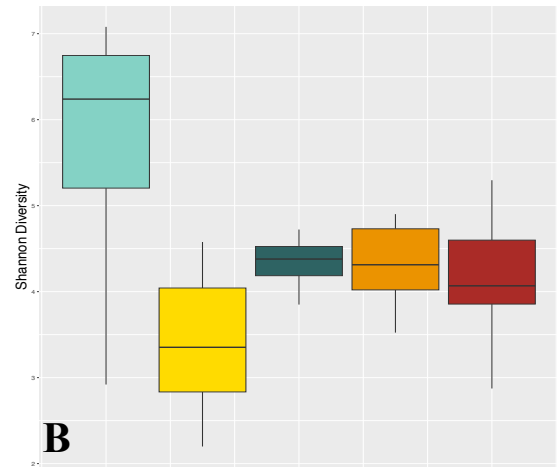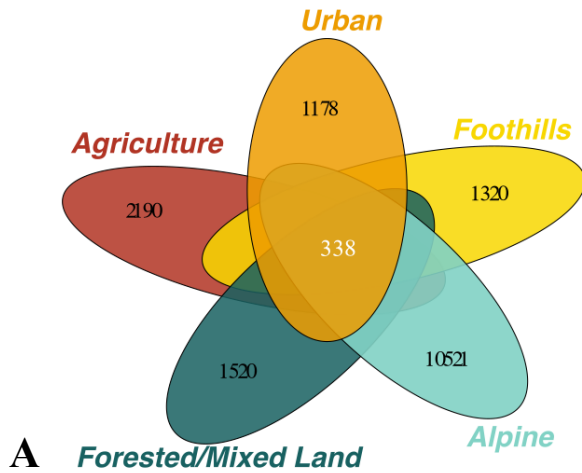
Figure 2.3 (A) Venn Diagram of shared and unique amplicon sequence variants (ASVs), (B) Shannon's diversity index for microbial assemblages, and (C) relative abundance of shared and unique ASVs across North Saskatchewan River tributary streams in the five land use and land cover (LULC) types sampled in this study. Colours represent LULC type (blue for alpine, yellow for foothills, green for forested/mixed land, orange for urban, and red for agriculture). In (C), sites are grouped by LULC and ordered by longitude (west to east) within each cluster. ASVs present in all LULC clusters are labelled 'Core' and ASVs shared among 2-4 land use clusters are labelled as 'Other'.

2.3.5   Indicator species

Indicator species analysis illustrated further separation of the alpine sites from other LULC classes, where 10 ASVs were strong indicators for the alpine (indicator value > 0.85, p < 0.05). In comparison, four ASVs were indicators for the foothills, two ASVs were indicators for the forested/mixed land, and three ASVs were indicators for the agriculture/urban (ag/urban) at a more moderate criterion (indicator value > 0.65, p < 0.05). The high diversity in the alpine and low diversity in the foothills is represented in the indicator species as well where the 10 indicator ASVs for the alpine sites include a diverse range of families including *VadinHA49, Comamonadaceae, Yersiniaceae, Nitrosopumilaceae, Flavobacteriaceae*, and *Nitrospiraceae*, and of the four foothills indicator ASVs, three are from the family *Flavobacteriaceae*. Finally, across our entire dataset, ASVs from the family *Comamonadaceae* were fairly ubiquitous with indicators in the alpine, forested/mixed land, and ag/urban clusters.

All 10 alpine indicators show similar correlation to the water chemistry and DOM variables, with negative correlation to parameters that were higher outside of the alpine streams (Figure 2.2A and 2.2B), including δ^{18}O, turbidity, PP, Al, K, Cl, Fe, As, TDN, humic-like Comp 1, and HIX and positive correlation to humic-like Comp 2 (Figure 2.4). Foothills and forested/mixed land indicators show little to no correlation to the water chemistry and OM parameters. Ag/urban indicators generally show strongest positive correlation to δ^{18}O, TDN, K, Cl, and As, and a negative correlation to humic-like Comp 2. There is slight variation within the ag/urban cluster, where genus *C39* in the family *Rhodocyclaceae* has no correlation to protein-like Comp 3, and a less negative and more positive correlation to Comp 2 and Comp 1, respectively, than the other ag/urban indicators of genus *Hydrogenophaga* and *Algoriphagus* (Figure 2.4).

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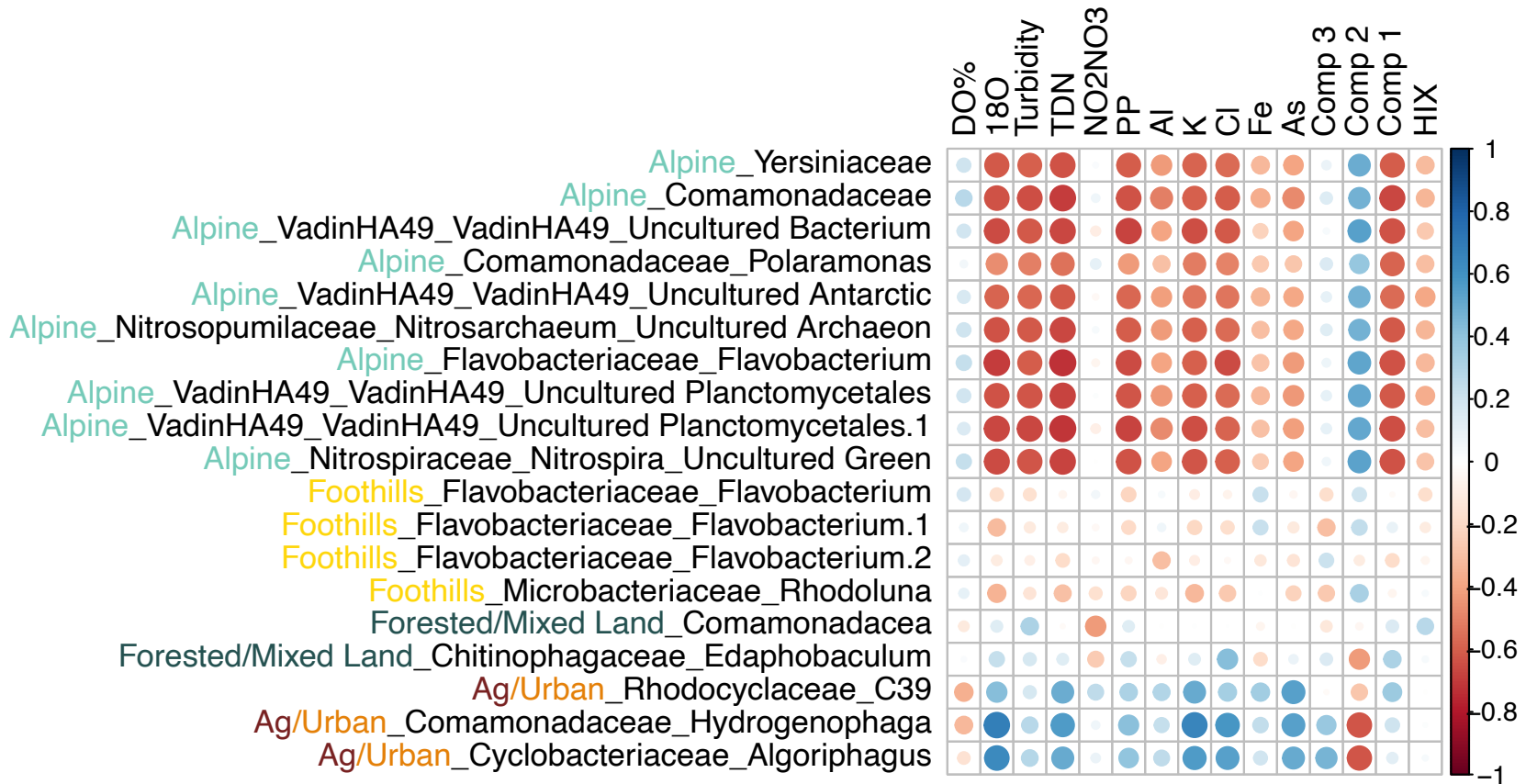
Figure 2.4 Indicator species and their correlation (spearman) to water chemistry and organic matter (OM) parameters. Indicator species for alpine sites had an indicator value of > 0.85 and Foothills, Forested/Mixed Land, and Ag/Urban had an indicator value > 0.65. Agriculture and urban land use and land cover (LULC) clusters were combined for indicator species analysis since the microbial communities were not significantly different (See Results and Figure 2.2C). Water chemistry and OM parameters were selected to represent unique trends across the basin (Figure A2.3). The colour of each dot represents either a positive (blue) or negative (red) correlation, and the size of each dot represents the strength of the correlation co-efficient (Spearman's Rho). Indicator species naming format lists the LULC cluster followed by the Family_Genus_Species of each amplicon sequence variant when all are defined.

