MACROINVERTEBRATE AND BACTERIA COMMUNITY RESPONSES TO TRIBUTARY INPUTS AT COASTAL WETLANDS OF THE DETROIT RIVER, ONTARIO, CANADA

By

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ABSTRACT

The Detroit River is a Great Lakes Area of Concern with five monitored wetlands in the Canadian jurisdiction. Habitat assessments have indicated possible stress at wetlands receiving inflow from Turkey Creek and River Canard tributaries. These assessments are made within the tributaries. Yet, macrophyte beds extend into the river upstream and downstream and are important biodiversity habitats. This thesis examines benthic macroinvertebrate and sediment bacteria community compositions for differences with respect to tributaries by two means. First, we examine inter-wetland differences for resemblance to water quality. We had found by NMDS and PERMANOVA that neither taxonomic group resembled water quality index scores. Second, we perform an intra-wetland comparison for Turkey Creek and River Canard to analyze for differences along tributary inputs. Wetland communities were delineated by position into upstream, downstream, and tributary plume strata and analyzed by NMDS and PERMANOVA. Additionally, to detect potentially impaired sample sites and support community differences along tributaries, a multivariate reference approach was applied by dividing sample sites by similar habitat characteristics to contrast River Canard and Turkey Creek to reference wetlands. At neither River Canard nor Turkey Creek we observed significant tributary influence on river communities but had found the Turkey Creek tributary communities significantly differed from the river. Multiple lines of evidence suggest community impairment in Turkey Creek likely from upstream waters rather than tributary inputs.

DEDICATION

The loving memory of my brother, Michael James Robson.

"*I am your friend still – time has not estranged.*" - *Ella Wheeler Wilcox*

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LIST OF ABBREVIATIONS/SYMBOLS

α - alpha

AOA - Ammonia-oxidizing archaea

AOB - Ammonia-oxidizing bacteria

AOC – area of concern

ANOVA - analysis of variance

β- beta

BUI – beneficial use impairment

CSS - cumulative sum scaling

δ - delta

DNA - deoxyribonucleic acid

DRM – Detroit River Marshes

ECCC - Environment and Climate Change Canada

EPT – Ephemeroptera, Plecoptera, Trichoptera

γ - gamma

GI – Grass Island

GLWQA- Great Lakes Water Quality Agreement

MRA – multivariate reference approach

NA – not applicable

NMDS – non-metric multidimensional scaling

PCA - principal component analysis

PCR - polymerase chain reaction

PERMANOVA - permutational multivariate analysis of variance

PI – Peche Island

RC – River Canard

RDA – redundancy analysis

SRM – sulfate-reducing microorganism

TC – Turkey Creek

TRC – River Canard tributary

TTC – Turkey Creek tributary

WQI - Water quality indices

NOMENCLATURE

CHIRON – Chironomidae

OLIGO – Oligochaeta

VALTID – Valvatidae

NEMATO – Nematoda

RISSOO – Rissooidea

DREISS – Dreissenidae

SPHAER – Sphaeriidae

CERATO – Ceratopogonidae

HYALEL – *Hyalella*

LEPTO – *Leptocerus*

HELOBD – *Helobdella*

PLANOR – Planorbidae

VALTRI – *Valvata tricarinata*

HEXAGN – *Hexagenia*

CRAMBI – Crambidae

PLYCNTR – *Polycentropus*

ERPOBD – Erpobdellidae

HYDPTIL – Hydroptilidae

ALBOGL – *Alboglossiphonia heteroclita*

CAECID – Caecidotea

COENAG – Coenagrionidae

NECTO – *Nectopsyche*

DIPSUEDO – Dipseudopsidae

TURBEL – Turbellaria

CHAPTER 1

GENERAL INTRODUCTION

1.1: Great Lakes Conservation

Freshwater ecosystems cover 0.8% of the earth's surface and are estimated to contain 10% of global species (Balian et al 2008). They are highly threatened systems whose contribution to biodiversity is disproportionate to their area covered globally. Five major global contributors threatening freshwater biodiversity are over-exploitation, water pollution, flow modifications, biotic introductions, and habitat degradation (Dudgeon et al 2005).

The Laurentian Great Lakes represent 20% of global freshwater and are a critical resource to both US and Canadian economies and First Peoples. The population of the Great Lakes basin is expected to increase with a concomitant rise in stressors to these ecosystems as landscape alterations and use invariably increase (Johnston 2006; Méthot et al 2015). The extensive use of the lakes as drinking water, fisheries, irrigation source, industrial uses, trade route and energy production have strained the natural ecosystem in the forms of fisheries exploitations, nutrient and hazard pollution, biotic introductions, damming, water diversions and landscape modifications. (Changnon and Changnon 1996; Rothlisberger et al 2010; Escobar et al 2017; Maguire et al 2019; Wilson et al 2022).

The Great Lakes Water Quality Agreement (GLWQA) is a bilateral agreement between Canada and the United States to manage restoration and protections of the lakes. Initially, the focus of this agreement had centered around issues regarding water quality (GLWQA 1972). However, later amendments to the agreement recognized the need for an ecosystem management approach, one that not only assesses and regulates environmental chemistry, but also adopts ecosystem indicators into assessments to generate cause-effect linkages between physical (e.g. habitat quality) and chemical stressors (nutrients and toxic pollutants) with

ecosystem structure and functional attributes (Teiter and Mander 2005). The GLWQA was an early actualization of ecosystem-based management by incorporating the above described physical, chemical, and biological approaches. Biological organisms respond to the accumulation of environmental changes and thusly analysis of biodiversity and species compositions from many levels of biological organization can be used in assessments (Markert et al 2003; Li et al 2010).

The most recent GLWQA (2012) amendment includes ecosystem health metrics in the form of indicator species and community composition metrics across several of its annexes. They include Annex 1 – Areas of Concern which directs the assessment and restoration of aquatic ecosystem services across 14 standardized Beneficial Use Impairments (BUI) in areas identified as highly impacted by anthropogenic stressors; Annex 2 – Lakewide Management which establishes chemical and biological benchmarks for ecosystem health on a lake-by-lake basis; Annex 7 concerned with habitat and species assemblages and Annex 6 focusing on the impacts of aquatic invasive species. Each of these annexes are concerned with linking aquatic biodiversity with ecosystem services and ecosystem sustainability.

1.2: Aquatic Biodiversity

A simplified definition of biodiversity is the aggregate of all biotic variation in an ecosystem. However, biodiversity is a multi-dimensional concept composed of functional, phylogenetic, temporal, and spatial components of a community (Purvis and Hector 2000; Wittebolle et al 2009). Biodiversity has linkages to ecosystem stability by providing ecosystem resistance and resilience by stabilizing ecosystem functions (Naeem and Li 1997). Community organization can be described in terms of alpha, beta, and gamma diversity (Whittaker 1960). Gamma diversity considers regional (coarse scale) diversity. On the other hand, alpha diversity examines local (fine scale) diversity describing species richness and species evenness. Species richness is the number of species in an ecosystem whereas species evenness considers the distribution of relative abundance of species. A species rich community offers ecosystem stability by increasing the probability that an ecosystem function can be maintained due to overlap in functional roles shared by multiple species (Hooper et al 2005). Species evenness can be a significant factor in ecosystem stability when a species functional characteristics strongly influence ecosystem functions (Hillebrand et al 2008). Studies in microbial microcosms suggest that species evenness within functionally redundant taxa offer ecosystems with increased resistance to stress (Wittebolle et al 2009). Species richness in macroinvertebrate communities supports rich functional diversity (Schmera et al 2016). Species richness and evenness can be synthesized into diversity metrics such as the Shannon-Weaver represented as H' to compare diversity temporally or spatially (Shannon and Weaver 1949; Clarke and Warwick 2001). Beta-diversity refers to change in assemblages across a space and it is comprised of two components, turnover and nestedness (Harrison et al 1992; Williams et al 2004; Baselga 2010; Lennon and Jones 2011). Species turnover, also referred to as species replacement, is a concurrent gain and loss of a species. Turnover may represent differences in inter- and intraspecific pressures on

community structures in response to habitat heterogeneity, local disturbance, or stress (Leprieur et al 2011; Legendre 2014). Nestedness considers species richness through commonalities - collections of taxa which are shared subsets between sites. Nestedness and beta-diversity share a negative relationship as beta-diversity is a measure of differences. Reductions in beta-diversity are associated to urbanization and agriculture land-use as both stressors homogenize the environment (McKinney 2006; Knop 2016; Buhk et al 2017).

The Laurentian Great Lakes is a biodiversity hot spot and home to 2200 aquatic taxa which includes 1433 benthic invertebrates (Trebitz et al 2019). Benthic macroinvertebrate communities consist of mature and immature stages of invertebrates greater than 500um inhabiting the benthic space of aquatic environments which consist of the substrate surface and oxic underlying strata (~10 to 15 cm) below the sediment-water interface. Benthic macroinvertebrates strongly influence nutrient and energy transfers from sediment to the water column and higher trophic organisms contributing to benthic-pelagic coupling (Grimm 1988; Wallace and Webster 1996; Demars et al 2021). Given that surface sediments reflect of a multi-year repository of accumulated pelagic (autochthonous) production and allochthonous carbon inputs, food web contributions from benthic production can buffer year-to-year variance in water column primary production acting as a temporal stabilizer of ecosystem energetics in support of ecosystem resistance (Covich et al 1999; Ashton et al 2014).

Habitat characteristics can influence community assemblage patterns such as water depth, velocity, temperature, food sources, flow rates and substrate (Mackay 1992; Collier 1995; Buss et al 2004; Jonsson et al 2017; Demi et al 2019). Species traits such as mobility (drift, crawling, swimming, flight) and species interactions (competition and predation) also effects community compositions (Mackay 1992; Clements 1999; Kelly et al 2006; Bonada et al 2007).

Anthropogenic stressors alter benthic community structures by altering hydraulic regimes, changing physical substrate characteristics (e.g., siltation of formerly granular substrates), altering oxygenation status and oxygen penetration within sediments and by contributing to toxicity via accumulation of toxic chemicals in sediment (Buss et al 2004; Larsen et al 2011; Larson et al 2013; Scavia et al 2014; Desrosiers et al 2018). Many of the above stressors contribute to chronic effects such as reduced growth and reproduction and acute effects such as mortality leading to loss of sensitive species (reduced biodiversity) and in some cases proliferation of a limited number of tolerant species (reduced species evenness) (Evans-White et al 2009; Ligeiro et al 2013; Barnum et al 2017).

Macroinvertebrates make good ecosystem indicators because of the wide range of sensitive and tolerant species with different substrate affiliations that facilitates an indicator species approach and whose communities can show quantifiable change to community structure in response to stress. For example, macroinvertebrates present a variety of respiratory adaptions that affect oxygen uptake capacities and their ability to cope with hypoxia such that dissolved oxygen concentrations can be a strong predictor of respiratory grouping abundances (Chapman et al 2004). Thus, changes to oxygen status of a waterbody induced by altered organic load and biological oxygen demand translates into shift from sensitive to low oxygen tolerant species. Macroinvertebrates also show wide ranges in between taxa differences in their sensitivity to metals, organic contaminants and ammonia that can translate into altered species richness, evenness, and community composition because of the progressive losses and reduced relative abundance of sensitive species (Lock and Goethals 2011; Malaj et al 2012; Rawi et al 2014; Windsor et al 2019). Ephemeroptera, Plecoptera, and Trichoptera (EPT) are grouped as indicator species of good habitat quality and are commonly incorporated as a metric in environmental monitoring (Brasil et al 2020). Although benthic macroinvertebrates have

demonstrated to respond to disturbances, it can be difficult to assess highly impacted sites where diversity and indicator species are low (Ostermiller and Hawkins 2004).

Species distributions and community structures are directed by their varying tolerances to their environment (Dudgeon 1984). Benthic macroinvertebrates are often used in biomonitoring as ranges in environmental tolerances have been well studied and developed into biotic indices (Resh et al 1995; Reynoldson et al 1995; Townsend et al 1997) and established to respond to water and sediment quality conditions (Lock and Goethals 2011; Beermann et al 2018; Akyildiz and Duran 2021). Different levels of biological organization vary in environmental tolerances and contributions to ecosystem functioning. Multi-species approaches to biomonitoring provide more comprehensive overview of habitat conditions (Catteau et al 2022). Microbial communities have the potential to be useful indicators as they have short generation times when compared to multicellular organisms. Microbial indicators currently focus on ecosystem functioning and have not been as extensively developed for habitat assessments but are of interest to the scientific community (Sims et al 2013; Bouchez et al 2016; Astudillo-García et al 2019). Many studies measure biomass or chemical activities such as respiration, metabolic processes, nutrient cycling, and contaminant degradation (Karimi et al 2017). The development and advancements of genetic techniques to sample and characterize microbial communities based on environmental DNA (e-DNA) collected from water, soils and sediments makes adoption of microbial community biomonitoring approaches highly promising.

Microbial activities mediate biogeochemical cycles including the cycling of elements such as carbon, nitrogen, and sulfur. Rock, sediment, leaf litter and plant surfaces become colonized by biofilms composed of complex microbial communities of bacteria, archaea, protozoa, fungi, and algae held together in a matrix of extracellular polymeric substances (Pusch

et al 1998). These self-organizing communities can play enhanced roles in nutrient cycling but also form a major food resource for benthic macroinvertebrates (Hall and Meyer 1998; Flemming and Wingender 2001; Kiyashko et al 2001). Below the sediment surface, wetland soils are characterized as low-oxygen environments to which bacterial metabolism has become adapted to and plays critical roles in biogeochemical cycles in wetlands. Oxygen availability and penetration with sediment depth will depend on variety of factors including water flow and oxygen delivery via overlying water, porewater exchange influenced by sediment porosity and macroinvertebrate bioturbation, oxygen leaching from macrophyte roots and the quantity and composition of organic material contributing to microbial metabolism and biological oxygen demand (Holmer et al 2002; Hand et al 2008; Pischedda et al 2008; Klayn et al 2021). Where oxygen is present, aerobic metabolism generally dominates, but as oxygen becomes depleted anaerobic respiration associated with methanogens, denitrifying bacteria, and bacteria capable of reducing sulphates or metals commences (Canfield and Thamdrup 2009; Hernandez et al 2015; Aldunate et al 2018). Thus, unlike macroinvertebrates, microbial community composition biomonitoring tools extend into anoxic habitats. The ability for nitrification and denitrification of microbes in wetland sediments relies on gene diversity and abiotic factors such as pH, dissolved oxygen. (Ligi et al 2014; Lai et al 2019; Bowen et al 2020). Ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) engage in the oxidation of ammonia to nitrate. Sensitivities to dissolved oxygen and ammonium concentrations vary between groups. AOAs tolerate low oxygen and low ammonium conditions whereas AOBs are associated with high oxygen and a higher affinity for ammonium (Di et al 2009; Liu et al 2011). The relative abundances of AOAs and AOBs has the potential to indicate dissolved oxygen and nutrient enrichment conditions. Methanogenesis is a metabolic pathway carried out by methanogens, an anaerobic group of archaea that utilizes acetate or carbon dioxide to produce methane and contribute to

greenhouse gas emissions. Methane emissions from wetlands are regulated by methanotrophs in aerobic and anaerobic soils (Hanson and Hanson 1996). Sulfate-reducing microorganisms (SRMs) efficiently use sulfate in their metabolic pathways with hydrogen sulfide and carbon dioxide as by-products. Often wetlands are low-sulfate environments and detection of SRMs were rarely detected with the use of gene libraries. However, some studies using targeted molecular analyses have detected the presence of SRMs in low-sulfate environments (Pester et al 2012). Microbial diversity is driven by a complex array of biological traits coupled with chemical environments operating within biofilms and in sediments. These known traits reflecting community response to various environmental gradients highlight the strong promise of microbial community biomonitoring tools. Coupling such tools with other indicators such as macroinvertebrates could improve environmental stress detection while also providing causative inferences about the nature of stressors.

1.3: Detroit River Wetlands

Since the twentieth century, more than half of total wetlands have been lost globally (Davidson 2014). Difficulty arises in assessing the true loss as wetlands are subject to natural variations in their areal extent and are often limited in the availability of baseline ecological data on which to compare temporal trends (Maltby and Acreman 2011). A large volume of literature discusses their importance to nutrient cycling (Fennessy et al 2008; Chandra et al 2019), flood control (Mitsch and Gosselink 2000; Pattison-Williams et al 2018), water quality (Ghermandi et al 2010) and to global biodiversity (Mensing et al 1998; Gibbs 2000).

Canadian wetlands are broadly classified by hydrology, surface morphology, soil characteristics and vegetation and are federally and provincially managed (Glooschenko 1983; Zoltai and Vitt 1995). The Ontario Ministry of Natural Resources developed the Wetland Evaluation System to rank significance of wetlands to ecosystem services. The coastal wetlands of the Great Lakes are provincially significant wetlands that include connecting channels and wetlands up to 2km inland (OMNR 2014). Under federal jurisdiction, objectives of the GLWQA include "support healthy and productive wetlands […] to sustain resilient populations of native species" (GLWQA 2012). These contributions of biodiversity support wetland productivity and resilience to direct anthropogenic stressors and climate change (Cardinale et al 2011; Pester et al 2012; Reich et al 2012; Isbell et al 2015; Yang et al 2018). The capacity to support ecosystem health and productivity are threatened by varying anthropogenic stressors and species loss. (Passy and Blanchet 2007; Geist and Hawkins 2016; Chen et al 2019).