2.4 Discussion

2.4.1 North Saskatchewan River tributary stream water chemistry is tightly connected to landscape conditions

Our findings demonstrate close connections between landscape conditions and stream water chemistry that are consistent with findings of numerous studies. As a key contributor to the water budget of stream ecosystems, runoff tightly links changing landscape conditions to stream water quality. As runoff traverses the watershed on its way to a stream, it accumulates compounds such as organic matter from the soil (Autio et al., 2016) or fertilizer from cropland (Carpenter et al., 1998). It is this passive integration of what is on the landscape that shapes runoff composition, influenced by both natural and anthropogenic landscape factors (Hamid et al., 2019). For example, vegetation can intercept runoff and utilize the water and nutrients (Peterjohn & Correll, 1984). The interaction of the runoff with the landscape is also greatly impacted by the slope of the watershed, where higher slopes transport runoff to the stream more rapidly, resulting in a lesser degree of interaction with the landscape, and often more dilute streams (Clow & Sueker, 2000). In addition to natural influence, anthropogenic land use, such as urbanization and agriculture, can significantly impact streams, and are of particular focus as these land uses become more widespread as populations increase regionally and globally (Meyer & Turner, 1992). Expansion of urban and farmland areas is often tied to watershed changes, including degrading water quality, nutrient enrichment, increased sedimentation, and contaminant pollution (Allan, 2004). In particular, urban watersheds increase the number of impervious surfaces across a landscape, which not only increase the transport of compounds to streams but also replaces vegetation that would intercept the runoff, thereby increasing discharge downstream (Walsh et al., 2005). Agricultural landscapes are, by comparison, often correlated with increased nutrient concentrations of both nitrogen and phosphorus in soils, due to an over-application of fertilizer/manure to crops, and thus flushing of these nutrients to streams (Carpenter et al., 1998). Additionally, higher amounts of ions can be found in anthropogenically influenced areas where removal of vegetation result in increased ground water that transports ions to the soil surface (Kaushal et al., 2021). In sum, many factors can shape runoff composition, ultimately resulting in the close connection often observed between landscape type and stream water chemistry, a relationship which has motivated water quality monitoring efforts for decades (Puckett, 1995).

Across the NSR basin in Alberta, landscapes are highly variable, starting in pristine alpine conditions in the west, and moving through forested, urban and agricultural landscapes in the east. Thus, variations in slope, soil, geology, vegetation, and land use all likely influence runoff and water chemistry of tributaries and the main river stem throughout the basin. In a watershed of a similar landscape diversity in Michigan (USA), 62 streams of stream orders 1-6 across catchments of undisturbed landscapes, heavy agriculture, and major cities were assessed, with landscape and geomorphology characteristics found to explain 56% of the observed variance across eight water chemistry parameters (Johnson et al., 1997). Similarly, across small (i.e., stream order 2-6) NSR tributaries, with a much larger water chemistry dataset (48 parameters measured) we see a close connection between landscape type and water chemistry, where each LULC cluster (i.e., alpine, foothills, forested/mixed land, urban, and agriculture) has unique water chemistry conditions (Figure 2.2A), and we see degrading water quality (e.g., nutrient enrichment) with increasing anthropogenic influence.

Starting in the west, alpine streams are typically dilute, attributed to limited soil water interaction in the basin because of high slope, slow weathering bedrock, and limited soil development within the watershed (Clow & Sueker, 2000). Together, these factors result in runoff quickly flowing over the landscape and mostly traversing over rock, as opposed to soil, thereby entraining few dissolved particles from the landscape. These characteristics have been attributed to the dilute streams in the alpine and sub-alpine streams in the Canadian and American Rocky Mountains (Hauer et al., 2007). A study in the Colorado Rocky Mountains when comparing nine sub basins, of various slopes, vegetation, and geology, found higher slopes and limited vegetation to be positively correlated to runoff, and negatively correlated to ions, silica, and alkalinity (Clow & Sueker, 2000). The role of slope alone has also been emphasized in non-alpine studies as well where a basin wide study of approximately 800 sites in North West England found slope had a negative correlation to concentrations of base cations, nutrients, and metals (Rothwell et al., 2010). We similarly observe this trend in our water chemistry characterization of the upland alpine and foothills streams, where streams were universally low in nutrients, ions, turbidity and temperature (Figure 2.2A).

Coming down from alpine regions, the presence of forests across watersheds also plays a role in inhibiting transport to streams, particularly with regards to dissolved ions, like SO_4^{2-} and NO_3^- (MacDonald et al., 2012), as well as nutrients, like nitrogen and phosphorus (Peterjohn &

Correll, 1984). This can occur because plant roots tend to preferentially uptake both ions and nutrients in runoff before they are able to reach stream networks, namely plant macronutrients such as nitrogen, phosphorus, and potassium (Chapin et al., 2011), effectively modifying the runoff. This is facilitated by the high porosity of forest soils from animal burrowing and deeper root growth, resulting in most runoff infiltrating the soil, thus interacting with plant roots, and reduced overland flow to streams as a result (Neary et al., 2009). Additionally, biogeochemical processes removing nutrients can be enhanced in low lying forests in riparian areas where water can accumulate, resulting in anoxic saturated soils. In studies on the riparian areas of the Canadian Boreal Plain which traverses Alberta, nutrient uptake from nitrogenous fertilizers was attributed to denitrification, where nitrate is transformed to nitrogen gas. The authors state that this is amplified in forest soils where anaerobic conditions can form with soil saturation, and where there is high carbon availability from the organic rich forest floor, that serves as an energy source to the denitrifying bacteria (Luke et al., 2007). In contrast, soils in the Canadian Boreal that are drier, favour nitrification resulting in the soils as a possible source of nitrogen to the streams (Luke et al., 2007). This filtering ability of forest ecosystems has been observed in many systems, resulting in protection of natural forests or replanting of trees as a recommendation for water quality conservation, particularly with regards to the riparian zone, where the trees would be in the optimal location to intercept runoff and the soils more prone to saturation (Luke et al., 2007; Sweeney & Newbold, 2014). It is this mitigative effect of forest cover on streams in the forested/mixed land tributaries, that we propose explains nutrient levels of TN, TDN, TP, and TDP remaining relatively low compared to the urban and agriculture sites (Figure 2.2A). Where most sites across these three clusters have a predominant agricultural presence, the forested/mixed land also has deciduous tree coverage, particularly in the riparian zone, where the urban and agriculture sites have little to no tree coverage. It is thus likely that these forests in the watershed and the riparian zone are maintaining the water quality of the nearby streams that would otherwise be compromised from agricultural influences.

Consequently, where forest cover declines in the urban and agriculture sites, water quality parameters such as turbidity, nutrient, and ion concentrations start to rise. Between urban and agricultural streams, there are few water quality differences, as they tend to have similar water chemistry profiles, characterized by high nutrients, turbidity, contaminants, and temperatures (Allan, 2004). Several processes combine to result in this observed similarity,

including the aforementioned clearing of vegetation, which also results in decreased canopy cover and more direct exposure to sunlight to heat up streams (Wehrly et al., 2006). Additionally, alteration of flow paths for both urban and agricultural streams results in increased runoff and erosion, leading to greater sediment loads (Russell et al., 2017). Runoff also integrates fertilizers applied to landscapes, which is thought to be the primary cause of higher nutrient levels within these types of watersheds (Carpenter et al., 1998). Similar to previous studies, we found that, compared to the other LULC clusters, the urban and agriculture sites have the greatest similarity amongst their water chemistry parameters.

Urban sites have been found to differ slightly from agriculture watersheds in that they tend to have higher contaminants and turbidity (Ai et al., 2015; Walsh et al., 2005). Indeed, when comparing agriculture and urban watersheds from across the world, urban catchments transported more than double the amount of sediment than agricultural catchments (Russell et al., 2017). This observation was attributed to increased runoff from the watershed due to the greater coverage of impervious surfaces, and thus more efficient erosion of exposed surfaces such as infills and gravel. It is also common to find higher amounts of chloride and in urban areas due to de-icing mechanisms using sand and road salts (which are often calcium chloride), as specifically seen in the North Saskatchewan River basin (Laceby et al., 2019). Despite their similarities, it is likely these mechanisms that result in the significant differences in our urban and agriculture streams, where urban sites have higher particulates (TSS), as well as higher amounts of chloride and calcium ions.

The water chemistry of the agricultural streams also shows the greatest variation between locations with some sites more similar to the urban sites with higher particulates, DOC, temperature, nitrogen, and metals, and others with higher pH, ions, and phosphorus (Figure 2.2A and A2.2). In contrast, we do not observe larger-scale differences in landscape variables between these sites, thus it is possible that unique conditions at individual stream locations are driving these differences. Possible differences in the sites include precipitation events, represented by differences in flow, where after a precipitation event, we would expect to see higher amounts of particulates and nutrients flushed into the stream, versus during periods of drought we would expect increased concentrations of ions due to greater relative input from groundwater sources and increased evaporation (Mosley, 2015). Using flow data from monitoring stations nearby to each sampling location (Environment and Climate Change Canada, 2023; Government of

Alberta, 2023), no differences were identified between the two groups (Figure A2.5), but it is possible that the assessment was too coarse to encapsulate site specific events. Where for example, the flow data collected from the nearest station is not representative of the sampled stream as the precipitation event was more localized, or flow was altered by natural differences such as beaver dams upstream. Other restraints on our flow analysis include that we only sampled each site once. Another possibility for this difference is the erosivity of the landscape, where we would expect higher particulates in more erosive landscapes. Regardless, our findings support literature consensus that stream water chemistry is largely influenced by watershed landscapes including factors such as slope, vegetation, and land use and degrading water quality is seen with increasing anthropogenic influence.

2.4.2   Stream dissolved organic matter composition shifts with differences in soil type

Similar to general water quality, differences in landscapes can significantly influence the composition of organic matter delivered to streams from the surrounding watershed. Allochthonous organic matter, which is produced outside of the stream, is generally derived from surrounding plant and soil matter, typically has a higher molecular weight and aromaticity, and is thought to be the dominant source of OM in stream ecosystems (Brooks et al. 1999). Differences across natural landscapes can source different types of OM to streams, as has been observed in Alaska where unique DOM signatures were identified across terrestrial soils of four different wetland and forested landscapes (bog, fen, forested wetland, and upland forested) (Fellman et al., 2008). Across our sites in the NSR basin, soil types are generally brunisols in the alpine, luvisols in the foothills, and chernozems in the forested/mixed land, urban, and agriculture sites (Alberta Soil Information Centre, 2016). Across the NSR basin, we find OM composition to be distinct across the alpine and foothills sites, but not between the forested/mixed land, urban and agriculture sites. These differences appear to be driven primarily by allochthonous OM composition and quantity (Figure 2.2B) possibly as a result of variation in soil types across the basin that follow this same pattern. Compositionally, the watersheds with chernozemic soils, forested/mixed land, urban, and agriculture, have higher proportions of humic-like Comp 1 and greater quantity of OM, as seen through the positive association with humic-like Peaks A, M, and C (Figure 2.2B). Chernozems have been found to be high in OM, attributed to the clay like properties of the soil that can physically protect organic matter from decomposition (Pennock et

al., 2011). It is also possible increased agriculture in these areas plays a role in organic matter dynamics; for example, in a study in northern Alabama (USA) agriculture watersheds were found to have greater delivery of soil derived OM, and the authors suggested that agriculture can compact the soil, allowing flow paths to become more shallow, ultimately resulting in runoff that traverses soil that is more rich in organics (S. Chen et al., 2021).