The Detroit River is a binational Annex 1 – Area of Concern within the GLWQA managed by the US Environmental Protections Agency and Environmental and Climate Change Canada (ECCC). The designation of Areas of Concern (AOCs) is defined by Beneficial Use Impairments

(BUI) which are measurable impacts of poor habitat quality broadly attributed to water quality and includes impairments attributed to sediment quality. Issues regarding poor sediment quality from metals and persistent organic compounds had adversely affected benthic invertebrate communities in the Detroit River. Sediment contamination impairs benthic community assemblages (Tang et al 2018), functional diversity (Desrosiers et al 2018), and causes physiological deformities (Hudson and Ciborowski 1996). River-wide sediment contamination surveys conducted every 5-10 years since the 1960s are indicating that overall contaminant concentrations in surface sediments have been declining (Szalinska et al 2013). The general status of degradation of benthos in the Detroit River has been recently upgraded to "not impaired" in Canadian waters yet it remains impaired in the US jurisdiction of the AOC (DRCC 2020). It should however be noted that the latter assessments reflect a system-wide status as a whole and it is acknowledged in the remedial action plan process that when a BUI is delisted as unimpaired this does not necessarily mean complete elimination of all hotspots and/or elimination highly localized areas of impact (Green et al 2010).

The quintessential theoretical model of aquatic habitat gradients informing community compositions is the River Continuum Concept which describes river networks as a continuous gradient of physical and chemical conditions that predictably characterize biological communities along a gradient (Vannote et al 1980). The Detroit River, being a connecting channel, has more homogenous flow conditions compared to multi-ordered streams implicit in the river continuum model. As a connecting channel, the Detroit River receives >98% of its flow from the upstream Lake St. Clair and water quality tends to remain consistent along most waters from the Canadian upper to lower reaches of the river. Furthermore, deep, cooler, high velocity waters flowing through navigation channels throughout the river length have for the most part maintained hydraulic separation between nearshore water masses along Canadian

and U.S. shorelines reducing the impact of U.S. legacy and point sources inputs on Canadian nearshore habitats (Drouillard et al 2006). Nearshore habitats and areas receiving inflow from tributaries may have localized impacts from water quality with potential implications to ecosystem quality. Tributaries receiving urban and agricultural stress are subject to increased erosion that alters grain size distributions of deposited particles, increased loadings of suspended solids, turbidity, and organic carbon as well as pollutants (Johnes 1996; Lammert and Allan 1999; Wilkinson and McElroy 2007; Baldwin et al 2016b; Baldwin et al 2016a; Montiel-León et al 2019; Pronschinske et al 2022). As tributaries join into rivers, areas of confluence can create physiochemical gradients depending on flow characteristics. These areas can increase biodiversity by creating more heterogeneity in a habitat. However, in stressed habitats benthic community diversity can be negatively impacted by homogenized habitats (Hayward et al 2022).

There are three Canadian tributaries that contribute water and local watershed loadings to the Detroit River: Little River that enters in the upper reach, Turkey Creek which empties in the middle reach and River Canard that drains into the lower reach. Turkey Creek and River Canard freshwater estuaries support wetlands within the Detroit River and differ with respect to source of stressor from the watershed. River Canard is subject to agricultural stress while Turkey Creek is subject to urban stressors influenced by its course through the City of Windsor, Town of LaSalle, and a recipient of direct storm drainage discharge from highway 401. There is a large municipal wastewater plant discharge source operated by City of Windsor located approximately 3.5 km upstream of the Turkey Creek wetland as well as a rock salt mine and associated ship dock operating at the upstream margin of the Turkey Creek wetland.

ECCC routinely surveys five provincially designated significant wetlands in the Detroit River AOC; Canard River Marsh (River Canard), Detroit River Marshes (DRM), Detroit River

Marshes-North (Grass), Peche Island Marsh (Peche) and Turkey Creek Marsh (Turkey Creek). Water quality at these sites is assessed using the Chow-Fraser Water Quality Index (WQI) based on water quality parameters of turbidity, conductivity, temperature, and pH. WQI scores ranged from *good* to *very degraded*. Peche, DRM and Grass scored as good. Peche has the highest year-to-year water quality average in the river, followed by DRM and Grass. Turkey Creek had scored moderately degraded whereas River Canard scored very degraded. Data collected from 2006-2016 indicates water quality is improving across all wetlands with exception to River Canard. Suspended solids and turbidity are the major factors contributing to poor water quality in these tributary-influenced Canadian nearshore wetlands (Environment Canada 2017). ECCC's sampling design of tributary-impacted wetlands concentrates its benthic sampling efforts to the wadable portions located within the tributary. However, the wetlands along Turkey Creek and River Canard have continuous bed segments upstream and downstream of the tributary input point with portions of the wetland found within the confluence of the tributary/Detroit River mixing zone. By focusing their sampling efforts primarily within the tributary plume, the ECCC surveys potentially overemphasize the tributary impact on these two wetlands as a whole. In addition, there remains a lack of research investigating beta-diversity patterns of the Detroit River and whether differences between benthic communities reflect water quality assessments.

1.4 Thesis Objectives

The purpose of this thesis is to compare benthic macroinvertebrate and bacteria community compositions across the 5 provincially significant wetlands identified for the Detroit River. The objectives of this thesis are to address whether:

1) benthic macroinvertebrate and bacteria communities reflect differences in water quality previously identified across the wetlands.
2) benthic macroinvertebrate and bacteria communities show altered structure within the tributary plume, and potentially downstream of the plume, compared to portions of the wetland located upstream of the tributary drainage point.

Although these smaller tributaries generally fall outside the management scope of GLWQA Annex 1, they have local and potentially regional impacts on Detroit River water quality while also acting as vectors of macroinvertebrate and microbial species dispersal. Tributary confluences are areas where water and sediment quality and habitat characteristics can vary from the mixing of water sources. Areas of confluences can disrupt community organizations by creating a patchwork of varying habitats (Best 1987; Bravard and Gilvear 1996; Strungaru et al 2021). Monitoring of benthic macroinvertebrate communities in the Detroit River currently does not distinguish tributary communities from the river's wetlands. Yet, the movement of water and suspended solids through tributaries have the potential to alter both water quality and sediment characteristics at tributary/connecting channel confluences altering habitat characteristics. Therefore, tributary mixing zones should be an important consideration to the examination of spatial arrangements of benthic communities (Rice et al 2001). Information gathered in this study will describe benthic community distributions in the Detroit River which can inform current biomonitoring practices of macroinvertebrates. There is limited information

about microbial communities in sediments of the Detroit River and no published information comparing Detroit River wetland bacteria compositions. The observations on microbial composition in Detroit River wetlands generated by this thesis can therefore inform future adoption of microbial community biomonitoring strategies for the Canadian waters of this AOC.

REFERENCES

Akyildiz GK, Duran M (2021) Evaluation of the impact of heterogeneous environmental pollutants on benthic macroinvertebrates and water quality by long-term monitoring of the buyuk menderes river basin. Environmental Monitoring and Assessment 193:280. doi: 10.1007/s10661-021-08981-8

Aldunate M, Iglesia RD la, Bertagnolli AD, Ulloa O (2018) Oxygen modulates bacterial community composition in the coastal upwelling waters off central Chile. Deep Sea Research Part II: Topical Studies in Oceanography 156:68–79. doi: 10.1016/j.dsr2.2018.02.001

Ashton MJ, Morgan RP, Stranko S (2014) Relations between macroinvertebrates, nutrients, and water quality criteria in wadeable streams of Maryland, USA. Environmental Monitoring and Assessment 186:1167–1182. doi: 10.1007/s10661-013-3447-1

Astudillo-García C, Hermans SM, Stevenson B, et al (2019) Microbial assemblages and bioindicators as proxies for ecosystem health status: potential and limitations. Applied Microbiology and Biotechnology 103:6407–6421. doi: 10.1007/s00253-019-09963-0

Baldwin AK, Corsi SR, Cicco LAD, et al (2016a) Organic contaminants in Great Lakes tributaries: Prevalence and potential aquatic toxicity. Science of The Total Environment 554:42–52. doi: 10.1016/j.scitotenv.2016.02.137

Baldwin AK, Corsi SR, Mason SA (2016b) Plastic Debris in 29 Great Lakes Tributaries: Relations to Watershed Attributes and Hydrology. Environmental Science & Technology 50:10377–10385. doi: 10.1021/acs.est.6b02917

Balian EV, Segers H, Lévèque C, Martens K (2008) The Freshwater Animal Diversity Assessment: an overview of the results. Hydrobiologia 595:627–637. doi: 10.1007/s10750-007-9246-3

Barnum TR, Weller DE, Williams M (2017) Urbanization reduces and homogenizes trait diversity in stream macroinvertebrate communities. Ecological Applications 27:2428–2442. doi: 10.1002/eap.1619

Baselga A (2010) Partitioning the turnover and nestedness components of beta diversity. Global Ecology and Biogeography 19:134–143. doi: 10.1111/j.1466-8238.2009.00490.x

Beermann AJ, Elbrecht V, Karnatz S, et al (2018) Multiple-stressor effects on stream macroinvertebrate communities: A mesocosm experiment manipulating salinity, fine sediment and flow velocity. Science of The Total Environment 610:961–971. doi: 10.1016/j.scitotenv.2017.08.084

Best J (1987) Flow Dynamics at River Channel Confluences: Implications for Sediment Transport and Bed Morphology. SEPM Special Publication: Recent Developments in Fluvial Sedimentology 27–35. doi: 10.2110/pec.87.39.0027

Bonada N, Dolédec S, Statzner B (2007) Taxonomic and biological trait differences of stream macroinvertebrate communities between mediterranean and temperate regions: implications for future climatic scenarios. Global Change Biology 13:1658–1671. doi: 10.1111/j.1365-2486.2007.01375.x

Bouchez T, Blieux AL, Dequiedt S, et al (2016) Molecular microbiology methods for environmental diagnosis. Environmental Chemistry Letters 14:423–441. doi: 10.1007/s10311-016-0581-3

Bowen H, Maul JE, Cavigelli MA, Yarwood S (2020) Denitrifier abundance and community composition linked to denitrification activity in an agricultural and wetland soil. Applied Soil Ecology 151:103521. doi: 10.1016/j.apsoil.2020.103521

Brasil LS, Luiza-Andrade A, Calvão LB, et al (2020) Aquatic insects and their environmental predictors: a scientometric study focused on environmental monitoring in lotic environmental. Environmental Monitoring and Assessment 192:194. doi: 10.1007/s10661-020-8147-z

Bravard J-P, Gilvear DJ (1996) The Fluvial Hydrosystems. 98–116. doi: 10.1007/978-94-009-1491-9_5

Buhk C, Alt M, Steinbauer MJ, et al (2017) Homogenizing and diversifying effects of intensive agricultural land-use on plant species beta diversity in Central Europe — A call to adapt our conservation measures. Science of The Total Environment 576:225–233. doi: 10.1016/j.scitotenv.2016.10.106

Buss DF, Baptista DF, Nessimian JL, Egler M (2004) Substrate specificity, environmental degradation and disturbance structuring macroinvertebrate assemblages in neotropical streams. Hydrobiologia 518:179–188. doi: 10.1023/b:hydr.0000025067.66126.1c

Canfield DE, Thamdrup B (2009) Towards a consistent classification scheme for geochemical environments, or, why we wish the term 'suboxic' would go away. Geobiology 7:385–392. doi: 10.1111/j.1472-4669.2009.00214.x

Cardinale BJ, Matulich KL, Hooper DU, et al (2011) The functional role of producer diversity in ecosystems. American Journal of Botany 98:572–592. doi: 10.3732/ajb.1000364

Catteau A, Porcher J-M, Bado-Nilles A, et al (2022) Interest of a multispecies approach in active biomonitoring: Application in the Meuse watershed. Science of The Total Environment 808:152148. doi: 10.1016/j.scitotenv.2021.152148

Chandra P, Enespa, Kumar M (2019) Restoration of Wetland Ecosystem: A Trajectory Towards a Sustainable Environment. 101–124. doi: 10.1007/978-981-13-7665-8_8

Changnon SA, Changnon JM (1996) History of the Chicago Diversion and Future Implications. Journal of Great Lakes Research 22:100–118. doi: 10.1016/s0380-1330(96)70940-1

Chapman LJ, Schneider KR, Apodaca C, Chapman CA (2004) Respiratory Ecology of Macroinvertebrates in a SwampRiver System of East Africa. BIOTROPICA 36:572–585. doi: 10.1646/1598

Chen J, Gao H, Wang P, et al (2019) Effects of decabromodiphenyl ether on activity, abundance, and community composition of phosphorus mineralizing bacteria in eutrophic lake sediments. Science of The Total Environment 695:133785. doi: 10.1016/j.scitotenv.2019.133785

Clarke K, Warwick R (2001) Change in marine communities: an approach to statistical analysis and interpretation, 2nd edition. PRIMER-E Ltd, Plymouth

Clements WH (1999) Metal tolerance and predator–prey interactions in benthic macroinvertebrate stream communities. Ecological Applications 9:1073–1084. doi: 10.1890/1051-0761(1999)009[1073:mtappi]2.0.co;2

Collier KJ (1995) Environmental factors affecting the taxonomic composition of aquatic macroinvertebrate communities in lowland waterways of Northland, New Zealand. New Zealand Journal of Marine and Freshwater Research 29:453–465. doi: 10.1080/00288330.1995.9516679

Covich AP, Palmer MA, Crowl TA (1999) The Role of Benthic Invertebrate Species in Freshwater Ecosystems. BioScience 49:119. doi: 10.2307/1313537

Davidson NC (2014) How much wetland has the world lost? Long-term and recent trends in global wetland area. Marine and Freshwater Research 65:934–941. doi: 10.1071/mf14173

Demars BOL, Kemp JL, Marteau B, et al (2021) Stream Macroinvertebrates and Carbon Cycling in Tangled Food Webs. Ecosystems 24:1944–1961. doi: 10.1007/s10021-021-00626-8

Demi LM, Benstead JP, Rosemond AD, Maerz JC (2019) Experimental N and P additions alter stream macroinvertebrate community composition via taxon-level responses to shifts in detrital resource stoichiometry. Functional Ecology 33:855–867. doi: 10.1111/1365-2435.13289

Desrosiers M, Usseglio-polatera P, Archaimbault V, et al (2018) Assessing anthropogenic pressure in the St. Lawrence River using traits of benthic macroinvertebrates. Science of The Total Environment 649:233–246. doi: 10.1016/j.scitotenv.2018.08.267

Di HJ, Cameron KC, Shen JP, et al (2009) Nitrification driven by bacteria and not archaea in nitrogen-rich grassland soils. Nature Geoscience 2:621–624. doi: 10.1038/ngeo613

DRCC (2020) Re-designation Report: Assessment of Benthos (BUI #6) in the Detroit River Canadian Area of Concern. Windsor, ON, Canada

Drouillard KG, Tomczak M, Reitsma S, Haffner GD (2006) A River-wide Survey of Polychlorinated Biphenyls (PCBs), Polycylic Aromatic Hydrocarbons (PAHs), and Selected Organochlorine Pesticide Residues in Sediments of the Detroit River1999. Journal of Great Lakes Research 32:209–226. doi: 10.3394/0380-1330(2006)32[209:arsopb]2.0.co;2

Dudgeon D (1984) Longitudinal and temporal changes in functional organization of macroinvertebrate communities in the Lam Tsuen River, Hong Kong. Hydrobiologia 111:207–217. doi: 10.1007/bf00007201

Dudgeon D, Arthington AH, Gessner MO, et al (2005) Freshwater biodiversity: importance, threats, status and conservation challenges. Biological Reviews 81:163. doi: 10.1017/s1464793105006950

Environment Canada (2017) Detroit River Area of Concern: Coastal Wetland Habitat Assessment Report 2016 Update. CWS-ON, Downsview, Ontario

Escobar LE, Mallez S, McCartney M, et al (2017) Aquatic Invasive Species in the Great Lakes Region: An Overview. Reviews in Fisheries Science & Aquaculture 26:1–18. doi: 10.1080/23308249.2017.1363715

Evans-White MA, Dodds WK, Huggins DG, Baker DS (2009) Thresholds in macroinvertebrate biodiversity and stoichiometry across water-quality gradients in Central Plains (USA) streams. Journal of the North American Benthological Society 28:855–868. doi: 10.1899/08-113.1

Fennessy MS, Rokosch A, Mack JJ (2008) Patterns of plant decomposition and nutrient cycling in natural and created wetlands. Wetlands 28:300–310. doi: 10.1672/06-97.1

Flemming H-C, Wingender J (2001) Relevance of microbial extracellular polymeric substances (EPSs) - Part I: Structural and ecological aspects. Water Science and Technology 43:1–8. doi: 10.2166/wst.2001.0326

Geist J, Hawkins SJ (2016) Habitat recovery and restoration in aquatic ecosystems: current progress and future challenges. Aquatic Conservation: Marine and Freshwater Ecosystems 26:942–962. doi: 10.1002/aqc.2702

Ghermandi A, Bergh JCJM van den, Brander LM, et al (2010) Values of natural and human-made wetlands: A meta-analysis. Water Resources Research. doi: 10.1029/2010wr009071

Gibbs JP (2000) Wetland Loss and Biodiversity Conservation. Conservation Biology 14:314–317. doi: 10.1046/j.1523-1739.2000.98608.x

Glooschenko V (1983) Development of an evaluation system for wetlands in Southern Ontario. Wetlands 3:192–200. doi: 10.1007/bf03160741

GLWQA (1972) Great Lakes Water Quality Agreement. IJC, Ottawa, ON and Washington, DC

GLWQA (2012) Great Lakes Water Quality Agreement: Protocol Amending the Agreement Between Canada and the United States of America on Great Lakes Water Quality, 1978, as Amended on October 16, 1983, and on November 18, 1987.