In contrast to watershed-derived OM, autochthonous OM, which is produced within the stream by microorganisms and macrophytes, is generally less structurally complex, and often has more nitrogen molecules (Fellman et al., 2008; Yamashita et al., 2011), both of which result in autochthonous organic matter being more biologically available, or labile. Microbially derived autochthonous OM can be representative of in stream productivity that results in biological assimilation, identified as protein-like OM, or representative of humic by-products of in stream decomposition of detritus identified as microbial- humic like OM (Fellman et al., 2010). In this study we observe a microbial-like Comp 3 that is associated with a subset of streams in the forested/mixedland, urban, and agriculture clusters (Figure 2.2B). The top four matches in OpenFluor for the microbial-like Comp 3, identified this component as either protein like, or microbial humic-like (Table 2.2). BIX values, when greater than 1, are typically indicative of fresh autochthonous organic matter (Guarch-Ribot & Butturini, 2016). In this study, our highest BIX value across all landscapes was 0.87, thus, taken together, it is likely that Comp 3 in this study is more representative of microbial-humic like organic matter, but it is difficult to definitively discern without further analysis of the organic matter (e.g., mass spectrometry).

Autochthonous OM can also be influenced by varying landscapes as they alter nutrient concentrations (Wilson & Xenopoulos, 2009), light and temperature (Reche et al., 1998), and OM character sourced into the stream (Amon & Benner, 1996). Agriculture has been associated with increased microbially-derived stream OM where explanations include that nutrients from agriculture runoff stimulate autochthonous production (Wilson & Xenopoulos, 2009) or alternatively that agriculture processes such as tillage increase soil microbial activity that are eventually transported to the stream (S. Chen et al., 2021). Moreover, we did not find microbially-derived OM to be higher across all agriculture sites, but only in a cluster of sites spread between the forested/mixed land, urban, and agriculture clusters (Figure 2.2B). Although each of these have agriculture within their watersheds, what differentiates these streams from the others in our dataset which also contain agriculture, but are not associated with microbial-like

Comp 3, remains unclear. Previous findings that found microbially-derived OM to be higher in agriculture catchments that suggest it to be a product of either higher nitrogen levels or temperature (Liu et al., 2022; Wilson & Xenopoulos, 2009), as each can stimulate microbial productivity, but we did not observe either of these to be higher in this cluster of sites (Figure A2.6). Agricultural practices have been found to increase microbial derived soil OM due to practices that aerate the soil (e.g., ploughing, tillage), which in turn facilitates microbial OM transformation of in situ soil OM that is then transported downstream. (Heinz et al., 2015). Presumably, the release of OM varies with intensity of these processes, which could possibly explain this subset of sites associated with greater microbially derived OM. Other suggestions for increased microbially-derived OM in streams attribute decreased canopy cover, as this can result in photodegradation resulting in less complex organic matter (Masese et al., 2017). This conclusion could possibly explain the increased autochthonous OM in anthropogenic systems as decreased canopy cover could result in more labile carbon for microbial consumption. Thus, it is possible our study did not capture more important landscape differences, such as canopy cover or intensity of agricultural practices, in explaining microbially derived OM in the select cluster of sites. Our findings conclude that OM is distinct across major landscape differences largely driven by differences in allochthonous OM, and that finer scale landscape differences are possibly important controls on microbially-derived OM.

2.4.3   *Microbial communities distinct across land use and land cover clusters*

Closely connected to both water chemistry and organic matter, microbial communities in streams have been shown to be unique across different landscape types (Jones et al., 2020; Staley et al., 2014; Zeglin, 2015). These differences are often characterized by a shift in relative abundance of common freshwater microorganisms such as a core community (i.e., taxa which are universally shared across all samples in a study) (Staley et al., 2015). For example, over a 1-year study in the English Channel, 93 sequences were found across all samples collected (n = 12), while accounting for 54% of all the sequence reads (n = 17,673) (Gilbert et al., 2009). In another study in the upper Mississippi River, 552 taxa (out of a total of 16,400) accounted for 90.5% of all sequence reads (Staley et al., 2013). In this study, we similarly see a core community of 338 ASVs shared across all tributaries, which is found in a large range of relative abundances (6-98%) across the NSR watershed (Figure 2.3C). This core community was made

up of common freshwater families found across the basin including *Comamondaceae, Spirosomaceae, Flavobacteriacea, Burkholderiaceae, Chitinophagaceae, Sporichthyaceae*, and *Crocinitomicaceae* (Zwart et al., 2002) (Figure A2.4). Despite its universal presence across the NSR tributaries the core community fluctuates greatly, having the lowest relative abundance (6%) in the alpine sites, and the highest (98%) in the foothills.

Differences in microbial communities across stream ecosystems are also characterized by the presence/absence of specific species (Jones et al., 2020; Simonin et al., 2019). We observe this trend across our LULC clusters in this study, with all of the clusters containing ASVs found only within that particular landscape type (Figure 2.3A, C). To investigate the taxa associated with these sites, indicator species analysis was used to identify strong taxonomic indicators (IV > 0.85) for the alpine region, who are presumably adapted to these streams (Figure 2.4). Notably, taxa of the *VadinHA49* family were discovered to be indicative of our alpine streams, and previously, members of this group have been shown to have low organic matter uptake in an incubation experiment (Coskun et al., 2018). Additionally, an indicator of the genus *Polaromonas* a psychrophile that is commonly found on glacier surfaces (Gawor et al., 2016), was also found in our alpine streams. Weaker indicators (IV > 0.65) were identified for the other LULC clusters, including members from the genus *Hydrogenophaga*, facultative autotrophs known to be hydrogen oxidizers (Willems et al., 1989), for the urban/agriculture sites. Using hydrogen as an electron donor is a metabolic process exclusive to anoxic conditions, thus it is possible that anoxic conditions form in these lower flowing urban and agriculture streams. Together, our findings corroborate current literature that finds both a shift in relative abundance of common freshwater taxa as well as the presence of specific species to be characterizing differences in microbial community composition across different landscapes.

2.4.4 *Possible importance of dispersal mechanisms in shaping community composition and diversity*

Controls on microbial community can be investigated through a metacommunity framework (Leibold et al., 2004), where a metacommunity is defined as separate communities linked through dispersal. Within this framework the primary mechanisms driving these differences in microbial community composition in stream ecosystems are dispersal from upstream or surrounding environments (e.g., soil) (Ruiz-González et al., 2015) and species

sorting, whereby selection for specific taxa occurs as stream conditions change (i.e., as microorganisms move downstream) (Staley et al., 2016; Van der Gucht et al., 2007). Proponents for the species sorting hypothesis argue that microorganisms respond rapidly to changing environmental conditions (Van der Gucht et al., 2007), and thus that community structure is an outcome of the abiotic environmental conditions, where different taxa are found in specific environments because they are better suited to live there. In our system, the species sorting approach would entail that differences in microbial community composition across the LULC clusters occur as a result of differences in stream conditions. In contrast, the dispersal approach advocates that microbial community structure is primarily a consequence of dispersal from surrounding communities. In our study this mechanism of driving microbial community composition would entail that differences across the LULC clusters are a result of different microbial communities inoculating the streams through runoff. Disentangling the dominant mechanism in stream environments is often challenging due to the inherent correlation between changing landscape types and changing stream water chemistry (Carpenter et al., 1998; Z. Chen et al., 2021; Clow & Sueker, 2000).

Consistent with the metacommunity framework, it is likely that both are occurring at some level, as investigated in a study across Arctic river surface waters, where headwater (thus minimal dispersal from upstream) stream and soil communities were most similar, and suggested that initial dispersal from soils is a major source to the river community, with selection and adaptation happening as conditions change downstream (Crump et al., 2012). A similar study across an entire boreal watershed in Quebec had similar findings, attributing >75% on average of aquatic taxa to terrestrial origin across all sites (Ruiz-González et al., 2015). Other studies that similarly found freshwater and soil communities to significantly overlap (Lozupone & Knight, 2007), explain that despite the differences in habitat, soils can develop micro-niches where planktonic microorganisms can survive, such as within interstitial waters (Crump et al., 2012). The transferring of these planktonic communities from soils to streams has been suggested to primarily occur during hydrological events, when high flow conditions can result in an increase in bacterial cells in streams (Caillon & Schelker, 2020), providing a mechanism of transferring the soil community from soils to the stream (Staley et al. 2015). In a study in the upper Mississippi River, when comparing across sediment, water, and soil microbial communities surrounding the Mississippi, a ubiquitous core community was found to be present and comprise

at least 40% relative abundance in all samples (Staley et al., 2015). It is this dispersal mechanism from soils followed by species sorting after the dispersal event, that we propose explains the differences in core community relative abundance across the NSR basin for a few reasons (Figure 2.3C). First, the alpine sites have the lowest relative abundance of the core community, and also the least amount of soil interaction due to increased slope and bedrock in the watershed. Second, the foothills sites have the highest relative abundance of the core community, well-developed soils, and high amounts of precipitation compared to downstream landscapes, thus flushing events of soil microorganisms to the streams would be the most frequent in these regions (Figure A2.1). Third, we see moderate relative abundance of the core community in the forested/mixed land, urban, and agriculture sites as soil influence in the watershed is maintained, but precipitation declines. It is possible that when there is not as frequent inoculation from the soil, other species can flourish, outcompeting the generalist core. Finally, we also see higher relative abundance of microorganisms within the *Actinobacteriota* phylum outside of the alpine sites, which has been found to be more prevalent in soil communities compared to surface water (Staley et al., 2016).