Green N, Cargnelli L, Briggs T, et al (2010) Detroit River Canadian Remedial Action Plan Stage 2 Report. Detroit River Canadian Cleanup, Essex, Ontario, Canada

Grimm NB (1988) Role of Macroinvertebrates in Nitrogen Dynamics of a Desert Stream. Ecology 69:1884–1893. doi: 10.2307/1941165

Hall RO, Meyer JL (1998) The Trophic Significance of Bacteria in a Detritus-based Stream Food Web. Ecology 79:1995–2012. doi: 10.1890/0012-9658(1998)079[1995:ttsobi]2.0.co;2

Hand VL, Lloyd JR, Vaughan DJ, et al (2008) Experimental Studies of the Influence of Grain Size, Oxygen Availability and Organic Carbon Availability on Bioclogging in Porous Media. Environmental Science & Technology 42:1485–1491. doi: 10.1021/es072022s

Hanson RS, Hanson TE (1996) Methanotrophic bacteria. Microbiological Reviews 60:439–471. doi: 10.1128/mr.60.2.439-471.1996

Harrison S, Ross SJ, Lawton JH (1992) Beta Diversity on Geographic Gradients in Britain. The Journal of Animal Ecology 61:151. doi: 10.2307/5518

Hayward EE, Gillis PL, Bennett CJ, et al (2022) Freshwater mussels in an impacted watershed: Influences of pollution from point and non-point sources. Chemosphere 307:135966. doi: 10.1016/j.chemosphere.2022.135966

Hernandez ME, Beck DAC, Lidstrom ME, Chistoserdova L (2015) Oxygen availability is a major factor in determining the composition of microbial communities involved in methane oxidation. PeerJ 3:e801. doi: 10.7717/peerj.801

Hillebrand H, Bennett DM, Cadotte MW (2008) Consequences of Dominance: a Review of Evenness Effects on Local and Regional Ecosystem Processes. Ecology 89:1510–1520. doi: 10.1890/07-1053.1

Holmer M, Gribsholt B, Kristensen E (2002) Effects of sea level rise on growth of *Spartina anglica* and oxygen dynamics in rhizosphere and salt marsh sediments. Marine Ecology Progress Series 225:197–204. doi: 10.3354/meps225197

Hooper DU, Chapin FS, Ewel JJ, et al (2005) Effects of Biodiversity on Ecosystem Functioning: a Consensus of Current Knowledge. Ecological Monographs 75:3–35. doi: 10.1890/04-0922

Hudson LA, Ciborowski JJ (1996) Spatial and taxonomic variation in incidence of mouthpart deformities in midge larvae (Diptera: Chironomidae: Chironomini). Canadian Journal of Fisheries and Aquatic Sciences 53:297–304. doi: 10.1139/f95-199

Isbell F, Craven D, Connolly J, et al (2015) Biodiversity increases the resistance of ecosystem productivity to climate extremes. Nature 526:574–577. doi: 10.1038/nature15374

Johnes PJ (1996) Evaluation and management of the impact of land use change on the nitrogen and phosphorus load delivered to surface waters: the export coefficient modelling approach. Journal of Hydrology 183:323–349. doi: 10.1016/0022-1694(95)02951-6

Johnston D (2006) Connecting People to Place: Great Lakes Aboriginal History in Cultural Context. Ipperwash Commission of Inquiry

Jonsson M, Burrows RM, Lidman J, et al (2017) Land use influences macroinvertebrate community composition in boreal headwaters through altered stream conditions. Ambio 46:311–323. doi: 10.1007/s13280-016-0837-y

Karimi B, Maron PA, Boure NC-P, et al (2017) Microbial diversity and ecological networks as indicators of environmental quality. Environmental Chemistry Letters 15:265–281. doi: 10.1007/s10311-017-0614-6

Kelly DW, Bailey RJ, MacNeil C, et al (2006) Invasion by the amphipod *Gammarus pulex* alters community composition of native freshwater macroinvertebrates. Diversity and Distributions 12:525–534. doi: 10.1111/j.1366-9516.2006.00275.x

Kiyashko S, Narita T, Wada E (2001) Contribution of methanotrophs to freshwater macroinvertebrates: evidence from stable isotope ratios. Aquatic Microbial Ecology 24:203–207. doi: 10.3354/ame024203

Klayn S, Berov D, Karamfilov V (2021) Environmental Protection and Disaster Risks, Selected Papers from the 1st International Conference on Environmental Protection and Disaster RISKs (EnviroRISKs). Studies in Systems, Decision and Control 371–382. doi: 10.1007/978-3-030-70190-1_25

Knop E (2016) Biotic homogenization of three insect groups due to urbanization. Global Change Biology 22:228–236. doi: 10.1111/gcb.13091

Lai X, Zhao Y, Pan F, et al (2019) Enhanced optimal removal of nitrogen and organics from intermittently aerated vertical flow constructed wetlands: Relative COD/N ratios and microbial responses. Chemosphere 244:125556. doi: 10.1016/j.chemosphere.2019.125556

Lammert M, Allan JD (1999) Assessing Biotic Integrity of Streams: Effects of Scale in Measuring the Influence of Land Use/Cover and Habitat Structure on Fish and Macroinvertebrates. Environmental Management 23:257–270. doi: 10.1007/s002679900184

Larsen S, Pace G, Ormerod SJ (2011) Experimental effects of sediment deposition on the structure and function of macroinvertebrate assemblages in temperate streams. River Research and Applications 27:257–267. doi: 10.1002/rra.1361

Larson JH, Trebitz AS, Steinman AD, et al (2013) Great Lakes rivermouth ecosystems: Scientific synthesis and management implications. Journal of Great Lakes Research 39:513–524. doi: 10.1016/j.jglr.2013.06.002

Legendre P (2014) Interpreting the replacement and richness difference components of beta diversity. Global Ecology and Biogeography 23:1324–1334. doi: 10.1111/geb.12207

Lennon JT, Jones SE (2011) Microbial seed banks: the ecological and evolutionary implications of dormancy. Nature Reviews Microbiology 9:119–130. doi: 10.1038/nrmicro2504

Leprieur F, Tedesco PA, Hugueny B, et al (2011) Partitioning global patterns of freshwater fish beta diversity reveals contrasting signatures of past climate changes. Ecology Letters 14:325–334. doi: 10.1111/j.1461-0248.2011.01589.x

Li L, Zheng B, Liu L (2010) Biomonitoring and Bioindicators Used for River Ecosystems: Definitions, Approaches and Trends. Procedia Environmental Sciences 2:1510–1524. doi: 10.1016/j.proenv.2010.10.164

Ligeiro R, Hughes RM, Kaufmann PR, et al (2013) Defining quantitative stream disturbance gradients and the additive role of habitat variation to explain macroinvertebrate taxa richness. Ecological Indicators 25:45–57. doi: 10.1016/j.ecolind.2012.09.004

Ligi T, Oopkaup K, Truu M, et al (2014) Characterization of bacterial communities in soil and sediment of a created riverine wetland complex using high-throughput 16S rRNA amplicon sequencing. Ecological Engineering 72:56–66. doi: 10.1016/j.ecoleng.2013.09.007

Liu Z, Huang S, Sun G, et al (2011) Diversity and abundance of ammonia-oxidizing archaea in the Dongjiang River, China. Microbiological Research 166:337–345. doi: 10.1016/j.micres.2010.08.002

Lock K, Goethals PLM (2011) Distribution and ecology of the mayflies (Ephemeroptera) of Flanders (Belgium). Annales de Limnologie - International Journal of Limnology 47:159–165. doi: 10.1051/limn/2011011

Mackay RJ (1992) Colonization by Lotic Macroinvertebrates: A Review of Processes and Patterns. Canadian Journal of Fisheries and Aquatic Sciences 49:617–628. doi: 10.1139/f92-071

Maguire TJ, Spencer C, Grgicak-Mannion A, et al (2019) Distinguishing point and non-point sources of dissolved nutrients, metals, and legacy contaminants in the Detroit River. Science of The Total Environment 681:1–8. doi: 10.1016/j.scitotenv.2019.04.311

Malaj E, Grote M, Schäfer RB, et al (2012) Physiological sensitivity of freshwater macroinvertebrates to heavy metals. Environmental Toxicology and Chemistry 31:1754–1764. doi: 10.1002/etc.1868

Maltby E, Acreman MC (2011) Ecosystem services of wetlands: pathfinder for a new paradigm. Hydrological Sciences Journal 56:1341–1359. doi: 10.1080/02626667.2011.631014

Markert BA, Breure AM, Zechmeister HG (2003) Chapter 1 Definitions, strategies and principles for bioindication/biomonitoring of the environment. Trace Metals and other Contaminants in the Environment 6:3–39. doi: 10.1016/s0927-5215(03)80131-5

McKinney ML (2006) Urbanization as a major cause of biotic homogenization. Biological Conservation 127:247–260. doi: 10.1016/j.biocon.2005.09.005

Mensing DM, Galatowitsch SM, Tester JR (1998) Anthropogenic effects on the biodiversity of riparian wetlands of a northern temperate landscape. Journal of Environmental Management 53:349–377. doi: 10.1006/jema.1998.0215

Méthot J, Huang X, Grover H (2015) Demographics and societal values as drivers of change in the Great Lakes–St. Lawrence River basin. Journal of Great Lakes Research 41:30–44. doi: 10.1016/j.jglr.2014.11.001

Mitsch WJ, Gosselink JG (2000) The value of wetlands: importance of scale and landscape setting. Ecological Economics 35:25–33. doi: 10.1016/s0921-8009(00)00165-8

Montiel-León JM, Munoz G, Duy SV, et al (2019) Widespread occurrence and spatial distribution of glyphosate, atrazine, and neonicotinoids pesticides in the St. Lawrence and tributary rivers. Environmental Pollution 250:29–39. doi: 10.1016/j.envpol.2019.03.125

Naeem S, Li S (1997) Biodiversity enhances ecosystem reliability. Nature 390:507–509. doi: 10.1038/37348

OMNR (2014) Ontario Wetland Evaluation System, Southern Manual, 3rd Edition. Queen's Printer for Ontario, Ontario, Canada

Ostermiller JD, Hawkins CP (2004) Effects of sampling error on bioassessments of stream ecosystems: application to RIVPACS-type models. Journal of the North American Benthological Society 33:363–382. doi: 10.1899/0887-3593(2004)023<0363:eoseob>2.0.co;2

Passy SI, Blanchet FG (2007) Algal communities in human-impacted stream ecosystems suffer beta-diversity decline. Diversity and Distributions 13:670–679. doi: 10.1111/j.1472-4642.2007.00361.x

Pattison-Williams JK, Pomeroy JW, Badiou P, Gabor S (2018) Wetlands, Flood Control and Ecosystem Services in the Smith Creek Drainage Basin: A Case Study in Saskatchewan, Canada. Ecological Economics 147:36–47. doi: 10.1016/j.ecolecon.2017.12.026

Pester M, Knorr K-H, Friedrich MW, et al (2012) Sulfate-Reducing Microorganisms in Wetlands – Fameless Actors in Carbon Cycling and Climate Change. Frontiers in Microbiology 3:72. doi: 10.3389/fmicb.2012.00072

Pischedda L, Poggiale JC, Cuny P, Gilbert F (2008) Imaging Oxygen Distribution in Marine Sediments. The Importance of Bioturbation and Sediment Heterogeneity. Acta Biotheoretica 56:123–135. doi: 10.1007/s10441-008-9033-1

Pronschinske MA, Corsi SR, DeCicco LA, et al (2022) Prioritizing Pharmaceutical Contaminants in Great Lakes Tributaries Using Risk-Based Screening Techniques. Environmental Toxicology and Chemistry 41:2221–2239. doi: 10.1002/etc.5403

Purvis A, Hector A (2000) Getting the measure of biodiversity. Nature 405:212–219. doi: 10.1038/35012221

Pusch M, Fiebig D, Brettar I, et al (1998) The role of micro-organisms in the ecological connectivity of running waters. Freshwater Biology 40:453–495. doi: 10.1046/j.1365-2427.1998.00372.x

Rawi CSM, Al-Shami SA, Madrus MR, Ahmad AH (2014) Biological and ecological diversity of aquatic macroinvertebrates in response to hydrological and physicochemical parameters in tropical forest streams of Gunung Tebu, Malaysia: implications for ecohydrological assessment. Ecohydrology 7:496–507. doi: 10.1002/eco.1368

Reich PB, Tilman D, Isbell F, et al (2012) Impacts of Biodiversity Loss Escalate Through Time as Redundancy Fades. Science 336:589–592. doi: 10.1126/science.1217909

Resh V, Norris RH, Barbour MT (1995) Design and implementation of rapid assessment approaches for water resource monitoring using benthic macroinvertebrates. Austral Ecology 20:108–121. doi: 10.1111/j.1442-9993.1995.tb00525.x

Reynoldson TB, Bailey R, Day K, Norris RH (1995) Biological guidelines for freshwater sediment based on BEnthic Assessment of SedimenT (the BEAST) using a multivariate approach for predicting biological state. Australian Journal of Ecology 20:198–219. doi: 10.1111/j.1442-9993.1995.tb00532.x

Rice SP, Greenwood MT, Joyce CB (2001) Tributaries, sediment sources, and the longitudinal organisation of macroinvertebrate fauna along river systems. Canadian Journal of Fisheries and Aquatic Sciences 58:824–840. doi: 10.1139/f01-022

Rothlisberger JD, Lodge DM, Cooke RM, Finnoff DC (2010) Future declines of the binational Laurentian Great Lakes fisheries: the importance of environmental and cultural change. Frontiers in Ecology and the Environment 8:239–244. doi: 10.1890/090002

Scavia D, Allan JD, Arend KK, et al (2014) Assessing and addressing the re-eutrophication of Lake Erie: Central basin hypoxia. Journal of Great Lakes Research 40:226–246. doi: 10.1016/j.jglr.2014.02.004

Schmera D, Heino J, Podani J, et al (2016) Functional diversity: a review of methodology and current knowledge in freshwater macroinvertebrate research. Hydrobiologia 787:27–44. doi: 10.1007/s10750-016-2974-5

Shannon C, Weaver W (1949) The Mathematical Theory of Communication. The University of Illinois Press, Urbana, IL

Sims A, Zhang Y, Gajaraj S, et al (2013) Toward the development of microbial indicators for wetland assessment. Water Research 47:1711–1725. doi: 10.1016/j.watres.2013.01.023

Strungaru S-A, Pohontiu CM, Nicoara M, et al (2021) Response of aquatic macroinvertebrates communities to multiple anthropogenic stressors in a lowland tributary river. Environmental Toxicology and Pharmacology 87:103687. doi: 10.1016/j.etap.2021.103687

Szalinska E, Grgicak-Mannion A, Haffner GD, Drouillard KG (2013) Assessment of decadal changes in sediment contamination in a large connecting channel (Detroit River, North America). Chemosphere 93:1773–1781. doi: 10.1016/j.chemosphere.2013.06.009

Tang D, Liu X, Wang X, Yin K (2018) Relationship between the Main Communities and Environments of an Urban River and Reservoir: Considering Integrated Structural and Functional Assessments of Ecosystems. International Journal of Environmental Research and Public Health 15:2302. doi: 10.3390/ijerph15102302

Teiter S, Mander Ü (2005) Emission of N2O, N2, CH4, and CO2 from constructed wetlands for wastewater treatment and from riparian buffer zones. Ecological Engineering 25:528–541. doi: 10.1016/j.ecoleng.2005.07.011

Townsend CR, Scarsbrook MR, Dolédec S (1997) The intermediate disturbance hypothesis, refugia, and biodiversity in streams. Limnology and Oceanography 42:938–949. doi: 10.4319/lo.1997.42.5.0938

Trebitz A, Sykes M, Barge J (2019) A reference inventory for aquatic fauna of the Laurentian Great Lakes. Journal of Great Lakes Research 45:1036–1046. doi: 10.1016/j.jglr.2019.10.004

Vannote RL, Minshall GW, Cummins KW, et al (1980) The River Continuum Concept. Canadian Journal of Fisheries and Aquatic Sciences 37:130–137. doi: 10.1139/f80-017

Wallace JB, Webster JR (1996) The Role of Macroinvertebrates in Stream Ecosystem Function. Annual Review of Entomology 41:115–139. doi: 10.1146/annurev.en.41.010196.000555

Whittaker RH (1960) Vegetation of the Siskiyou Mountains, Oregon and California. Ecological Monographs 30:279–338. doi: 10.2307/1943563

Wilkinson BH, McElroy BJ (2007) The impact of humans on continental erosion and sedimentation. GSA Bulletin 119:140–156. doi: 10.1130/b25899.1

Williams P, Whitfield M, Biggs J, et al (2004) Comparative biodiversity of rivers, streams, ditches and ponds in an agricultural landscape in Southern England. Biological Conservation 115:329–341. doi: 10.1016/s0006-3207(03)00153-8

Wilson CC, Haxton T, Wozney KM, Friday M (2022) Historical genetic connectivity of lake sturgeon in a dammed Great Lakes tributary. Journal of Great Lakes Research 48:798–805. doi: 10.1016/j.jglr.2022.03.007

Windsor FM, Pereira MG, Tyler CR, Ormerod SJ (2019) River organisms as indicators of the distribution and sources of persistent organic pollutants in contrasting catchments. Environmental Pollution 255:113144. doi: 10.1016/j.envpol.2019.113144

Wittebolle L, Marzorati M, Clement L, et al (2009) Initial community evenness favours functionality under selective stress. Nature 458:623–626. doi: 10.1038/nature07840

Yang G, Wagg C, Veresoglou SD, et al (2018) How Soil Biota Drive Ecosystem Stability. Trends in Plant Science 23:1057–1067. doi: 10.1016/j.tplants.2018.09.007

Zoltai SC, Vitt DH (1995) Canadian wetlands: Environmental gradients and classification. Vegetatio 118:131–137. doi: 10.1007/bf00045195

CHAPTER 2

DETROIT RIVER BENTHIC MACROINVERTEBRATE RESPONSE ALONG TRIBUTARY JUNCTIONS

2.1: Introduction

Wetlands of the Detroit River are internationally recognized as important habitat to global biodiversity (Hartig and Bennion 2017). Anthropogenic stresses have strained the river since European settlement resulting in losses of 97% of coastal wetland habitat (Hartig and Bennion 2017). Wetland loss was indicated as a driver of fish species loss and extinction debt within Lake St. Clair and Lake Erie where the Detroit River's River Canard wetland had been identified as requiring the greatest need of restoration (Montgomery et al 2020). Multiple taxa in the Great Lakes region respond to surrounding land use (Kovalenko et al 2014). Land-use surrounding River Canard is predominantly agricultural. Agricultural practices alter habitats such that changes in fish and macroinvertebrate species assemblages are observed within watershed, riparian-corridors and reaches (Stewart et al 2001; Scharold et al 2015). Land-use surrounding Turkey Creek is predominately under urban pressures.