In addition, contrasting the species sorting hypothesis, which would argue distinct environmental conditions result in distinct microbial communities (Van der Gucht et al., 2007), we do not see distinct microbial communities between the urban and agriculture clusters despite their distinct water chemistry composition (Figure 2.2). This could support that dispersal mechanisms are important for these LULC clusters particularly because these two clusters are the most similar in geographic location (Figure 2.1A). Together, our study supports the metacommunity framework suggesting both species sorting and dispersal to be important mechanisms in determining microbial community structure, with a possible greater influence of dispersal across the NSR basin.

2.4.5   *Differences in alpha diversity driven by presence of core community*

Other studies that have emphasized the importance of soils in inoculating stream microbial communities have also attributed the soil community to be a source of diversity. Previous work in the Arctic found alpha diversity to be the highest for the pelagic community in headwater streams when compared to downstream rivers and lakes (Crump et al., 2012), as a result of the increased importance of terrestrial inputs to headwater regions. Similarly, a study in

Austria also attributed increased organic matter terrestrial inputs to be the source of increased diversity observed in headwater biofilm communities (Besemer et al., 2013). In contrast to these works, even though our alpine sites had the highest microbial diversity, they were also the sites with limited terrestrial and organic matter input. Comparatively, our foothills sites exhibited the lowest diversity despite being those with the highest amount of precipitation and presumably the greatest terrestrial input. A recent paper assessing the relationship between nutrient supply and diversity in aquatic systems concluded that there was no single relationship for microorganisms as there are so many differences in regional community composition, connectivity, disturbances, and food web structure (Smith, 2007). Across our NSR tributaries, we propose that microbial diversity is directly related to the degree with which the core community is transported from the soil, with the alpine sites exhibiting the highest diversity due limited soil input, resulting in a limited dominant core community presence, allowing other taxa to flourish. In comparison, our foothill sites have the lowest diversity because they are dominated by the core community, which is effectively dispersed into tributary streams during high precipitation events. Although soil microbial community diversity patterns have been shown to be highly variable (Hendershot et al., 2017), a recent review found increased microbial community diversity in soils that underwent land use change (Zhou et al., 2020). This could provide an additional explanation as we see this same trend across the NSR watershed where greater diversity was observed in the more anthropogenically influenced forested/mixed land, urban, and agriculture sites, when compared to the more pristine foothills.

2.4.6   *Implications for a changing watershed*

Across the NSR basin the two primary mechanisms driving watershed change are: land use change (Martellozzo et al., 2015) and climate change (Newton et al., 2021). In a study assessing land use change from 1988-2010 in Alberta, urban and agriculture land use was found to have increased, with urban landscapes primarily replacing agriculture land use and agriculture, in turn, replacing natural vegetation (Martellozzo et al., 2015). If this trend continues, as it's expected to in the future, our study confirms that this particular landscape shifts towards agriculture and urban land use, will result in changes in water chemistry. Further, the shifts from agriculture to urban likely will not influence the microbial community, but further studies are needed to understand if a shift from natural vegetation to agriculture would impact community

structure. A recent analysis of Alberta's climate trends under potential IPCC scenarios, found rainfall to increase as snowfall declines and for high precipitation extremes to become more frequent (Newton et al., 2021). Our study suggests increases in precipitation will potentially decrease alpha diversity of stream microbial communities, as connectivity with the soil microbial community increases.

2.5   Conclusion

Our study aimed to understand the shifting patterns in water chemistry, organic matter composition, and microbial community composition across changing landscapes in the NSR basin. We found all three datasets to be impacted by natural and anthropogenic landscape differences including slope, vegetation, urbanization, and agriculture, with water chemistry being the most closely impacted. Organic matter composition was broadly driven by differences in terrestrial source pools, with differences in light and agriculture intensity possibly driving the presence of microbially derived OM. Microbial community composition differentiated across landscapes driven by presence of generalist and specialist communities, where alpine streams were the most distinct, with several indicator species identified. Furthermore, we found a possible large influence of soil community dispersal resulting in decreased diversity. Our study further clarified our understanding of the base of the food web in lotic ecosystems, and has important implications for both changing climate regimes and land use change.

Chapter 3. General Conclusions

3.1 Research Findings

       Motivated by shifting landscapes and climate regimes, our study sought to understand how these changes would influence water chemistry, organic matter composition, and microbial communities in stream ecosystems. We found water chemistry to be closely connected to landscape conditions, following well documented findings of dilute streams in undisturbed alpine and forested landscapes (Clow & Sueker, 2000; Peterjohn & Correll, 1984), and degrading water quality (i.e, higher nutrients, particulates) in streams with increasing agriculture and urban influence (Allan, 2004). The organic matter composition was also connected to landscape type but on broader scales, largely influenced by the differences in soil type across the basin. Finally, the microbial community composition was distinct across all landscape types, except for urban and agriculture, with the alpine community being the most different from the other landscapes. Community differences were driven by varying relative abundances of a core community, taxa shared across all samples composed of common freshwater microbes, as well as the presence/absence of specific species only found within a specific cluster. With the lowest relative abundance in the alpine streams and the highest in the foothill streams, we propose the core community to be sourced from the soil environment, as the foothills has the greatest soil interaction due to the presence of well-developed soils and increased precipitation which would allow for more frequent inoculation. Our findings build off of previous studies which emphasize the importance of the terrestrial environment in seeding the stream microbial communities (Crump et al., 2012; Ruiz-González et al., 2015). When this dispersal from soils is not as frequent from less precipitation, as in the sites further east, other species can flourish, resulting in a lower relative abundance of the core community. It is the presence of this core community that we propose results in a decreased diversity, as diversity is highest when the core community relative abundance is lowest. Our findings highlight the influence of landscape controls on the base of the aquatic food web and suggest shifting land uses and climate regimes to alter stream ecosystem functioning, specifically related to changing water chemistry and possibly decreasing microbial diversity.

3.2 Future Research

      This study identified distinct microbial communities across streams with varying watersheds and suggested possible mechanisms driving these differences. Our study highlighted the possibility of inoculation from soil communities to be driving differences in community composition and diversity, but no soil samples were collected to confirm this. Future studies focusing on the overlap of these communities and how this is influenced by precipitation events are needed to confirm our hypothesis. Additionally, our study assessed landscape influences across broad LULC classifications. To be of greater use to policy makers further research could focus on specific landscapes of concern (e.g., urban and agriculture), and assess how varying types and intensities impact the base of the aquatic food web.

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APPENDIX

Table A2.1 Land use and land cover (LULC) cluster, latitude, and longitude for all sampled tributaries. Sites ordered by longitude.

Tributary ID	LULC Cluster	Latitude	Longitude
CRD2	alpine	52.878326	-116.8979
UNM5	alpine	52.0030229	-116.48674
UNM7	alpine	52.2123682	-116.47777
BWN1	alpine	52.7638677	-116.36022
UNM8	alpine	52.3390242	-116.35423
BLA1	alpine	52.7071	-116.3232
CNG1	alpine	52.7129753	-116.3209
BIG1	alpine	52.369708	-116.30262
SHU3	alpine	52.4980461	-116.00314
NRM1	alpine	52.2830021	-116.00148
CRP3	alpine	52.1910577	-115.98406
ELK6	foothills	52.9904659	-115.98355
UNM30	foothills	52.9933695	-115.98098
SHU1	alpine	52.50101	-115.9157
UNM9	foothills	53.0165858	-115.76633
UNM1	foothills	52.6768227	-115.41571
BAP2	foothills	52.5863	-115.3573
UNM22	foothills	52.9806939	-115.35627
BAP3	foothills	52.488138	-115.31601
PRA4	alpine	52.24201	-115.3091
CLE2	alpine	51.9697	-115.2408
PRA3	foothills	52.2614457	-115.16903
PRA2	foothills	52.2317	-115.1134
COW1	foothills	52.3486	-115.09401
BUS1	foothills	52.4982	-115.0313
WOU1	forested/mixed land	53.0635	-115.0228
ROS1A	foothills	52.931	-115.01
ROS2	foothills	52.8586	-114.991
WOU2	forested/mixed land	53.018	-114.963
PRA1	foothills	52.2736	-114.9292
ROS3	foothills	52.7557	-114.9045
MIS3	forested/mixed land	53.3276442	-114.90385
STU4	forested/mixed land	53.5914	-114.8599
MIS1	forested/mixed land	53.3407389	-114.82888

Tributary ID	LULC Cluster	Latitude	Longitude
MUD1	foothills	52.1658	-114.7871
BUC1	forested/mixed land	53.2121	-114.7689
BKI1	forested/mixed land	52.931138	-114.76217
TOM1	forested/mixed land	53.3517	-114.6593
MOD2	forested/mixed land	53.10501	-114.5091
MOD3	forested/mixed land	53.0181012	-114.50373
MOD4	forested/mixed land	52.9746765	-114.47673
POP1	forested/mixed land	53.0802	-114.4648
STR2	forested/mixed land	53.2203712	-114.36739
STR1	forested/mixed land	53.3113	-114.0522
ATM1	urban	53.5902498	-113.88532
CON1	agriculture	53.2651	-113.8269
CAR1	urban	53.6368	-113.7064
STU1	urban	53.6355722	-113.62692
WMD2	urban	53.4565	-113.54801
LIT2	agriculture	53.74601	-113.5229
BMD3	urban	53.4456	-113.5172
BMD2	urban	53.415	-113.5164
MILL1	urban	53.5208	-113.4749
IRV1	urban	53.3744314	-113.46728
STU2	urban	53.8315	-113.3346
TRIB1	agriculture	54.03601	-113.212
RSS3	urban	53.6871	-113.07101
BEA1	agriculture	53.8886	-112.9494
WAS1	agriculture	54.0622	-112.7729
BEA2	agriculture	53.7452	-112.6822
SMO1	agriculture	54.0348	-112.3885
EGG2	agriculture	53.8896	-112.3487
WHI1	agriculture	54.0716	-112.2448
WSK5	agriculture	53.45901	-112.0909
VER5	agriculture	53.3089	-112.0632
VER4	agriculture	53.4915	-112.0412
VRT1	agriculture	53.6439	-111.9679
SAD1	agriculture	53.92101	-111.6961
UNN1	agriculture	53.8021	-111.6928
VER6	agriculture	53.6189589	-111.4515
BIR1	agriculture	53.3341	-111.2482
SLA2	agriculture	53.6972	-111.0627

Tributary ID	LULC Cluster	Latitude	Longitude
SIL1	agriculture	53.8289	-111.0333
SLA1	agriculture	53.6216	-110.9649
ATI1	agriculture	53.8667	-110.9122
MOO1	agriculture	53.8864	-110.676
FRO1	agriculture	53.7682	-110.4457
VER1A	agriculture	53.5103526	-110.3923

Table A2.2 Summary of data acquisition methods and the associated detection limit for all water chemistry parameters**.** Multi-parameter sonde data were acquired by deploying the instrument (Hydrolab DS5X Water Quality Multiprobe sonde) at each stream. Whole water and particulate samples were whole water samples collected directly from the stream, where particulate samples were then filtered through a GF/F filter or GF/C for chlorophyll *a*, and the filter was collected for analysis. Filtered samples were samples filtered through a syringe on site and the filtrate was collected for analysis.

Parameter	Sample type	Sample bottle	Analysis	Detection limit
Temperature	Sonde	-	Hydrolab DS5X Water Quality Multiprobe sonde	0.01 °C
pH	Sonde	-	Hydrolab DS5X Water Quality Multiprobe sonde	0.01
Conductivity	Sonde	-	Hydrolab DS5X Water Quality Multiprobe sonde	1 uS/cm
DO (mg/L)	Sonde	-	Hydrolab DS5X Water Quality Multiprobe sonde	0.01 mg/L
DO (%)	Sonde	-	Hydrolab DS5X Water Quality Multiprobe sonde	-
Turbidity	Sonde	-	Hydrolab DS5X Water Quality Multiprobe sonde	0.1
ORP	Sonde	-	Hydrolab DS5X Water Quality Multiprobe sonde	1 mv
Total Nitrogen	Whole water	1L polypropylene bottle	Lachat QuikChem 8500 FIA automated ion analyzer	11 µg/L
Total Kjeldahl **Nitrogen**	Whole water	1L polypropylene bottle	Lachat QuikChem 8500 FIA automated ion analyzer	11 µg/L
Total Phosphorus	Whole water	1L polypropylene bottle	Lachat QuikChem 8500 FIA automated ion analyzer	1 µg/L
Total Suspended Solids	Particulate	2L polypropylene bottle	Value was calculated by dividing filter weight difference (before and after filtering) by volume filtered	blank filter weight
Particulate Carbon	Particulate	2L polytetrafluoroethylene bottle	CE440 Elemental Analyzer to get mass, then divide by volume filtered	0.10 µg
Particulate Nitrogen	Particulate	2L polytetrafluoroethylene bottle	CE440 Elemental Analyzer to get mass, then divide by volume filtered	0.15 µg
Particulate Phosphorus	Particulate	2L polypropylene bottle	CE440 Elemental Analyzer	1 µg/mg
$\delta^{18}O$	Filtered- 0.22 µm	20-mL HDPE scintillation vial	Picarro L2130 isotope and gas concentration analyzer	-
$\delta^{2}H$	Filtered- 0.22 µm	20-mL HDPE scintillation vial	Picarro L2130 isotope and gas concentration analyzer	-
Dissolved Organic Carbon	Filtered- 0.22 µm	40-mL amber EPA vial	Shimadzu TOC-L	0.004 ppm
Dissolved Inorganic Carbon	Filtered- 0.22 µm	12-mL glass exetainer	Apollo SciTech AS-C3 DIC Analyzer coupled to a LI-COR LI-7000 infrared CO_2 analyzer	-

Parameter	Sample type	Sample bottle	Analysis	Detection limit
Major Ions: **Al^{3+}** **Ca^{2+}** **K^{2+}** **Mg^{2+}** **Na^+**	Filtered- 0.22 μm	50-mL centrifuge tube	Inductively Coupled Plasma - Optical Emission Spectrometer (Thermo Scientific ICAP6300)	3.6 μg/L 0.01mg/L 0.01 mg/L 0.01 mg/L 0.02 mg/L
Total Dissolved Nitrogen	Filtered- 0.22 μm	15-mL centrifuge tube	Lachat QuikChem 8500 FIA automated ion analyzer	11 μg/L
Silica	Filtered- 0.22 μm	15-mL centrifuge tube	Lachat QuikChem 8500 FIA automated ion analyzer	0.02 μg/L
Total Dissolved Phosphorus	Filtered- 0.22 μm	15-mL centrifuge tube	Lachat QuikChem 8500 FIA automated ion analyzer	1 μg/L
Alkalinity	Filtered- 0.22 μm	15-mL centrifuge tube	Autotitrator (Man-Tech PC-Titrate with conductivity probe)	1 mg/L
Chloride	Filtered- 0.22 μm	15-mL centrifuge tube	Ion Chromatographer (Dionex DX600 and Dionex ICS 2500)	0.03 mg/L
Sulphate	Filtered- 0.22 μm	15-mL centrifuge tube	Ion Chromatographer (Dionex DX600 and Dionex ICS 2500)	0.04 mg/L
Nitrite	Filtered- 0.22 μm	15-mL centrifuge tube	Lachat QuikChem 8500 FIA automated ion analyzer	3 μg/L
Nitrite+Nitrate	Filtered- 0.22 μm	15-mL centrifuge tube	Lachat QuikChem 8500 FIA automated ion analyzer	2 μg/L
Ammonium	Filtered- 0.22 μm	15-mL centrifuge tube	Lachat QuikChem 8500 FIA automated ion analyzer	3 μg/L
Soluble Reactive Phosphorus	Filtered- 0.22 μm	15-mL centrifuge tube	Lachat QuikChem 8500 FIA automated ion analyzer	1 μg/L
Trace Metals: **Ag** **As** **Ba** **Be** **Cd** **Co** **Cu** **Fe** **Mg** **Mn** **Ni**	Filtered- 0.02 μm	15-mL centrifuge tube	Inductively Coupled Plasma - Mass Spectrometer (Agilent ICP-MS 7900)	0.01 μg/L 0.04 μg/L 0.05 μg/L 0.01 μg/L 0.02 μg/L 0.01 μg/L 0.01 μg/L 0.8 μg/L 0.05 μg/L 0.04 μg/L 0.02 μg/L

Parameter	Sample type	Sample bottle	Analysis	Detection limit
Pb				0.01 μg/L
Sb				0.02 μg/L
Se				0.01 μg/L
Sn				0.09 μg/L
Sr				0.04 μg/L
Ti				0.13 μg/L
Tl				0.01 μg/L
V				0.01 μg/L
Zn				0.87 μg/L
Chlorophyll *a*	Particulate		Agilent 1100 Series HPLC	-
Ultraviolet visible spectroscopy (absorbance and fluorescence)	Filtered- 0.22 μm	40-mL amber EPA vial	Horiba Aqualog	-

Table A2.3 Summary of land use and land cover (LULC) parameters used to define LULC clusters (see methods and Figure 2.1B), description, and data source. Asterisk (*) indicates anthropogenic LULC parameters.

Parameter	Type	Description	Data source
Slope	Slope	Mean slope	GOA – Phase II – Provincial DEM
Wind	Wind	Mean average wind speed	GOA – Data from GOA modelling team or Data from Alberta Agriculture
Precipitation	Precipitation	Mean annual precipitation	GOA – Data from GOA modelling team or Data from Alberta Agriculture
Temperature	Temperature	Mean temperature (May- Oct)	GOA – Data from GOA modelling team or Data from Alberta Agriculture
Lake	Lakes	Percent cover of lakes	GOA – Phase II ArcHydro Hydrology Base Features
Deciduous Trees	Canopy types	Percent cover deciduous	GOA – Alberta Vegetation Inventory
Coniferous Trees	Canopy types	Percent cover coniferous	GOA – Alberta Vegetation Inventory
Mixed Forest (C)	Canopy types	Percent cover mixed (dominant coniferous)	GOA - Alberta Vegetation Inventory
Mixed Forest (D)	Canopy types	Percent cover mixed (dominant deciduous)	GOA - Alberta Vegetation Inventory
Swamp	Wetland cover	Percent cover swamp	GOA Alberta Merged Wetland Inventory
Fen	Wetland cover	Percent cover fen	GOA Alberta Merged Wetland Inventory
Bog	Wetland cover	Percent cover bog	GOA Alberta Merged Wetland Inventory
Marsh	Wetland cover	Percent cover marsh	GOA Alberta Merged Wetland Inventory
Open Water	Wetland cover	Percent cover open water	GOA Alberta Merged Wetland Inventory
Bedrock	Surficial Geology	Percent cover of bedrock outcrops	AER/AGS - Bedrock Geology
Fluvial Deposits	Surficial Geology	Percent cover of fluvial deposits	AER/AGS - Bedrock Geology
Ice Thrust Moraine	Surficial Geology	Percent cover of ice-thrust moraine deposits	AER/AGS - Bedrock Geology
Moraine Deposits	Surficial Geology	Percent cover of moraine deposits	AER/AGS - Bedrock Geology
Colluvial Deposits	Surficial Geology	Percent cover of colluvial deposits	AER/AGS - Bedrock Geology
Eolian Deposits	Surficial Geology	Percent cover of eolian deposits	AER/AGS - Bedrock Geology
Organic Deposits	Surficial Geology	Percent cover of organic deposits	AER/AGS - Bedrock Geology