The Detroit River sediments had been adversely affected by long-term pollution from industrialization along its shoreline and from Lake St. Clair inflow. *Hexagenia*, a burrowing mayfly species, showed reduced populations as metals increased and oxygen decreased in river sediments (Edsall et al 1991) coupled with uptake of contaminants in their tissues and transfer of sediment-associated contaminants into other components of the aquatic food web (Kovats and Ciborowski 1989; Drouillard et al 1996; Corkum et al 1997). Sediment contamination along Canadian portions of the Detroit River has improved to where very few sediment samples exceed pollutant concentrations indicative of toxicity based on laboratory toxicity assays of sensitive indicators such as Ephemeroptera (DRCC 2020). However, macroinvertebrate

composition in Canadian zones may still be affected by habitat alterations and conditions attributed to sediment or water quality in proximity to tributary inputs.

In addition to sediments, overlying water is also a factor in the uptake of contaminants to macroinvertebrates (Li et al 2019a). Water quality encompasses pollutants that can be chemical, physical, or biological where the suitability of water to perform functions is restricted (Mostertman 1976). Water quality indices (WQIs) vary by selected parameters and allow for comparisons and monitoring of water integrity. ECCC water quality scores of the Detroit River AOC considers turbidity, conductivity, temperature, and pH. These parameters were originally selected as indicators of wetland quality based on multivariate model selection of variables across 300 Great Lakes coastal wetland sites reflecting a gradient of high quality to highly degraded sites (Chow-Fraser 2006). The above parameters have also been demonstrated to alter benthic macroinvertebrate communities (Lamberti and Resh 1985; Stewart and Downing 2008; Matlou et al 2017; Sabha et al 2022; Taborda et al 2022).

Benthic macroinvertebrate communities of the Detroit River have been primarily studied related to sediment contamination and research exploring local influences on community structures can inform further studies of community dynamics (Kovalenko et al 2014). This chapter seeks to examine patterns of macroinvertebrate community compositions at wetlands monitored in the Detroit River AOC with considerations made to tributary-impacted wetlands which are classified as impaired with respects to water quality.

H1: Macroinvertebrate community assemblages will respond to water quality differences between tributary-impacted and unimpacted wetlands.

H2: Macroinvertebrate assemblages at tributary-impacted sample sites demonstrate differences with respect to tributary plume position.

Variations in macroinvertebrate communities are expected at River Canard and Turkey Creek wetlands which have been previously assessed to have increased turbidity and conductivity arising from the tributary input (Environment Canada, 2018). River Canard is the most impacted wetland with respect to water quality and is therefore expected to vary in its macroinvertebrate composition most significantly. I further anticipate that the two tributary impacted wetlands will differ from one another due to differences in the tributary flow regime and respective tributary watershed land-use. River Canard tributary (TRC) courses through a predominately agricultural watershed whereas Turkey Creek Tributary (TTC) and wetland is affected by urban stressors such as mining, wastewater treatment, and storm water discharge from a major highway. I anticipate both wetlands will have reduced diversity metrics, low sensitive taxa (EPT%) which is commonly seen in anthropogenically stressed habitats (Manfrin et al 2013). Wetlands which received good water quality scores, Detroit River Marshes (DRM), Peche Island Marsh, and Grass Island Marsh are expected to show similarities with one another, and they are expected to have overall improved diversity metrics like higher species evenness, higher species diversity, and more pollution-intolerant species such as EPT taxa. For tributary-impacted sample sites with respects to position to the tributary, it is predicted that the community assemblage upstream of the tributary inflow will differ most significantly from downstream and tributary communities and resemble unimpaired communities as impacted communities are expected at the tributary and downstream of in flow.

2.2 Methodology:

2.2.1: Study Site and Site Selection

The Detroit River is a binational connecting channel in the Laurentian Great Lakes between Detroit, Michigan, USA and Windsor, Ontario, Canada. It flows southward 43.5km from Lake St. Clair to Lake Erie with a discharge rate ranging from 4400m^3s^{-1} in the winter to 5,700 m^3s^{-1} in the summer. Since river slope is uniform (3ft), water depth and flow largely depend on water levels of Lake St. Clair and Lake Erie which vary annually (Derecki 1984). River sediment deposition is contingent on flow velocities. As flow velocities decreases, finer particle sizes are deposited generating longitudinal gradients across the river as well as fine grained deposition zones in the vicinity of the numerous islands, near shorelines and in shallow areas (UGLCCS 1989). Fine sediment (sands and silt) is common in the upper and middle reaches where flow velocities are higher. Very fine sediments (silts and clay) deposit mainly in the lower reach and in narrow canals. Areas of finer sediment correspond with lower water depths and submerged aquatic vegetation (Fallon and Horvath 1985; Wood 2004; Szalinska et al 2013). Geological surveys of Windsor-Essex County determined the sedimentary rock of the Canadian shoreline, including tributary inlets to be limestone, stromatolitic calcarenite and anhydrite (Morris 1994).

Site selection in this study focuses on five coastal wetlands located in the Canadian waters of the Detroit River. They include Peche Island Marsh, Detroit River Marshes (DRM), Grass Island, Turkey Creek Marsh, and River Canard Marsh (**Figure 2.1**). The geographic extent of the above wetlands was delineated with the use of bathymetry data and July 2018 satellite imagery data provided by Google Earth Pro. A random stratified sampling design was implemented to select sample site locations. For each stratum, a grid of composed of 50m^2-

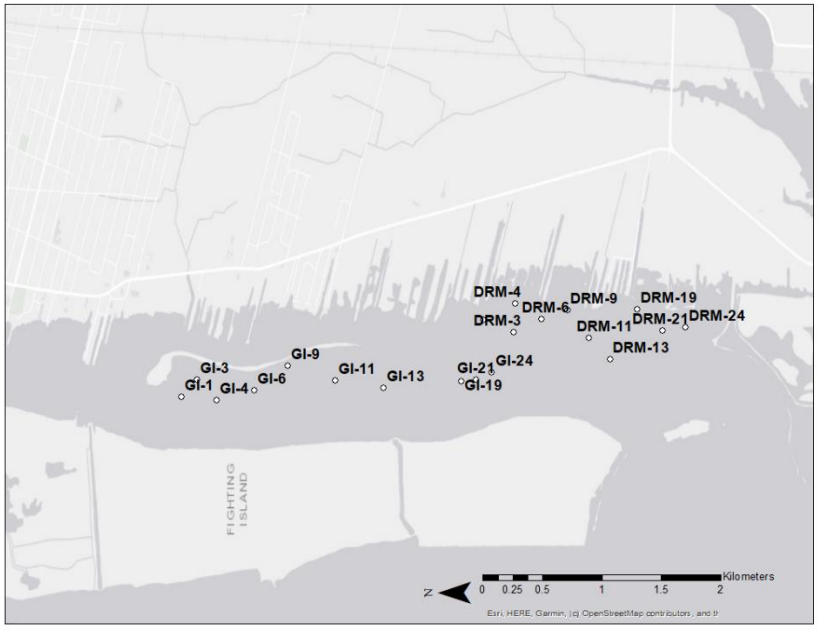
$100m^2$ quadrants were superimposed and each quadrant was assigned a location code. Each reference wetland had one stratum to which ten randomized sample sites were assigned based on the position coordinate at the center of the selected quadrant (**Figure 2.2**). Tributary-impacted wetlands, River Canard and Turkey Creek were subdivided into 3 strata; the first stratum encompassed wetland positions that were upstream of the tributary input. A second stratum was assigned within the tributary plume and a third stratum considered all wetland areas downstream of the tributary. The tributary plume area was delineated using turbidity measurements collected in 2018 and Google Earth Pro to visualize turbidity directions across time using the historical imagery feature. The plume at River Canard had extended into nearshore zones. Five randomized sample locations were assigned per strata for the tributary-impacted wetlands for a total of 15 locations at each wetland (**Figure 2.3**). The data used in this study was collected over a nine-day period in late September into October 2019. At the time of sampling, water level conditions in the Great Lakes, were increasing since 2012 and had peaked in 2018-2020 (USACE, 2023).

There are multiple terms used to describe sample sites in this study. This study examines communities by wetland and position for tributary-impacted sample sites, within each of these groupings contain strata – defined by the method of site selection, random stratified selection. Wetlands consist of five strata DRM, Grass Island (GI), Peche Island (PI), River Canard (RC), and Turkey Creek (TC).; unimpacted or unimpaired are DRM, Grass Island and Peche Island; these wetlands are referred to as reference wetlands in the multivariate reference approach (MRA) analyses where sample sites were divided into two sediment habitat groups. The tributary-impacted, also referred to as impaired, are River Canard and Turkey Creek and further divided into positional strata – upstream, downstream, and tributary plume. Site codes were

assigned by a wetland/tributary abbreviation followed by a site number. The tributary-impacted wetland site number is preceded by a letter to denote upstream (U) and downstream (D).

Figure 2.1: Detroit River wetland locations and boundaries map.

a)



b)

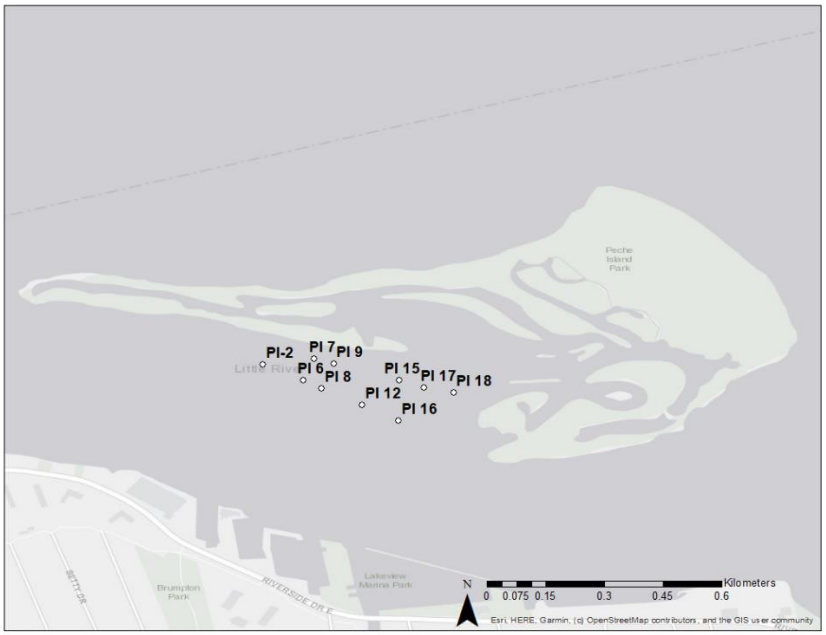
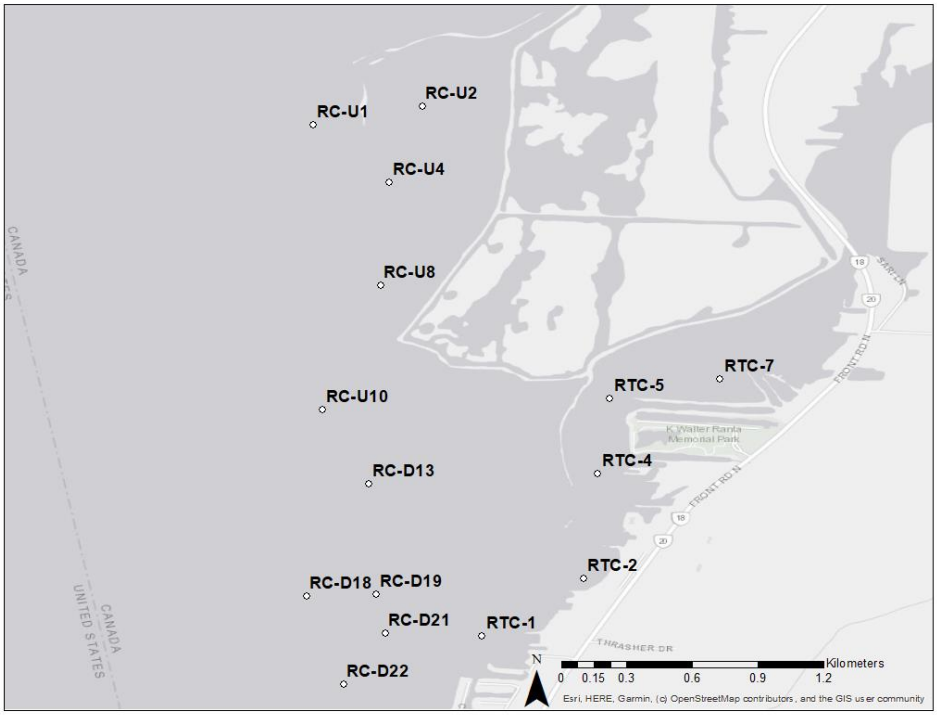


Figure 2.2: Sampling sites of unimpacted wetlands a) Grass Island and Detroit River Marshes b) Peche Island

a)



b)

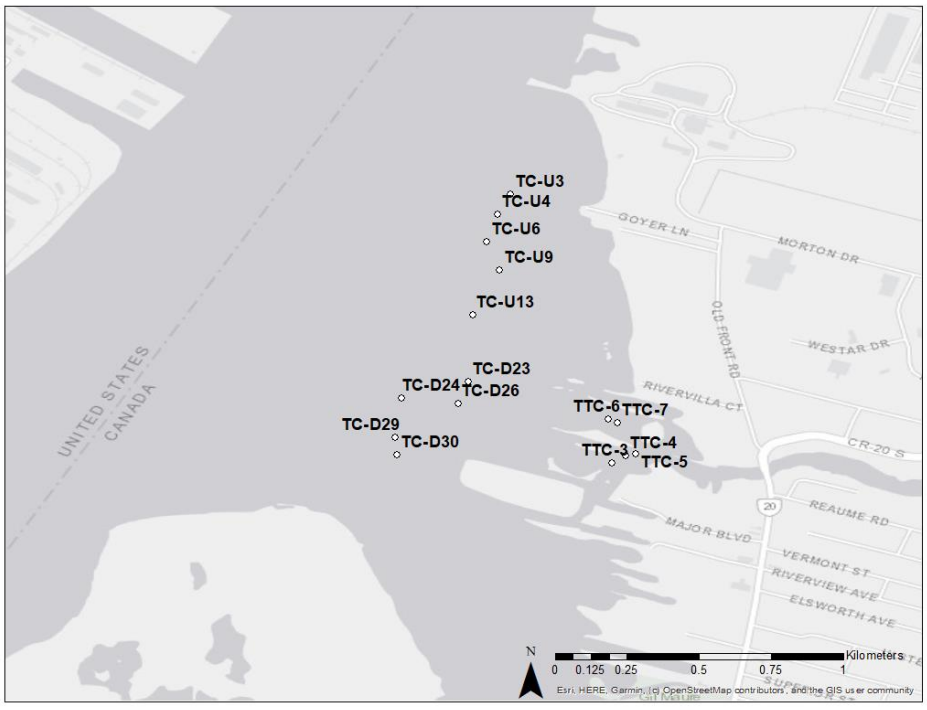


Figure 2.3: Sampling sites of tributary-impacted wetlands a) River Canard b) Turkey Creek.