Parameter	Type	Description	Data source
Stagnant Ice Deposits	Surficial Geology	Percent cover of stagnant ice moraine deposits	AER/AGS - Bedrock Geology
Glacio lacustrine Deposits	Surficial Geology	Percent cover of glaciolacustrine deposits	AER/AGS - Bedrock Geology
Riparian	Riparian area	Riparian area cover	GOA - Alberta Vegetation Inventory
Coniferous Riparian	Coniferous riparian area	Percent cover by riparian canopy type (coniferous)	GOA - Alberta Vegetation Inventory
Deciduous Riparian	Deciduous riparian area	Percent cover by riparian canopy type (deciduous)	GOA - Alberta Vegetation Inventory
Mixed forest (C) Riparian	Coniferous dominated riparian area	Percent cover by riparian canopy type (mixed-coniferous)	GOA - Alberta Vegetation Inventory
Mixed forest (D) Riparian	Deciduous dominated riparian area	Percent cover by riparian canopy type (mixed-deciduous)	GOA - Alberta Vegetation Inventory
Wildfire	Wildfire	Percent cover of burned area (last 10 years)	GOA - Wildfire History
Park Protected*	Parks and Protected Areas	Percent cover of parks and protected areas	GOA - Parks and Protected Areas Inventory
Agriculture*	Agriculture	Percent cover of agricultural footprint	ABMI Human Footprint Inventory 2018
Industrial*	Urban/Rural Industrial	Percent cover of rural industrial footprint	ABMI Human Footprint Inventory 2018
Residential*	Residential	Percent cover of residential footprint	ABMI Human Footprint Inventory 2018
Well Areas*	Oil and gas wells	Percent cover of oil and gas well sites	ABMI Human Footprint Inventory 2018
Waste Sites*	Landfills and lagoon, transfer station, sump	Percent cover of waste facilities	ABMI Human Footprint Inventory 2018
Harvest*	Harvesting (cutblocks)	Percent cover of harvested area	ABMI Human Footprint Inventory 2018
Canal*	Canals/ ditches	Percent cover of canals and ditches	ABMI Human Footprint Inventory 2018
Impervious*	Impervious Surfaces	Percent cover of industrial, residential, road, rail, and pipeline	ABMI Human Footprint Inventory 2018
Residential Riparian*	Riparian residential	Percent cover riparian residential footprint	ABMI Human Footprint Inventory 2018

Parameter	Type	Description	Data source
Agriculture Riparian*	Riparian agriculture	Percent cover of riparian agricultural footprint	ABMI Human Footprint Inventory 2018
Reservoir*	Reservoir	Percent cover of reservoirs	ABMI Human Footprint Inventory 2018

Table A2.4 Percent of samples below the detectable limit (BDL) ordered by percent of dataset BDL. Variables with values below the detection limit are included only. Variables in grey were excluded from analysis.