2.2.2 Environmental Analysis

In collecting environmental data, in-situ sonde readings were taken with Ruskin RBR profiler upon arrival to sample site for water depth (m), conductivity (mS/cm), pH, temperature (^{o}C), and turbidity (NTU). Bulk sediment was collected with petite ponar grabs (2.4 L), and a 100 mL subset was collected in plastic screw-cap jars. Jars were placed on ice in the field until transferred to a 4^{o}C laboratory storage area. Sediment was analyzed by the Organic Analytical and Nutrient Laboratory (OANL) at GLIER (University of Windsor) for grain-size and loss on ignition derived sediment organic carbon content; total organic carbon (TOC). TOC was measured by muffle drying sediments at 550^{o}C overnight and determining percentage of mass loss (Drouillard et al 2006). Grain size was measured by mechanically separating dry sediment through a sieve set (#5, #10, #35, #60, #100, #200) and calculating the proportion in each fraction. Gravel (>4mm), Sand (4.0-0.5mm), Fine Sand (0.5-0.149mm), Silt/Clay: <0.149mm).

2.2.3 Benthic Macroinvertebrates

Benthic macroinvertebrates were collected using a petite ponar grab (2.4 L) in triplicate. ponar grabs had to be a minimum of 60% full to be considered sufficient. Each grab sample was partially washed on-site through a 0.250 mm sieve bag, transferred into a polyethylene bag and preserved in a modified Kahle's solution (10:5:1 v/v/v water:95% ethanol:formalin). Samples were returned to laboratory, heat-sealed, and stored at room temperature until processing.

One of three samples collected at each site was selected for processing by randomization. The sample material was separated from preservative through a 0.250 mm sieve and washed with water through a nested sieve set (size fractions: 4 mm, 1 mm, 0.5 mm, 0.250 mm). Many samples from the Detroit River contained large amounts of plant material. To

facilitate the washing process, the sample was transferred into a tray with water and is spread apart with gentle agitation by forceps. Large-bodied invertebrates (>10 mm) could be collected with forceps to prevent damage to delicate structures in washing process. Each size-fraction was transferred to a petri-dish for sorting.

Special considerations were made with samples containing large volumes of sand. Sand has the potential to clog and prevent water from exiting the sieve-stack. When washing the sample, a panning technique is used to separate the less dense biological material from sand. Panning involves lightly agitating the contents of the pan with a side-to-side motion and pouring the suspended material through the sieve-set. Panning is repeated until it is visually apparent that all organic material has been removed. The excess sand was collected into petri dishes and inspected with a dissection scope before discarding.

Macroinvertebrates were sorted from detritus within respective size-fraction with a dissection microscope. The 4mm and 1mm size fractions were sorted entirely. The 0.500mm fraction was quartered and the 0.250 mm fraction was archived. Detritus was filtered from water into 0.7 µm filter paper and placed in a drying oven at 104^{o}C. Detritus weight was recorded for each size fraction and any quartered subfractions. For quality control, 10% of samples were verified by an independent taxonomist (A. Frazao, Biomonitoring Technician, GLIER, University of Windsor) and error could exceed 5%, otherwise the sample was re-counted. In this study, identification reached 99% accuracy.

Macroinvertebrates were identified using the following keys: Freshwater Invertebrates: Keys to Nearctic Fauna (Thorp and Rogers 2016); An Introduction to the Aquatic Insects of North America (Merritt et al 2008). Macroinvertebrates were identified to lowest taxonomic resolution with exception to Oligochaeta, Chironomidae, Nematoda, Turbellaria and Rissooidea.

Counts: Macroinvertebrate counts were compiled for all size fractions to represent the entire sample. Counts of the 0.500 mm quartered fraction was extrapolated to a full fraction sample according to the relative proportion of dried detritus weight.

0.500 mm fraction count =

(Count) (100% of detritus weight/detritus weight of quartered sample)

2.2.4 Statistical Analysis

The general approach to data interpretation and statistical analyses is presented as a flow chart in **Figure 2.4**. All statistical analyses were performed using R (R Core Team 2023) in RStudio (R Studio Team 2020) and plots were generated with ggplot2 (Wickham 2016) unless otherwise stated.

2.2.4.1: PCA Water Quality and Habitat

The wetland habitats were compared for differences in habitat given that physical and chemical environment is expected to influence the observed community assemblages. Results from these analyses were used when discussing potential explanatory factors on community variation. In accordance with parameters used to generate WQI scores, the PCA analysis (correlation; Euclidean distance) considered turbidity, conductivity, pH, and temperature. Physical characteristics included depth and sediment properties; TOC and grain-sizes (gravel, sand, fine sand, and silt). Pairwise-PERMANOVA of Euclidean distances were performed with a Bonferroni correction to p-values.

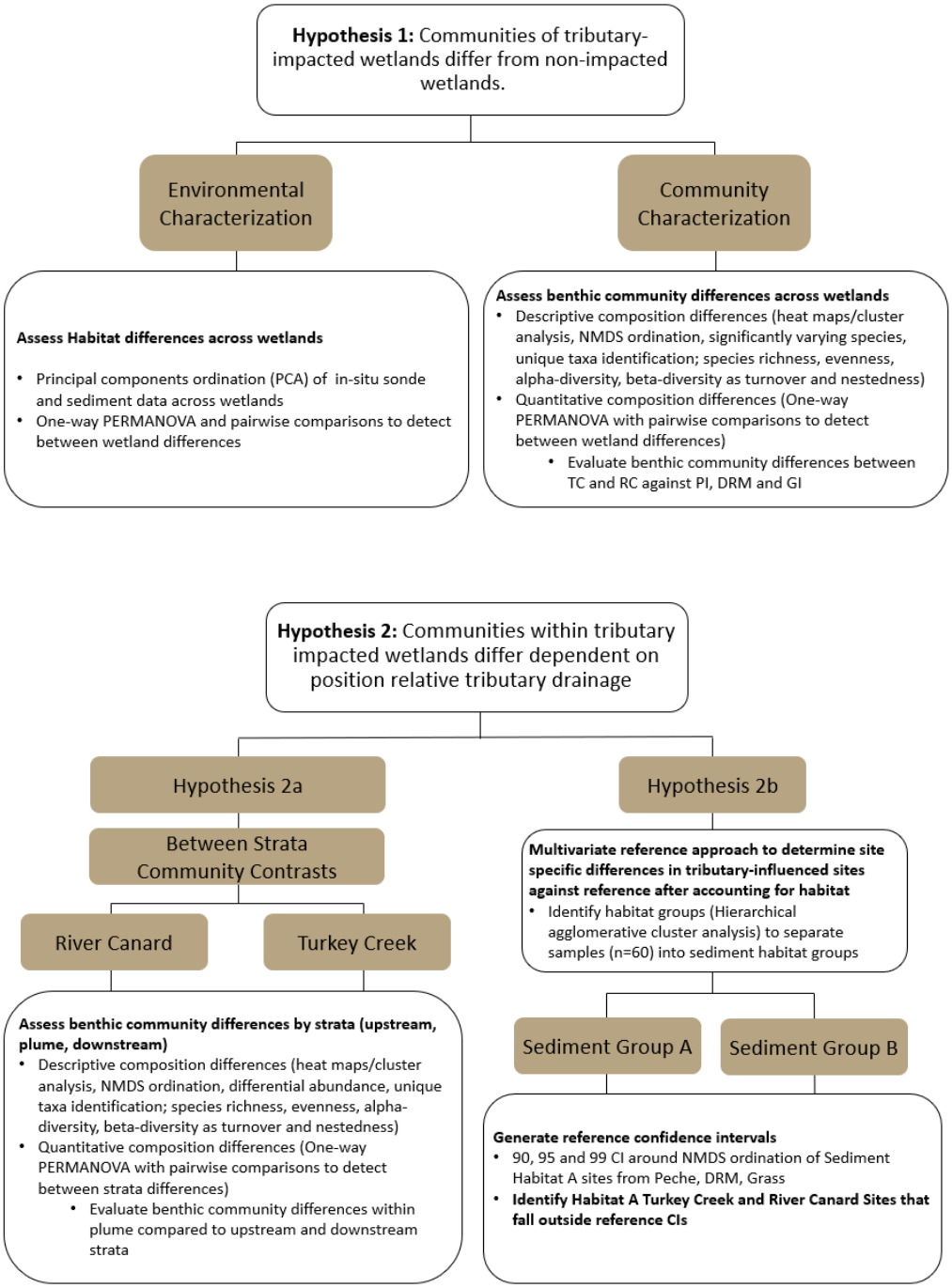


Figure 2.4: Statistical analysis schematic for each hypothesis tested.

2.2.4.2: Macroinvertebrate Community Data

Forty-one benthic macroinvertebrate taxa were identified from 14,694 observed individuals mostly to genus or species. Biodiversity surveys are limited by sampling efforts to capture very rare species (Cao et al 2021). To reduce noise in the dataset (Gauch 1982), rare taxa (occurrence in <10% of samples) were removed and where possible rare genera were combined to family level into a total of 28 taxa. This resulted in the removal of 26 individuals from 8 taxa that represented less than 3% of the relative abundance at each sample site (Resh et al 2005). Common species can provide a strong signal for bioassessments (Marchant 2002).

Relative abundance (RA) data was transformed to meet the assumptions of normality by an octave transformation of relative abundances (Preston 1948; Corkum et al 1997):

$$(3.5+\text{Log}_2(100*RA+0.001))$$

Independent t-tests, assuming unequal variances, were performed to compare the means of relative abundances between strata. Groups tested were commonly pollutant-tolerant taxa; Chironomidae, Ceratopogonidae, Oligochaeta, Nematoda)(Trauben and Olive 1984; Johnson and Kleve 2000; Meyer et al 2005; Arimoro et al 2007; Lin and Yo 2008), sensitive taxa grouped as EPT;. Ephemeroptera, Plecoptera, Tricoptera. T-tests were also performed on richness, evenness, and Shannon H' between strata.

Diversity metric tables were calculated on 28 taxa with *vegan* package (Oksanen et al 2022) for Shannon's index (H'), richness, Pielou Evenness (J) and beta-diversity: total, turnover and nestedness. Beta-diversity metrics were calculated using *adespatial* package (Dray et al 2022) from R statistical software and Podani family Sorenson-based indices. (Legendre 2014).

Boxplots and statistical tables that represent the distribution of taxon occurrences of Detroit River, wetlands (n=5) and positions (n=6) accompanied by statistical tables on relative abundance data.

Heatmaps of octave-transformed community relative abundances at each sample site were generated in R using *pheatmap* package (Kolde 2019). Cluster analysis was provided to generate additional information within the heat map to demonstrate community similarity by site. The heatmap and cluster analysis was not used in hypothesis testing as cluster analysis requires an *a priori* assumption of similarities between sites. Cluster analysis was included by generating a community dissimilarity matrix using *vegan* function *vegdist* of Bray-Curtis distances. Distances were clustered by agglomerative hierarchical clustering via Ward's method.

2.2.4.3: Community Ordination

NMDS ordinations were used to visualize community assemblage of macroinvertebrate by wetland, position, and multivariate reference approach (MRA) analyses in *vegan* (*function: adonis2*). The number of dimensions were selected by generating a scree plot representing stress values up to 25 dimensions (Goral and Schellenberg 2021). In general, the fewer the ordination dimensions, the more distortion is forced into the ordination solution. Only ordinations with stress values between 0.1-0.2 were selected. In general, a stress value of 0.2 is deemed acceptable but there is a potential to draw false inferences. A stress value of 0.1 is considered a good ordination where increasing the number of dimensions is unlikely to change the configuration of groupings Stress values of 0.35 are deemed unacceptable as it likely placement is random(Clarke 1993).

Examination of the ordination of individual wetlands used in the context of Hypothesis 1 was performed in four dimensions (Stress: 0.134) with wetlands groupings: DRM (n=10), Grass Island (n=10), Peche Island (n=10), Turkey Creek (n=10) and River Canard (n=10). Ordination by position for tributary-impacted wetlands used in hypothesis 2 were performed for strata: upstream (n=5), downstream (n=5), and tributary (n=5) for Turkey Creek (k = 3; Stress: 0.077), and River Canard (k=3; Stress: 0.0612).

NMDS performed was also performed on macroinvertebrate communities separated by sediment habitat groups as part of the MRA used in Hypothesis 2. All sites of a given habitat type were subject to the NMDS ordination. Sample sites from the unimpacted wetlands (DRM, Grass Island and Peche) were used to generate 90%, 95% and 99% confidence ellipses of the unimpacted condition and were plotted to compare against ordination position of tributary-impacted sites. The NMDS stress for sediment habitat group A (k=2) was 0.220. For sediment habitat group B (k=2) the stress was 0. 163.

RDAs were generated for later discussion for the purpose of exploring potential community assemblage associations to habitat characteristics (gravel, sand, fine sand, silt, TOC, depth) and in-situ water quality variables (conductivity, temperature, turbidity, pH). An RDA model with all locations (n=60) was produced in R statistical software with vegan package (Oksanen et al 2019). Silt was determined redundant by vegan function *rda* and was omitted (Alves et al 2017). High variance inflation factors were detected for pH, temperature, and conductivity. Thus, temperature and pH were removed. Analysis of Variance (ANOVA) was used to test the significance of the model.

2.2.4.4: PERMANOVA Procedure

Differences in community assemblages were detected using a two-factor permutation analysis of variance (PERMANOVA) which considers the distances of the mean of each grouping (Anderson 2001). The PERMANOVA was performed in R statistical software using the vegan package with 999 permutations at Bray-Curtis distances for relative abundance data and Euclidean for environmental data. PERMANOVA is susceptible to large within-group dispersions and generating Type II errors. A test for homogeneity of multivariate dispersions was performed using the *betadisper* function (*vegan*) followed by an ANOVA-like permutation test (*permutest*) and pairwise comparisons with Bonferroni-corrected p-values (*pairwise.adonis*) (Anderson 2006; Anderson and Walsh 2013).

2.2.4.5 Multivariate Reference Approach

In using biota for environmental assessments, temporal and spatial variability of habitat structure can confound stressor impact assessments given that biological communities can be structured by both habitat characteristics as well as environmental stressors. To circumvent this variability in habitat, approaches to detect anthropogenic stress have been developed where benchmark criteria is set on a comparative basis after first classifying and sub-dividing replicates into common habitat types. Tributary and tributary-impacted wetlands were examined for differences using a multivariate reference approach (MRA) analogous to Environment and Climate Change Canada's BEAST methodology (Milani et al 2013). The approach first subdivides reference and suspected impact sites into habitat standardized clusters based on environmental attributes of the sites by depth, TOC and grain-sizes by hierarchical agglomerative cluster analysis via Ward linkage on a Euclidean distance matrix with *cluster* package (Maechler et al 2022). Two main sediment habitat groups were determined to be optimal using within sum of

squares (elbow method) and supported by the silhouette method. A dissimilarity matrix was generated for each cluster based on Euclidean distances. Distances were clustered by agglomerative hierarchical clustering via Ward's method. Results of k-mean cluster means by grouping are listed below. Overall, the 60 sample sites from the present study were divided into two main habitat clusters. Group A consisting of slightly deeper sites with sediments containing a higher proportion of fine sand and lower TOC. Group B consisted of shallower sites with greater TOC content and a higher proportion of gravel and silt (**Table 2.1**)

Table 2.1: Sediment habitat group centroids (macroinvertebrate sample sites).

	Depth (m)	TOC (%)	GRAVEL	SAND	FINE SAND	SILT
Group A (n=27)	1.744	0.861	0.050	0.364	0.433	0.153
Group B (n=33)	0.937	1.112	0.070	0.375	0.377	0.178

2.3: Results

2.3.1: Water Quality and Habitat

Utilizing in-situ sonde readings of water quality and habitat characteristics at the time of collection, impacted sample sites were on average deeper with slightly higher conductivity and turbidity than unimpacted sample sites. Grain-size distributions slightly varied with coarser grain-sizes found at unimpacted wetland sites. At River Canard, contrasting upstream to downstream, the downstream positions were deeper and had reduced turbidity, TOC, and gravel with higher proportions of fine sand whereas the tributary compared to the river positions had higher conductivity and were shallower sites. The downstream of Turkey Creek compared to upstream had lower turbidity and depth with similar grain distributions. The tributary of Turkey Creek had higher conductivity, higher TOC and was the shallowest of all three positions. Tables of environmental measurements are provided in **Appendix A**. In the Principal Component Analysis, PC1 represented 27.17% of the variance with silt, temperature, conductivity and pH contributing substantially to PCA axis loadings. PC2 represented 20.19% of the habitat variance predominately attributed to habitat characteristics grain-sizes (Gravel to Fine Sand) and TOC (**Figure 2.5**). Pairwise PERMANOVA between wetland communities detected no significant habitat differences between wetlands with exception of Peche Island and Grass Island communities ($p<0.05$) (**Appendix A**).

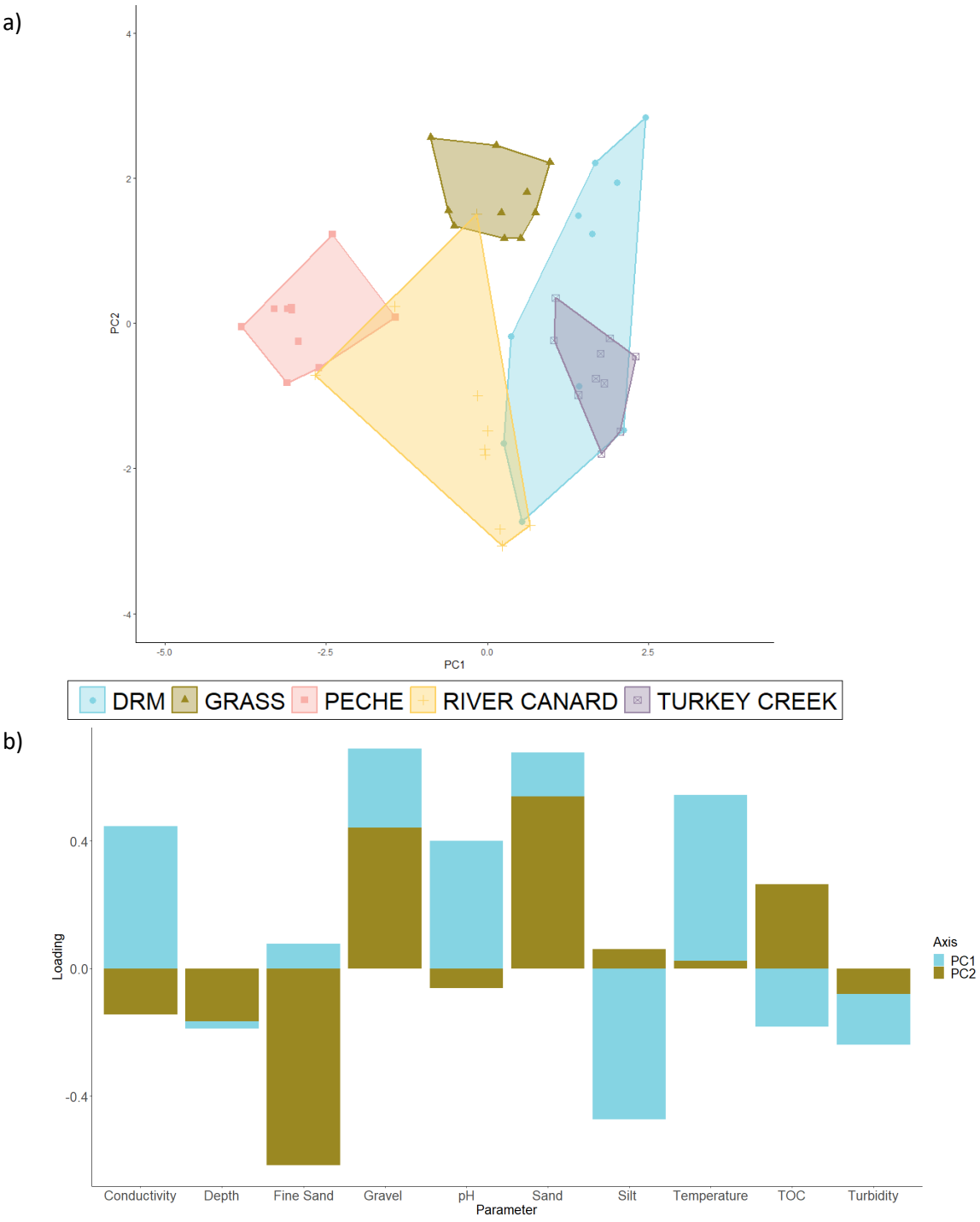


Figure 2.5: PCA ordination of PC1-PC2 of a) wetlands along water quality and sediment habitat parameters with b) axes loadings to parameter plot.

2.3.2: Macroinvertebrate Community

Across sampled wetlands, forty-one taxa were identified from 14,694 individuals. Taxa were upscaled to the family level generating a total of 28 taxa. Final taxonomic resolution belonged to 3 genus, 22 families, 1 subclass (Oligochaeta) and 1 phylum (Nematoda). For the purposes of this thesis, taxa will also be referred to as species in diversity measurements, i.e.: species richness, species turnover, species nestedness. The most common taxa observed were Chironomidae and Oligochaeta at 100% of sites, Nematoda (95%), Rissooidea (86.6%), Valvatidae (83.3%) and Dreissenidae (73.3%). Average species richness for the Detroit River wetlands was 13.2. EPT taxa (n = 8) made up 7.17% of individuals and were found at 60% of sample locations. Beta-diversity for the Detroit River as a whole was primarily species turnover (0.736).

T-test results (**Appendix B**) confirmed that pollution tolerant species had not significantly varied between wetlands yet significant differences were found with elevated EPT% at Grass Island contrasted to Peche Island and River Canard. DRM also had significantly higher EPT% than Peche Island communities. Alpha diversity metrics had displayed some differences between wetlands. DRM and Grass Island had significantly higher richness than both impacted wetlands. Differences were observed in Peche Island's evenness to DRM, Grass, and River Canard such that Peche Island communities were more even. In hypothesis two, communities were examined within River Canard and Turkey Creek wetlands to their respective tributaries. In pairwise t-tests of these groupings the tributary of Turkey Creek (TTC) significantly differed from the upstream strata with respects to richness, evenness, and Shannon's H'. TTC differed from the downstream strata only with respect to richness. These diversity metrics were qualitatively more optimal within the tributary plume than river wetland communities. No significant

differences were observed within River Canard nor were difference observed in the composition of pollution tolerant species at this wetland.

2.3.3: Hypothesis 1 - Wetland Community Contrasts

Community metrics are summarized in **Table 2.2** related to Shannon's diversity, richness, evenness, and EPT% and beta-diversity calculations can be found at **Table 2.3.** Community composition data including box plots and tables are available in **Appendix B**. Pairwise PERMANOVA results between wetlands are in **Table 2.4**. Summaries of main findings by wetland along with major taxa abundances are briefly described below.

PERMANOVA indicated significant differences between wetlands ($F_{5.30}$, R^2=0.320, p=0.001). Pairwise-PERMANOVA Bonferroni-corrected p-values indicated Peche Island was similar to Turkey Creek (p=0.074) and River Canard (p=0.056). When testing PERMANOVA assumptions of similar multivariate dispersions, River Canard had significantly different dispersion than DRM, GI, and TC. River Canard can only be compared to Peche Island with confidence (**Appendix B**). PERMANOVA pairwise comparisons are presented in **Table 2.4**.

Cluster analysis of benthic communities by wetland show there were two major clusters of communities (**Figure 2.6**). The first cluster indicated greater similarity between Peche, and Turkey Creek wetland sites. The second major cluster indicated more similarity between DRM and Grass Island sites. River Canard is found distributed across both clusters. These associations are similar to the NMDS wetland community ordinations of NMDS1-NMDS2 ordination (**Figure 2.7**). The wetland strata NMDS plot was performed in 4 dimensions. Additional NMDS axes are available in **Appendix B**. No additional differences were observed in these ordinations.

DRM sample sites had a species richness of 14.9, evenness (J) of 0.690, Shannon H' of 1.843 and the highest EPT abundance of 14.5%. Chironomidae (0.356 ± 0.038), Oligochaeta (0.256 ± 0.058), *Leptocerus* (0.100 ± 0.014), and Nematoda (0.078 ± 0.018) were most abundant species. Species turnover represented 0.652 of the total beta-diversity; nestedness accounts for 0.348.

Grass Island sample sites have the highest species richness, 15.2, Pielou J evenness of 0.683 and Shannon H' similar to DRM at 1.849. Most abundant taxa are Chironomidae (0.301 ± 0.038), Oligochaeta (0.204 ± 0.037), *Leptocerus* (0.082 ± 0.035) and *Hyalella* (0.089 ± 0.027). Beta-diversity is mostly attributed to species turnover (0.736); nestedness accounts for 0.264.

Peche Island sample sites have intermediate species richness, 13.0 and the highest evenness (Pielou J) of 0.773. Peche Island had the highest Shannon H' of all the wetlands, 1.962. EPT% represented 4.408% of the abundance. Chironomidae (0.286 ± 0.038), Oligochaeta (0.224 ± 0.017), Nematoda (0.083 ± 0.019), and Dreissenidae (0.062 ± 0.024). Species nestedness was the main contributor to beta-diversity (0.713); nestedness represented 0.287.

River Canard sample sites have richness of 11.8, Pielou J evenness of 0.663, Shannon H' at 1.652. Dominant taxa by relative abundance are Chironomidae (0.343 ± 0.057), Oligochaeta (0.232 ± 0.039), Nematoda (0.126 ± 0.040) and Rissooidea (1.03 ± 0.039). The EPT taxa was 5.61%. River Canard had the highest beta-diversity with species turnover representing 0.808 of the variance.

Turkey Creek sample sites have the lowest species richness (11.2), Pielou J species evenness 0.643, lowest Shannon H' of 1.543 and EPT% (1.88). Taxa with the highest abundances include Chironomidae (0.463 ± 0.043), Oligochaeta (0.205 ± 0.053), Nematoda (0.126 ± 0.031)

and Rissooidea (0.70 ± 0.014). Species turnover represents 0.723 of the variation in beta-diversity.

Table 2.2: Alpha diversity means by (a) Detroit River (b) wetland and (c) position.

	Shannon H'	Richness (n)	Pielou J	EPT%
a) Detroit River				
Wetlands (n=50)	1.764	13.220	0.690	7.17
b) **Wetland**				
Detroit River Marshes (DRM)	1.843	14.900	0.690	14.509
Grass Island (GI)	1.849	15.200	0.683	12.868
Peche Island (PI)	1.962	13.000	0.773	4.408
River Canard (RC)	1.625	11.800	0.663	5.610
Turkey Creek (TC)	1.543	11.200	0.643	1.883
c) Position				
RC - Tributary	1.490	9.800	0.745	7.570
RC – Downstream	1.611	11.800	0.658	3.822
RC - Upstream	1.640	11.800	0.668	7.398
TC – Tributary	2.090	16.400	0.750	11.271
TC – Downstream	1.639	11.600	0.669	2.545
TC – Upstream	1.447	10.800	0.617	1.221

Table 2.3: Beta-diversity metrics means by (a) Detroit River (b) wetland and (c) position – Sorenson.

	β-diversity Total (BDtotal)	Replenishment (Repl)	Richness Difference (RichDif)	Turnover (proportion) Repl/BDtotal	Nestedness (proportion) RichDif/BDtotal
a) Detroit River					
Wetlands (n=50)	0.192	0.141	0.051	0.736	0.264
b) Wetland					
DRM	0.158	0.103	0.055	0.652	0.348
Grass Island	0.144	0.106	0.038	0.736	0.264
Peche Island	0.176	0.125	0.050	0.713	0.287
River Canard (RC)	0.196	0.159	0.038	0.808	0.192
Turkey Creek (TC)	0.145	0.105	0.040	0.723	0.277
c) Position					
RC - Tributary	0.180	0.167	0.013	0.926	0.074
RC - Downstream	0.229	0.098	0.131	0.428	0.572
RC - Upstream	0.214	0.153	0.061	0.715	0.285
TC - Tributary	0.138	0.098	0.040	0.712	0.288
TC - Downstream	0.156	0.115	0.041	0.735	0.265
TC - Upstream	0.149	0.110	0.039	0.735	0.265

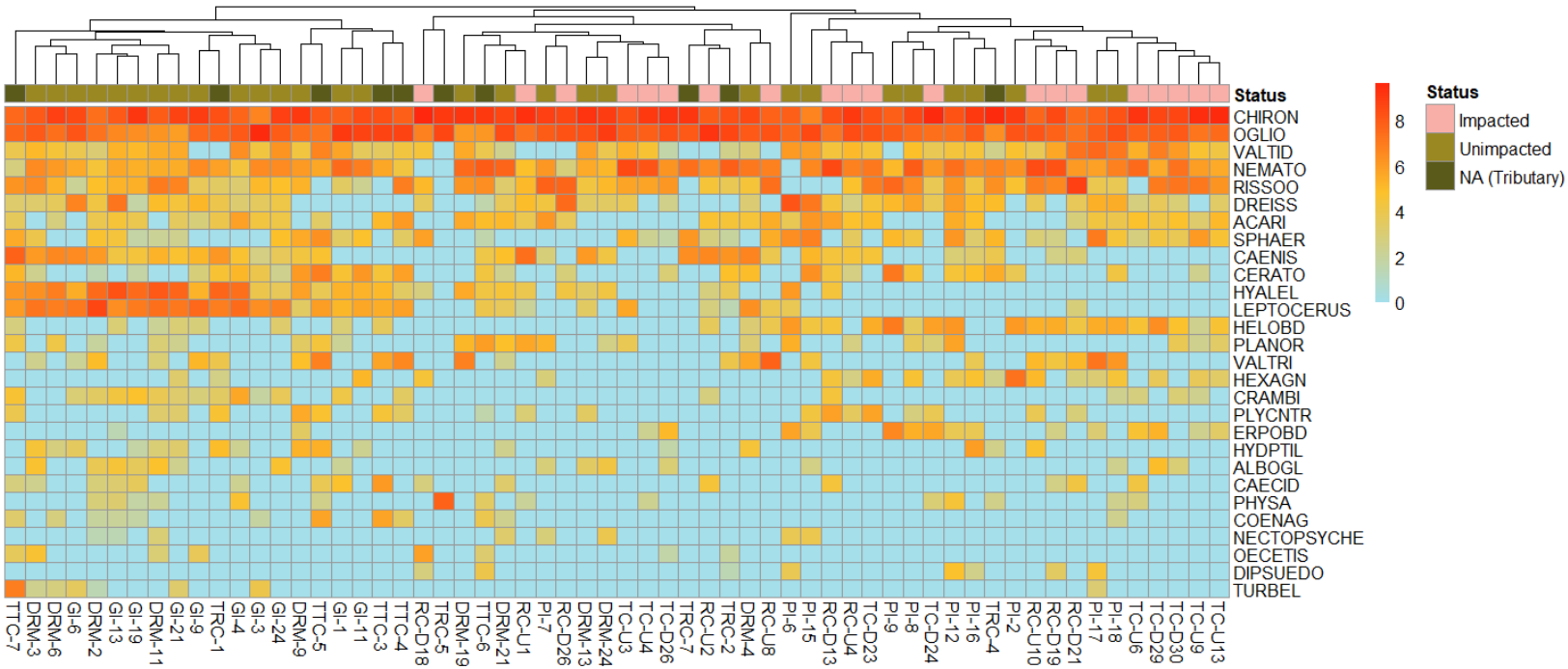


Figure 2.6: Heat map with cluster analysis of Detroit River and tributaries: benthic macroinvertebrate community assemblages and associated water quality status for wetlands.

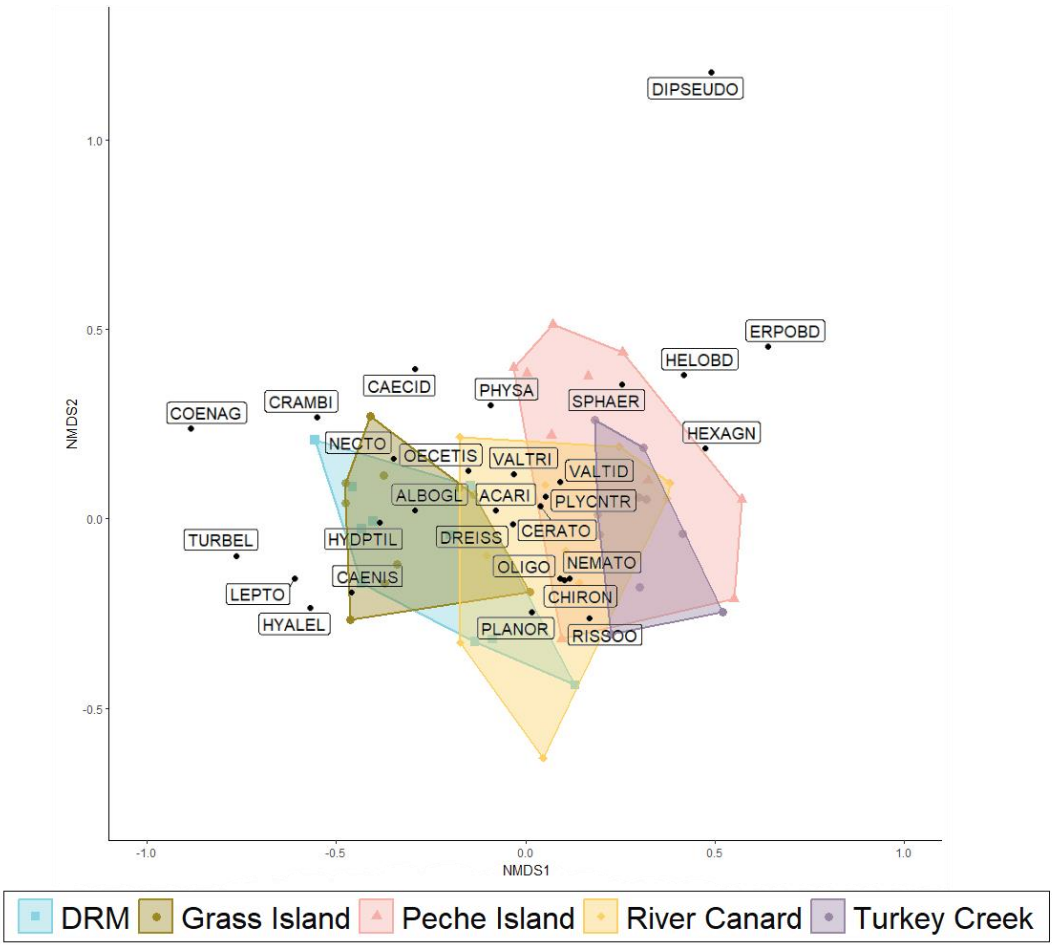


Figure 2.7: NMDS ordination: benthic macroinvertebrate communities by wetland with species scores plotted on NMDS1-NMDS2.