| Variable	Samples BDL	Total Samples	Percent of dataset BDL	Instrument Detectable Limit
TKN	1	81	1.23	11 (μg/L)
SRP	6	162	3.70	1 (μg/L)
TDP	5	81	6.17	1 (μg/L)
PP	9	81	11.11	1 (μg/mg)
Fe	9	81	11.11	0.001 (μg/L)
Ni	11	81	13.58	0.02 (μg/L)
As	13	81	16.04	0.04 (μg/L)
Al	14	81	17.28	3.6 (μg/L)
K	15	81	18.51	0.02 (mg/L)
Fe	20	81	24.69	0.02 (μg/L)
Pb	28	81	34.56	0.01 (μg/L)
NH_4	89	243	36.62	3 (μg/L)
NO_2NO_3	96	243	39.50	2 (μg/L)
Co	32	81	39.50	0.01 (μg/L)
Se	41	81	50.61	0.01 (μg/L)
V	41	81	50.61	0.01 (μg/L)
NO_2	124	243	51.02	3 (μg/L)
Sb	46	81	56.79	0.02 (μg/L)
Ti	50	81	61.72	0.13 (μg/L)
Zn	71	81	87.65	0.87 (μg/L)
Be	77	81	95.06	0.01 (μg/L)
Ag	80	81	98.76	0.01 (μg/L)
Cd	80	81	98.76	0.02 (μg/L)
Hg	81	81	100	0.01 (μg/L)
Sn	81	81	100	0.09 (μg/L)
Tl	81	81	100	0.01 (μg/L)
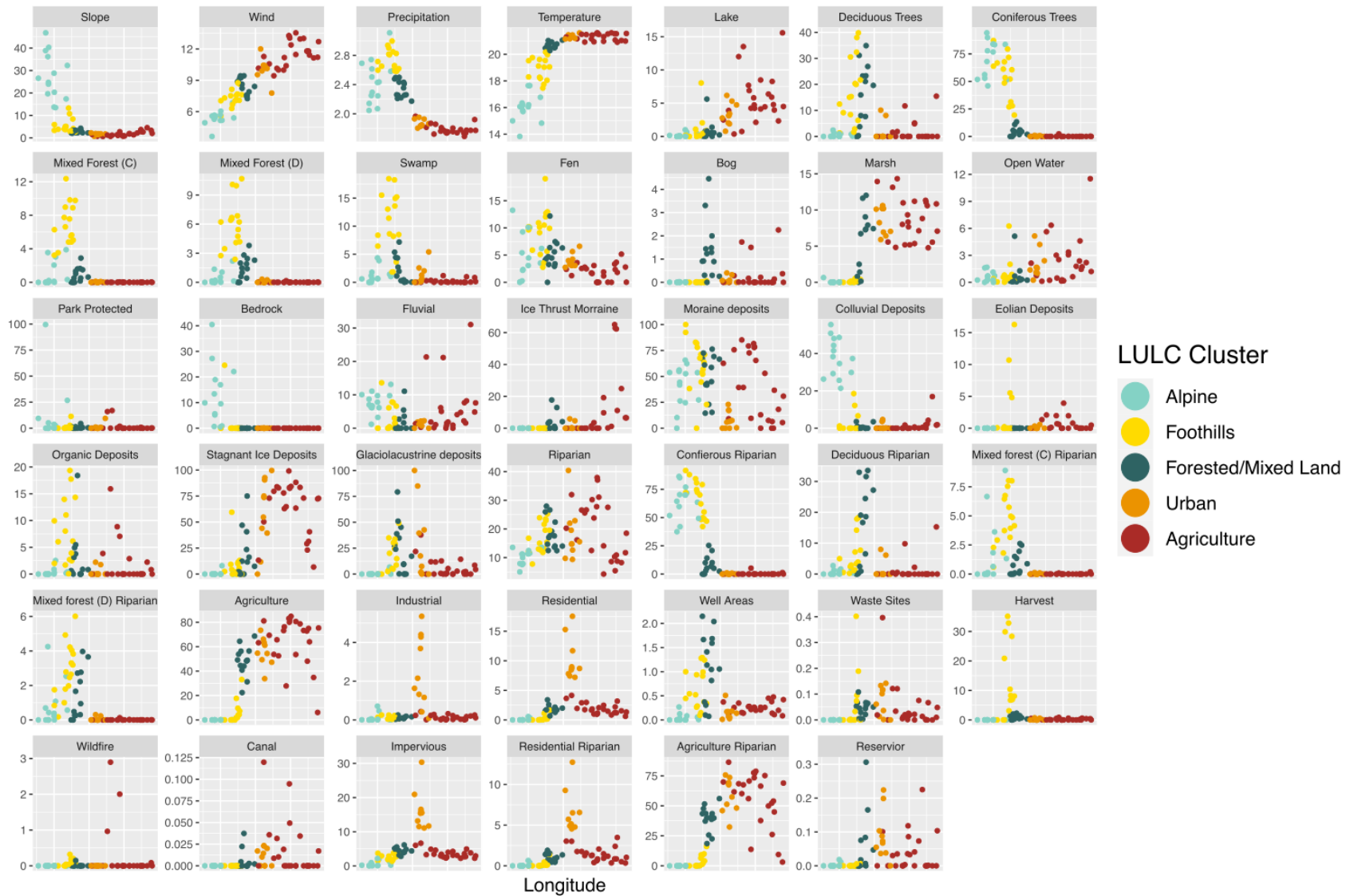
Figure A2.1 Climate and land use and land cover (LULC) variables plotted across longitude (From west to east). Colours are based on LULC cluster analysis (see Figure 2.1B). All LULC variables are areal percentages of their respective LULC coverages in the watersheds. Data sources detailed in Table A2.3.
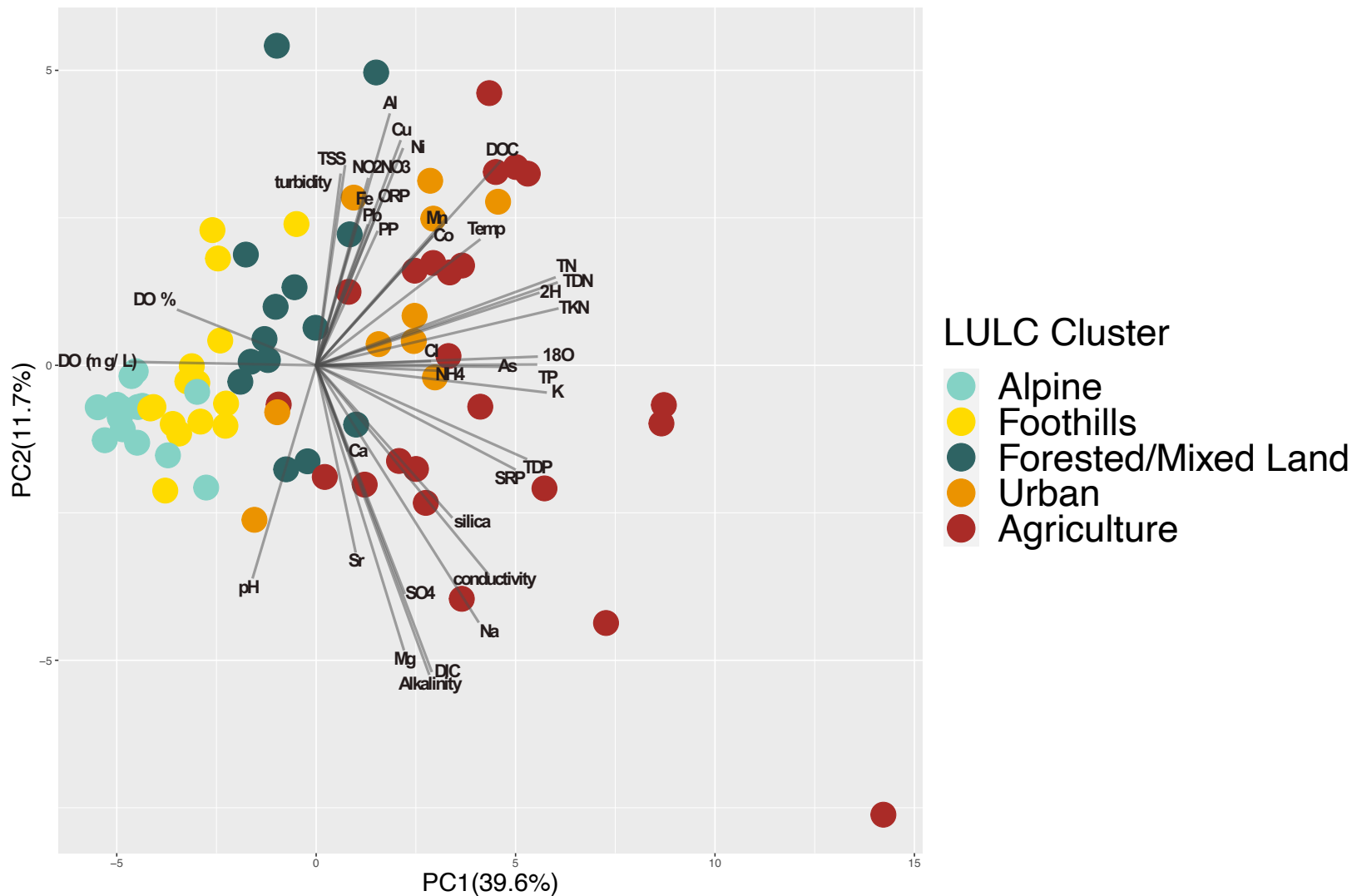
Figure A2.2 PCA of all water chemistry variables measured in this study. One point represents the full water chemistry dataset (see Table A2.2) for each tributary stream, with the exception of POC, PON, and chlorophyll *a* which were removed because of missing data. Colours are based on land use and land cover (LULC) clusters determined through hierarchical cluster analysis (see Methods and Figure 2.1B).