Table 2.4: Differences in benthic macroinvertebrate community by wetland based on PERMANOVA and Bonferroni-corrected pairwise comparisons.

	DRM	GRASS	PECHE	RIVER CANARD	TURKEY CREEK
DRM		**0.027**	**0.001**	**0.002**	**0.001**
GRASS	0.027		**0.002**	**0.001**	**0.001**
PECHE	0.001	0.002		0.056	0.074
RIVER CANARD	0.002	0.001	0.056		0.031
TURKEY CREEK	0.001	0.001	0.074	0.031	

2.3.4: Hypothesis 2a - Community Contrast by Tributary Position

Summaries of community diversity can be found in **Table 2.2** related to species diversity, richness, evenness. **Table 2.3** summarizes beta-diversity calculations. Full tables of community composition data including box plots and statistical tables are available in **Appendix B**. The tributary position strata NMDS plots were performed in 3 dimensions. Additional NMDS axes are available in **Appendix B**. No additional differences were observed in these ordination plots supporting differences.

2.3.4.1: River Canard

Sample sites in the upstream position of River Canard (n=5) had an average species richness of 11.8, Pielou J's evenness (0.658), Shannon H' of 1.640 and EPT% of 7.398. The most relatively abundant taxa and standard error were Chironomidae (0.321 ± 0.078), Oligochaeta (0.297 ± 0.063), Nematoda (0.121 ± 0.047) and *Caenis* (0.54 ± 0.029). Species turnover represented 0.715 of beta-diversity.

As with upstream sample sites, downstream sample sites (n=5) had a species richness of 11.8, evenness (Pielou J) 0.668, Shannon H' of 1.611 but lower EPT% of 3.82. Chironomidae (0.365 ± 0.092), Oligochaeta (0.168 ± 0.030), Rissooidea (0.142 ± 0.074) and Nematoda (0.132 ± 0.069). Species turnover represented 0.428 of beta-diversity and species nestedness represented 0.572.

Tributary position sample sites (n=5) had the lowest species richness 9.8, highest Pielou J's evenness 0.745, highest EPT% 7.570 within River Canard positions. Dominant species were Chironomidae (0.429 ± 0.068), Oligochaeta (0.243 ± 0.054), Nematoda (0.098 ± 0.035) and the

Ephemeropteran, *Caenis* (0.042 ± 0.015). Species turnover represented 0.926 of the beta-diversity.

One-way PERMANOVA analyses by tributary position demonstrated non-significant differences in community composition by strata ($F_{1.264}$, R^2=0.174, and p=0.212). NMDS reinforced the similarities in composition by strata and did not indicate any distinct separation of communities by strata (**Figure 2.8**).

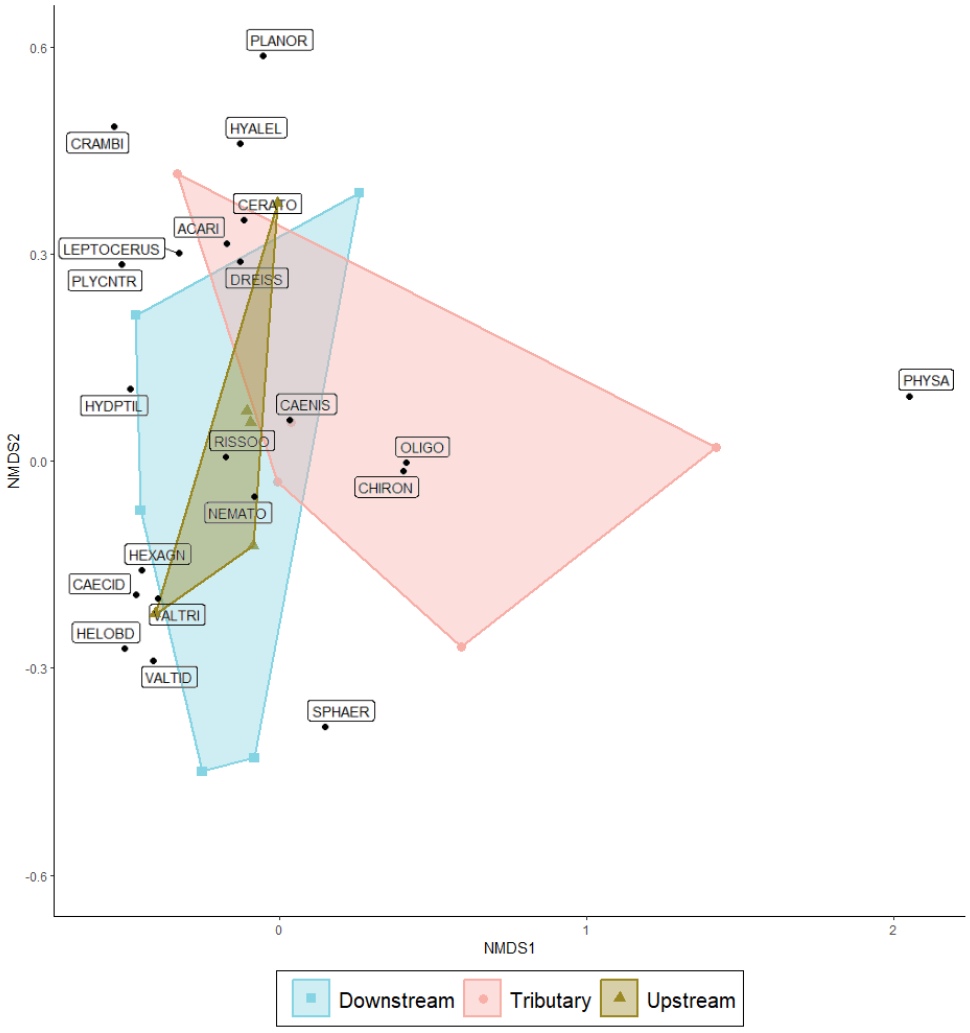


Figure 2.8: NMDS ordination: benthic macroinvertebrate communities at River Canard by tributary position with species scores plotted on NMDS1-NMDS2.

2.3.4.2 Turkey Creek

The downstream strata sites (n=5) had a species richness of 11.6, Pielou J's evenness of 0.669, Shannon (H') at 1.639 and EPT% of 2.55. The most prevalent taxa were Chironomidae (0.433 ± 0.066), Oligochaeta (0.230 ± 0.026), Nematoda (0.086 ± 0.018) and Rissooidea (0.085 ± 0.022). Species turnover represented 0.735 of beta-diversity and species nestedness represented 0.265.

Sample sites in the upstream strata of Turkey Creek (n=5) had a species richness of 10.8, Pielou J evenness at 0.617, Shannon H' at 1.447 and EPT% of 1.221 to the downstream position. Most abundant taxa were Chironomidae (0.493 ± 0.060), Oligochaeta (0.180 ± 0.017), Nematoda (0.166 ± 0.056) and Rissooidea (0.055 ± 0.019). Beta-diversity was represented primarily by species turnover (0.735) compared to species nestedness of (0.265).

Tributary position sample sites (n=5) had the highest species richness (16.4), Pielou J evenness (0.750), Shannon H' (2.090) and EPT% 11.271 within Turkey Creek plume. Dominant species were Chironomidae (0.268 ± 0.049), Oligochaeta (0.222 ± 0.017), Nematoda (0.066 ± 0.056) and Rissooidea (0.049 ± 0.021). Species turnover represented 0.712 of the beta-diversity.

One-way PERMANOVA analyses by position indicated significant differences in the benthic community by strata ($F_{5.803}$, R^2=0.492, p=0.001). P-values from pairwise PERMANOVA supported differences between tributary-to-downstream (p<0.01) and tributary to upstream (p<0.01) (**Table 2.5**). The NMDS supported the distinct separation of sites within the tributary plume compared to upstream and downstream strata as presented in **Figure 2.9**.

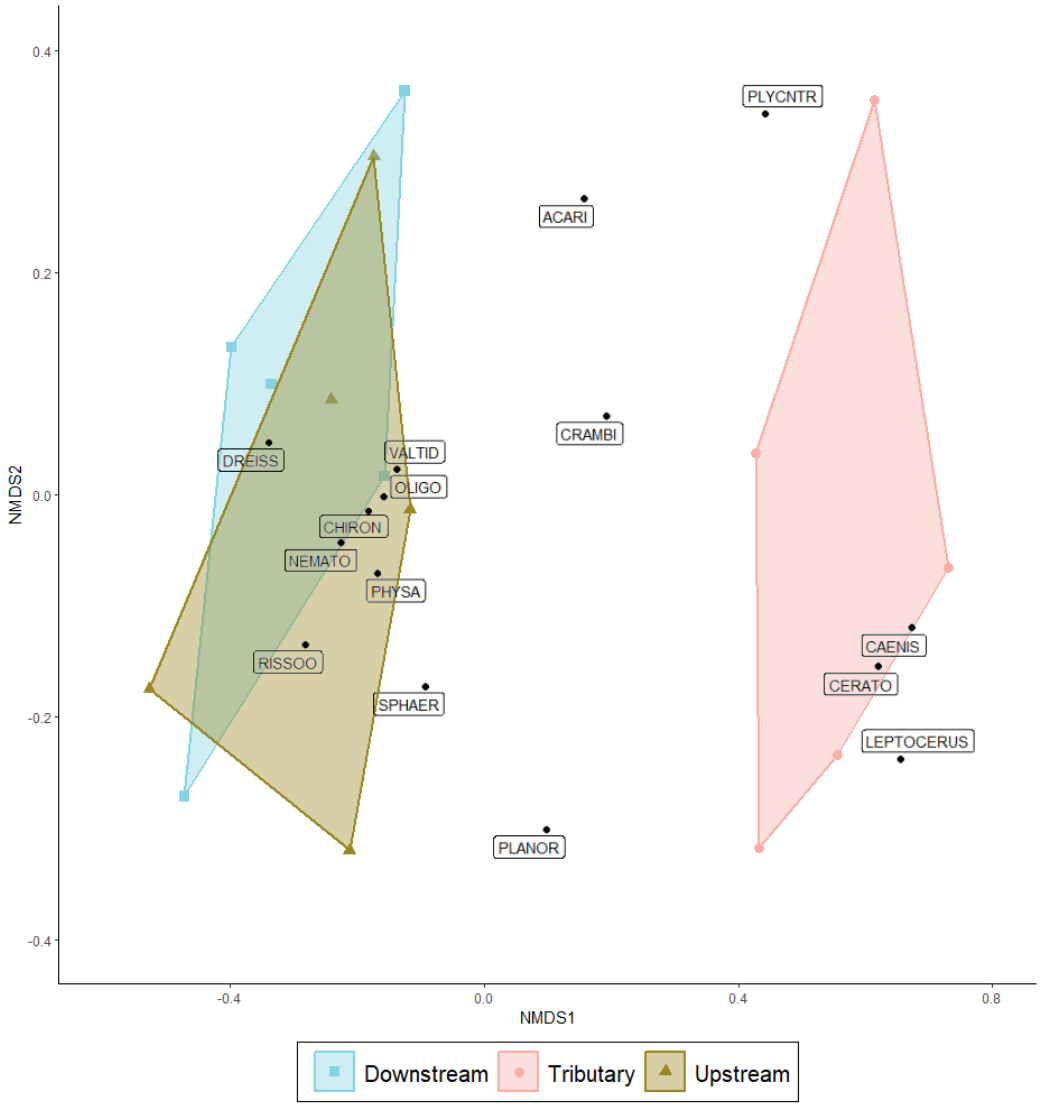


Figure 2.9: NMDS ordination: benthic macroinvertebrate communities at Turkey Creek by tributary position with species scores plotted on NMDS1-NMDS2.

Table 2.5: Differences in benthic invertebrate community by Turkey Creek position based on PERMANOVA and Bonferroni-corrected pairwise comparisons.

Turkey Creek	**Upstream**	**Downstream**	**Tributary**
Upstream		0.3528	0.0076
Downstream	0.3528		0.0077
Tributary	0.0076	0.0077	

2.3.5: Hypothesis 2b - Multivariate Reference Approach: Site-specific Differences at Tributary Impacted Wetlands

Sediment habitat group A was defined by deeper water, lower TOC, and greater prevalence of fine sands. Across the 60 sites tested, Habitat A consisted of 10 reference locations and 15 test sites located within the tributary-influenced wetlands. Reference condition confidence ellipses and test site position in the NMDS ordination space for Habitat A sites are presented in **Figure 2.10**.

Sediment habitat group B represents shallower sample sites with higher TOC, and larger proportions of gravel and silts. Habitat B contained 20 reference locations and 14 test sites. Reference condition confidence ellipses and test site position in the NMDS ordination space for Habitat A sites are presented in **Figure 2.11**.

The summation from sediment habitat groups had 5/15 River Canard sample sites exceeding 90% of macroinvertebrate community reference. The distinct sites were composed of 3/5 downstream sample sites (RC-D18, RC-D19, RC-D26) and 2/5 tributary plume locations (TRC-05 and TRC-07). Turkey Creek had 6/15, evenly distributed distinct stations between the downstream (TC-D24 and TC-D26); upstream (TC-U3 and TC-U4) and tributary plume (TTC-03 and TTC-05).

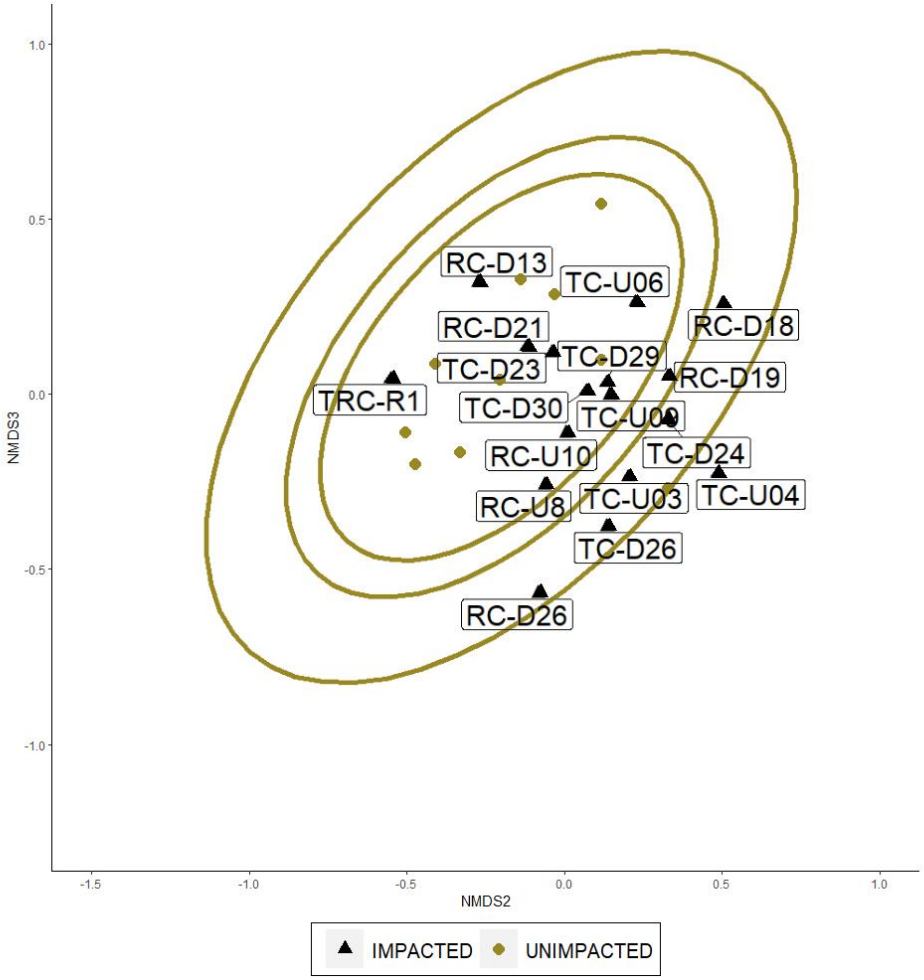


Figure 2.10: NMDS ordination of benthic macroinvertebrate communities showing MRA sediment habitat group A with unimpacted confidence ellipses from inner representing 90, 95, 99%.