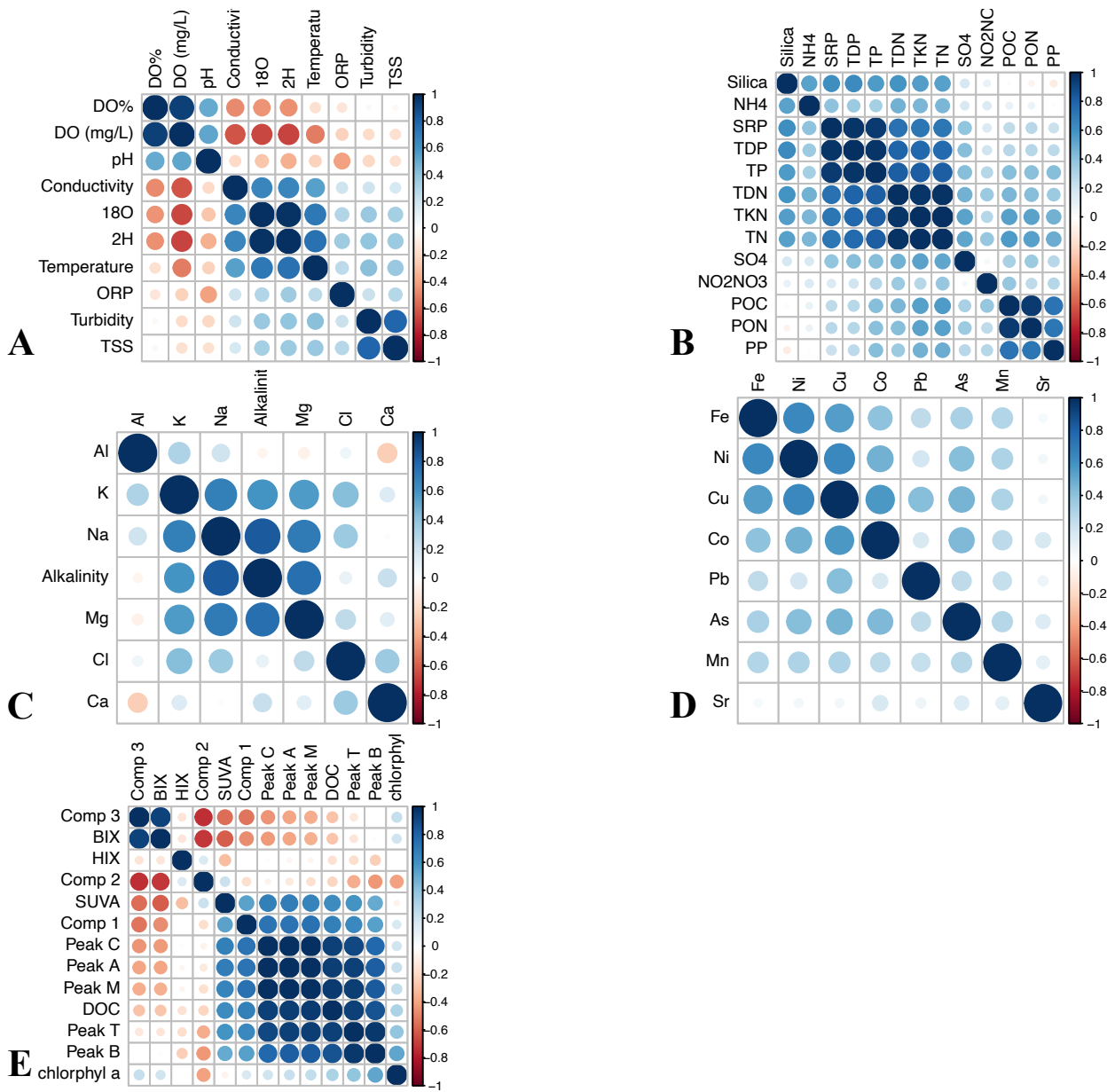
Figure A2.3 Pearson correlation matrices of (A) physical parameters and isotopes (B) nutrients, (C) ions, (D) metals, and (E) OM data. The colour of each dot represents either a positive (blue) or negative (red) correlation, and the size of each dot represents the strength of the correlation co-efficient (Spearman's Rho).
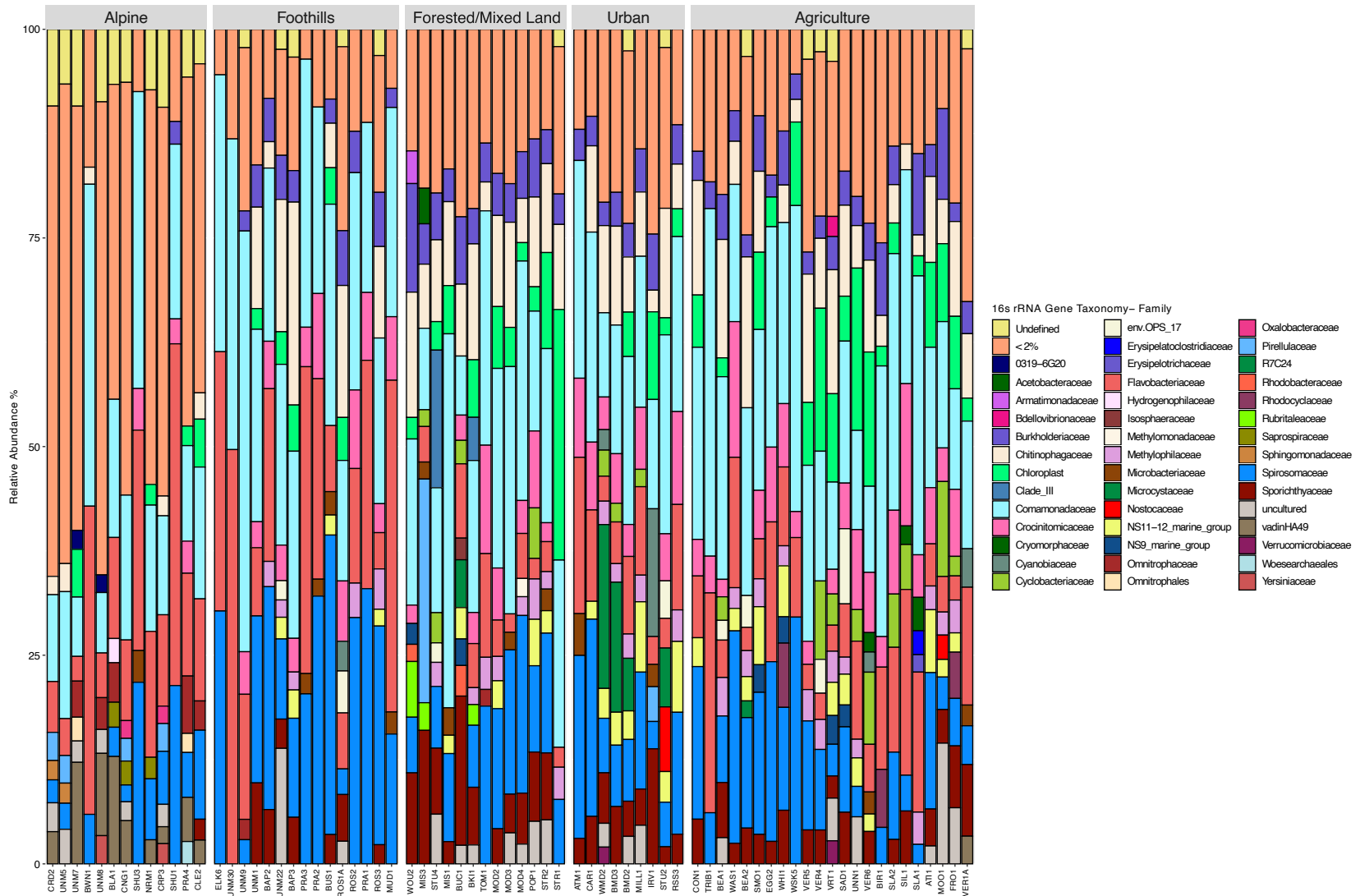
Figure A2.4 Taxanomic bar plot of the resolved microbial community composition across our North Saskatchewan River tributaries at the family level. Sites are separated by land use and land cover (LULC) cluster (see Figure 2.1B), and ordered by longitude (west to east) within each LULC type.
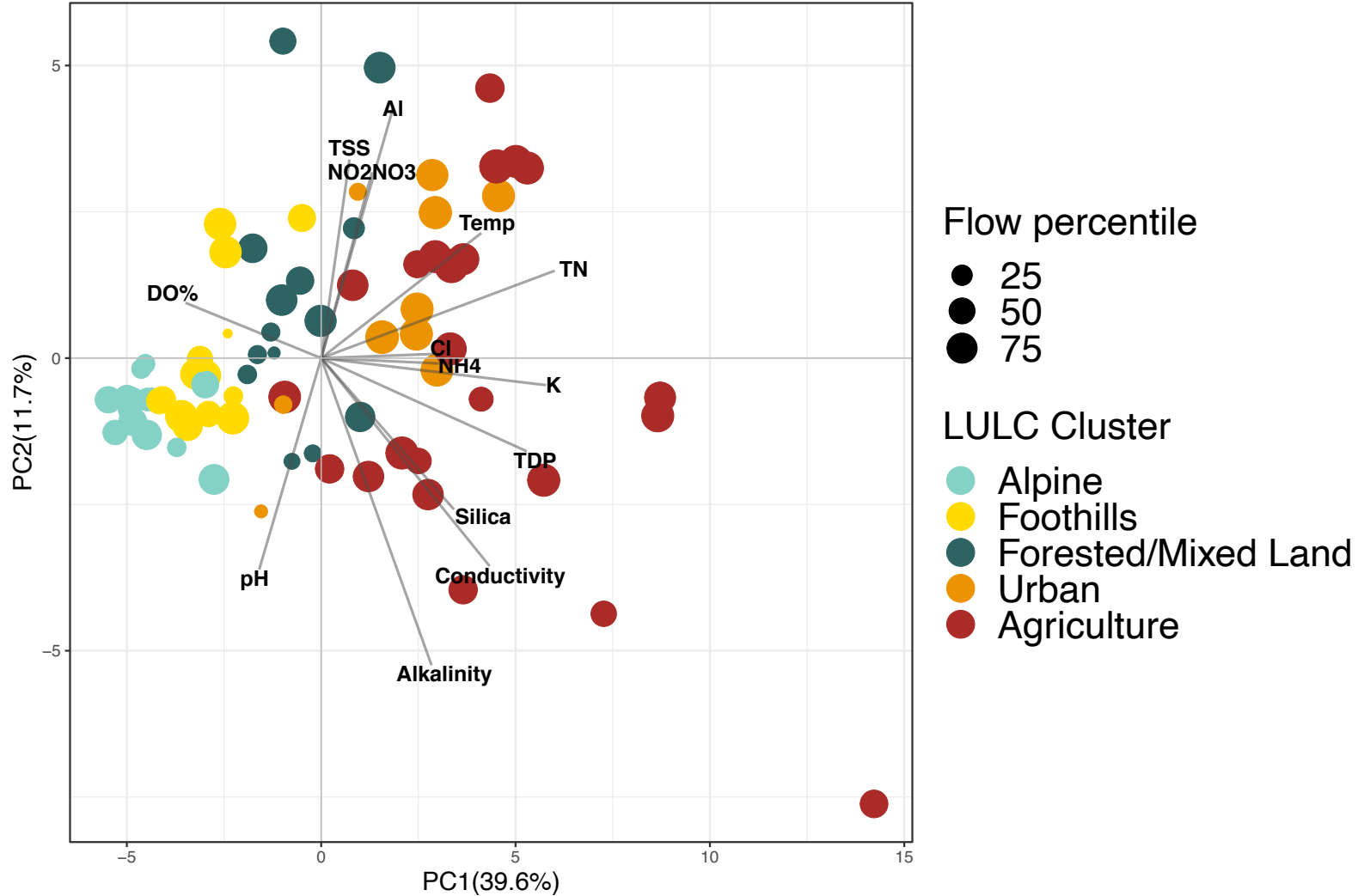
Figure A2.5 Principal components analysis of water chemistry where select vectors are plotted, but ordinations are based on the entirety of each. Each dot represents the water chemistry at one tributary stream, with the dot colouring indicative of land use and land cover (LULC) type as determined by the hierarchical cluster analysis shown in Figure 2.1B. Size represents flow at the nearest flow monitored station.
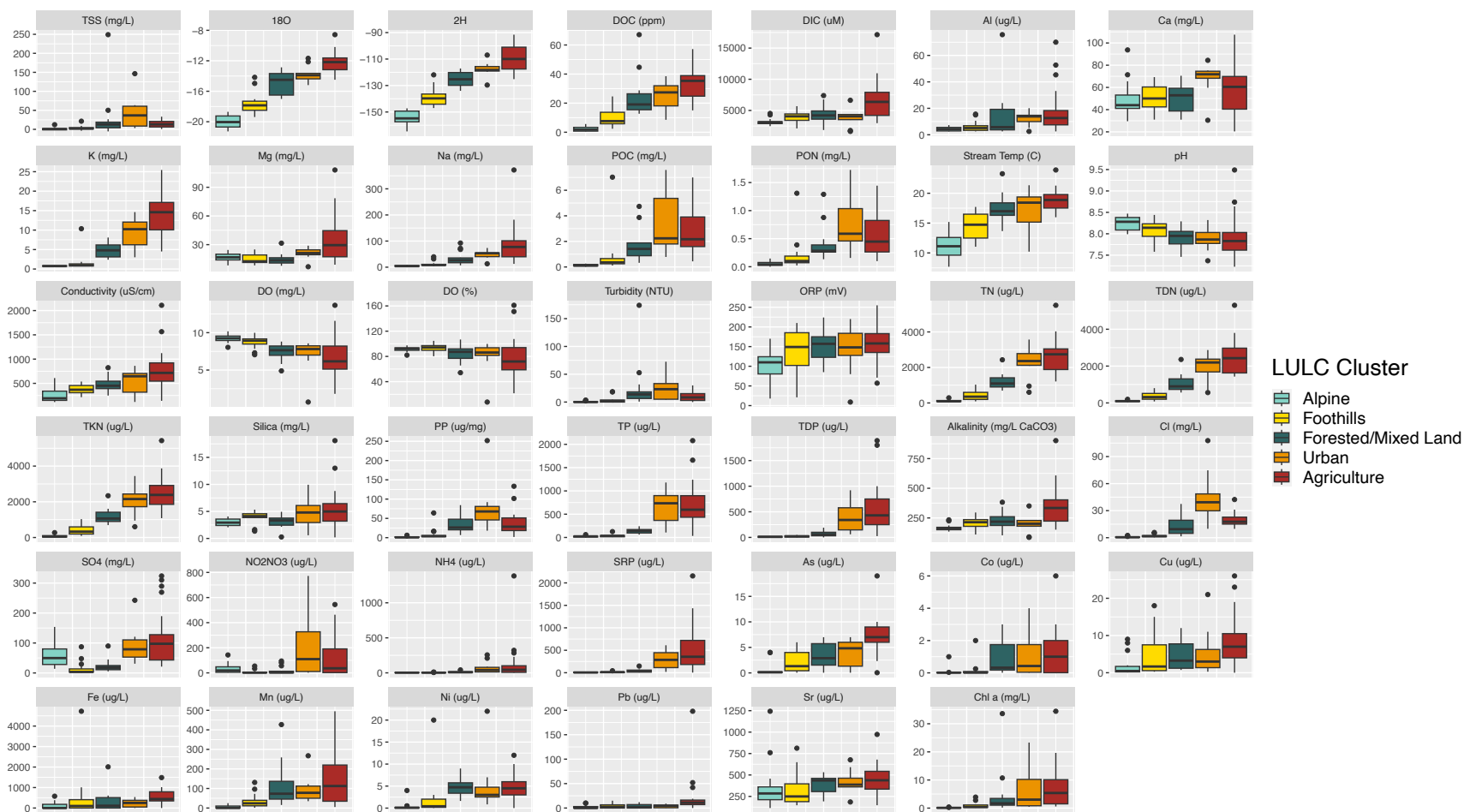
Figure A2.6 Boxplots of each water chemistry variable measured across the tributary streams plotted across the North Saskatchewan River basin. Colours are based on land use and land cover (LULC) clusters as determined by a hierarchical cluster analysis (shown in Figure 2.1B).