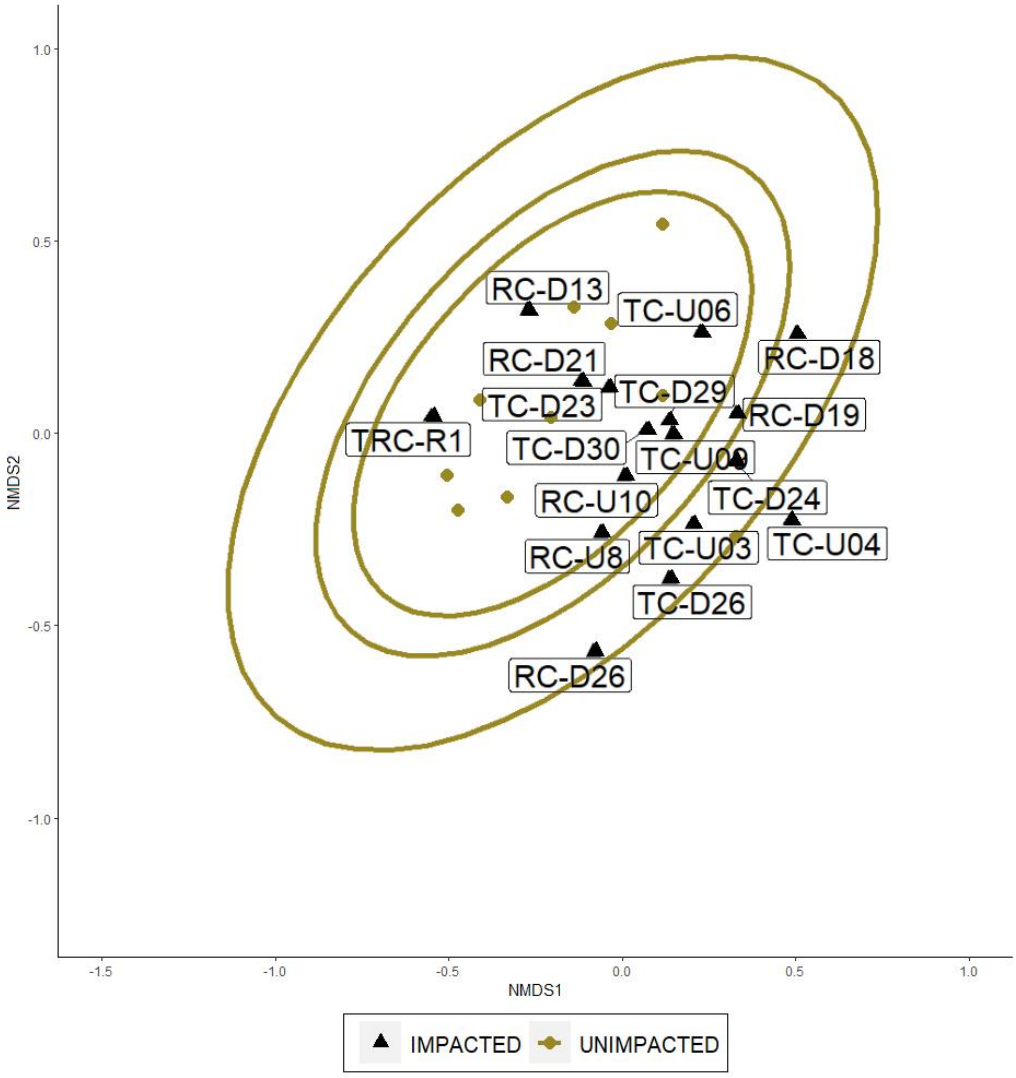


Figure 2.11: NMDS ordination: benthic macroinvertebrate communities – MRA sediment habitat group B with unimpacted confidence ellipses from inner representing 90, 95, 99%.

2.4 Discussion

In-situ readings taken at the time of collection demonstrated some differences in water quality and sediment characteristics across wetlands. However, these differences did not correspond with expected patterns of water quality differences described by prior studies (Environment Canada, 2017). In the PCA analyses, individual wetlands showed the greatest variation along the PC.1 axis which was predominately representative of the water quality parameters conductivity, temperature and pH. PC1 scores were also negatively associated with silt contents. Along this axis, Peche island exhibited the most negative scores followed by equivalent ordination of RC and Grass Island sites separated which were separated from DRM and Turkey Creek having the most positive scores. River Canard demonstrated the most variance along PC1. Along the PC2 axis, scores were most represented by fine sand, sand, and gravel along with sediment TOC contents. Sample sites showed less distinction by wetland with high degree of overlap between River Canard, DRM, Turkey Creek, and Peche Island distinct from Grass Island.

Notably, the basis of Hypothesis 1 was from prior monitoring data of water quality generated by Environment and Climate Change Canada (ECCC). Their data indicated that River Canard and Turkey Creek had consistently higher turbidity and the lowest water quality scores according to their water quality index compared to the other wetlands (Environment Canada, 2017). In the present study, turbidity, a chief component of the water quality score used by Environment and Climate Change Canada, poorly loaded onto either PC1 or PC2 axes and therefore did not strongly influence wetland position within the habitat ordination space. In addition, there were differences in the sampling design adopted by ECCC and that of the present study which may have strongly contributed to the differing results. In the present study,

wetlands were defined by the macrophyte edge boundaries. All portions of the wetland bed had an equal chance of being sampled as per the stratified random sampling design implemented. The ECCC approach used a directed sampling design with repeated sampling at specific multiple positions across different years. However, most significantly, wetlands were sampled mainly in the wadable portions of the wetland and near emergent vegetation. This constrained sampling efforts to near shore areas and precluded sampling in deeper sections of the wetland beds. These constraints seemed to be most prominent at the tributary influenced wetlands. Based on their maps of sampling positions, ECCC's sampling of the tributary influenced wetlands were confined to waters within the tributary proper rather than at the tributary/Detroit River mixing zones (referred to as tributary plume here). Their sampling excluded upstream and downstream strata from the plume. It would therefore appear that the water quality differences reported by ECCC are more reflective of the tributaries themselves than the receiving wetlands present within the Detroit River.

The most abundant taxa found in the Detroit River were Chironomidae, Oligochaeta, Nematoda and Rissooidea. High relative abundances of these taxa may be due to coarse taxonomic scale designation upon identification. Finer taxonomic resolution of Chironomidae, Oligochaeta and Nematoda require much more labour-intensive processes that involve mounting specimens and identification by compound microscopes. It is common practice to group these organisms at such coarse scales (Cooper et al 2007; Stewart and Schriever 2023). Finer resolution could provide more insights into ecosystems, however, there are general insights that can be made when communities are significantly different or uneven toward these taxa as they are associated with having tolerance for poor water quality and found in areas with elevated pollutants (Krieger 1984; Orzechowski and Steinman 2022). Three taxa were not found at tributary-impacted wetlands, *Nectopsyche* (Trichopteran), Turbellaria and Coenagrionidae.

Turbellaria abundances are negatively impacted by the presence of algal mats (Reise 1983). Agricultural and urban streams can have increases of nutrients which accumulate in the sediments, and it is possible that algal mats are affecting the ability of Turbellaria to colonize these areas. Damselfly, Coenagrionidae of the order Odonata, having exposed respiratory structures require high oxygen and are usually found in shallow, lotic water. They are moderately tolerant of metals, organic pollutants but are sensitive to nutrients and turbidity (Carchini et al 2009; Kefford et al 2010). Their absence from tributary impacted wetlands may be resulting from impacted wetlands being slightly deeper and outside physiological tolerances.

Wetland comparisons by PERMANOVA, also represented in the NMDS, showed dissimilarities between wetlands. Despite the close proximity of DRM to Grass Island wetlands, macroinvertebrate communities significantly differed between these two reference wetlands. Taxa detected at Grass Island (n=27) and DRM (n=26) only differed with *Hexagenia* being found at Grass Island. Notable differences in abundances were observed with *Nectopsyche*, Polycentropodiae, Planorbidae which were more prevalent at DRM and Crambidae, Caeidotea, Sphaeriidae more prevalent at Grass Island. These taxa are shredder-herbivores with exception to the predatory Polycentropodiae, suggesting similarities between macroinvertebrate ecosystem functions being maintained by different taxa. The mayfly, *Hexagenia*, feed on detritus and have substrate requirements for burrowing where clay and clay-sand sediments are most advantageous (Edsall et al 1991; Brunk et al 2014). Habitat characteristics did not vary significantly between Grass Island and DRM, though grain-sizes at Grass Island had slightly more coarse sand and silt than DRM.

Peche Island was determined to have similar communities to both the tributary impacted wetlands, demonstrating overlap in community assemblages. Peche Island

communities had higher richness and evenness than Turkey Creek and River Canard. EPT taxa was similar at Peche Island and River Canard but higher than at Turkey Creek. River Canard demonstrated similarity to all wetlands in the NMDS ordination. However, interpretation of the PERMANOVA must be made carefully for River Canard. PERMANOVA analysis can be sensitive to Type II errors due to local and dispersion differences between groups. Potential for dispersion effects increases with number of groups (Anderson and Walsh 2013). In testing for homogeneity of dispersions, River Canard failed only exhibiting similar dispersions to Peche Island, making further wetland community comparisons to River Canard dubious.

Beta-diversity of each wetland and tributary are more proportionately affected by species turnover. High species turnover in the Detroit River implies environmental sorting and historical constraints along a spatial gradient influencing community assemblages (Gaston et al 2007; Leprieur et al 2011). Peche Island demonstrates intermediate community assemblage between unimpacted and impacted wetlands. Peche Island receives inflow from Lake St. Clair and may be more reflective of Lake St. Clair than the remaining Detroit River or flow and sediment regimes resemble those of tributary junctions (Svendsen et al 2009).

In examining community differences with respects to upstream, downstream, and tributary positions, River Canard had shown no significant differences in community assemblages by PERMANOVA, nor NMDS. Distance to agricultural tributaries exhibit gradients of elevated conductivity, turbidity, and nitrates in wetlands (Schock et al 2014). However, there may not be a strong enough gradient in environmental pressures on communities. EPT% were higher in upstream and tributary sample sites with higher abundance of *Caenis* and *Leptocerus*. Leptoceridae are detritivore-shredders and periphyton scrapers with adaptations that facilitate swimming; elongated metathoracic legs and setae (Tindall 1964). These groups may benefit

from elevated nutrients as it increases periphyton abundances (Lamberti and Resh 1983; Katano et al 2007). Nutrients may not be responsible for differences in EPT% between tributary positions as macroinvertebrates can be depth-limited and downstream portions of River Canard wetland were deeper than upstream and tributary (Hieber et al 2005).

There were five locations that had varied significantly from the multivariate reference approach, RCD18, RC-D19, RC-D26 and RTC-05, RTC-07. The downstream sample site, RC-D26 had reduced species richness and no EPT taxa. The two tributary locations are marked by reduced species richness of 3-5 taxa; TRC-05 had three taxa (Chironomidae, Oligochaeta and Physa) and TRC-07 had five taxa detected (Chironomidae, Oligochaeta, Nematoda, Spharidae and *Caenis*). Physa are a scavenger/scrapper mollusc with preference for periphyton and benefit from elevated phosphorus (Stelzer and Lamberti 2001; Lombardo and Cooke 2002). Elevated nutrients are a well-established characteristic of agricultural streams (Heathwaite et al 1996). The mayfly, *Caenis* is also a consumer of periphyton. They are an EPT taxa associated with good-to-moderately degraded habitats and are common to agricultural streams (Zumberge et al 2003; Alhejoj et al 2014). TRC-05 and TRC-07 are both located furthest upstream in the River Canard tributary and are within 500m of one another. The remaining three tributary sites were located along nearshore zones of the tributary junction. Curiously, species richness had decreased in the upstream direction. Low species richness is common to degraded habitats and the agricultural drainage could be affecting community compositions in tributary-impacted communities (McCabe and Gotelli 2000; Park et al 2007). In contrast, the nearshore zones supported diverse communities in terms of richness and EPT%. EPT at these locations ranged from 2-20%.

Regarding beta-diversity, the River Canard tributary showed very high species turnover which is likely a sampling design artefact resultant of differences between the tributary inlet and the

nearshore zones. River Canard had the most nested beta-diversity across wetlands of this study. The upstream and downstream strata did not differ significantly with respects to alpha diversity but there was a reduction found in the EPT taxa in the downstream portions. Considering one sample site of ten had demonstrated significant differences in species compositions (RC-D26), we did not see evidence of significant impairment at River Canard and therefore reject hypothesis 1 with respect to this tributary location. However, River Canard is an agricultural stream with long-term water quality issues as related to nutrients, turbidity, and conductivity. The elevated degree of nestedness may be indicative of a pressure, natural or anthropogenic in the area that is contributing to more homogenized community compositions. Nested communities are generally interpreted as the effects of a differential local extinction resulting in subsets of species whose tolerances, recolonization, and dispersal capacities are congruent with conditions at the habitat (Cutler 1994). Examinations into species-area relationships of fish communities in the Lake Erie basin by Montgomery et al (2020) had detected extinction debt at River Canard being one of the most severe in the region and recommended wetland habitat restorations in support of local fisheries. Habitat restorations benefit macroinvertebrate communities and alterations in macroinvertebrate community compositions are reflected in fish community structures (Richardson 1993; Maloney et al 2008). A second explanation for high nestedness at River Canard may be due to a statistical phenomenon, passive sampling, where rare species are underestimated due to insufficient sampling or stochastic processes such as the placement of rare species in a habitat (Wright et al 1997; Higgins et al 2006). An issue arises from our study with respects to passive sampling phenomenon and rare species. Gammaridae had been detected at one site in River Canard and had been excluded due to rarity. Analysis of beta-diversity with the inclusion of Gammaridae had made no discernable difference on nestedness at River Canard. Nestedness and beta-diversity share a negative relationship as beta-

diversity is a measure of differences and nestedness considers commonalities between sites, therefore high nestedness can indicate reduced biodiversity. Reductions in beta-diversity are associated to urbanization and agriculture land-use as both stressors homogenize the environment (McKinney 2006; Knop 2016; Buhk et al 2017) and nestedness at River Canard may be indicating a significant pressure on community compositions.

The Turkey Creek tributary plume was significantly dissimilar to the wetland bed positions present at upstream and downstream strata. The up and downstream positions shared commonalties in the most abundant taxa; Chironomidae, Oligochaeta, Nematoda, Rissooidea. There were no distinguishable differences between upstream and downstream positions in diversity-metrics and prevalent taxa, yet potential impairment was detected at the Turkey Creek wetland in strata at the furthest upstream sample sites (TCU3, TC-U4) and sites downstream of the tributary plume sites (TC-D24 and TC-D26). Sites TC-D24, TC-D26 had pronounced abundances of Hirudinea taxa, *Helobdella* (*H. Stagnalis*) and Erpobdellidae. Hirudinea can be indicators of stress due to their moderate tolerances and are regularly associated with polluted areas (Kazancı et al 2015). *Helobdella stagnalis* demonstrates preference for areas with elevated organic nutrient pollution (Tavzes et al 2006; Kazancı et al 2015). The lack of distinction between upstream and downstream by pairwise PERMANOVA and the MRA approach do not allow for strong support of Hypothesis 2. Analysis into diversity show reduced sensitive taxa; the absence of sensitive taxa serves as better indicator of stress than presence of tolerant species establishing an inability to colonize an area. Turkey Creek wetland had low abundances of sensitive EPT taxa throughout. The lowest diversity metrics were found in TC-U3 and TC-U4 located in the most upstream portion of Turkey Creek and within 600m downstream of a salt-mine and 400m of the shipping dock. Mining activities involve altered topography by the removal of topsoil and vegetation and are areas of compacted soil due to industrial activity,

which are all detrimental characteristics contributing to storm run-off (Negley and Eshleman 2006). Mining operations have been demonstrated to alter water quality by increasing conductivity and turbidity (Bernhardt and Palmer 2011). Conductivity and turbidity measurements collected throughout Turkey Creek were consistent with the river making it unlikely that the proximity to the mine caused perpetually elevated water quality issues. It is suggested that the area be monitored for changes in water quality relationships with storm events where the effects of run off or wind-dispersal of eroded soils could be detected more readily. Changes monovalent and divalent ion concentrations can lead to toxic or lethal effects (Bayly 1969) and the effects of salinization on benthic macroinvertebrates have been reported to contribute to reduce species richness and EPT (Kefford et al 2016). Community compositions in areas near mining operations are regularly described as having low abundance of EPT taxa present (Maret et al 2003; Pond et al 2008). EPT taxa across Turkey Creek can be described as low, 1.88%, the upstream portion had only 1.22% in comparison to the downstream strata of 2.55%. However, in contrast to expectations, the tributary plume sites of Turkey Creek had both high species richness and EPT taxa comparable to the unimpacted wetlands of the Detroit River.

Differences between Turkey Creek wetland upstream-downstream strata and the tributary plume were observed in taxa presence and abundances. The tributary plume had the highest species richness, evenness, and Shannon diversity (H') in this study. EPT% taxa were comparable to unimpacted wetlands and more pronounced than those at upstream and downstream strata. High prevalence of EPT taxon richness is not expected for an urban tributary as they are prone to fluctuating hydrologic regimes and contaminated sediments which can reduce their richness (Konrad and Booth 2005). Urbanized areas have a range of habitat types that can resemble natural areas in flow velocities and saprobic conditions supporting richness (Goertzen et al 2022). Tributary taxa not found at bed sites include Coenagrionidae, and three

algal consumers, Dipseudopsidae, *Hyalella* and Turbellaria suggesting conditions more favourable in supporting diverse algal and macroinvertebrate communities.

A supplementary RDA was performed on water quality parameters (pH, Turbidity, Conductivity) and habitat characteristics of depth, grain-sizes, and TOC. The RDA suggested that water quality was not strongly influencing benthic macroinvertebrate community structure compared to depth and sediment characteristics (**Figure 2.12**). It is not unexpected that water chemistry in a large river does not pose a strong enough pressure gradient to affect macroinvertebrate composition (Williams et al 2004). When impacted sample sites were clustered by similar habitat characteristics and contrasted to comparable unimpacted sites, forty percent of Turkey Creek sample sites differed from unimpacted sites. Differences observed were the high presence of tolerant species and lack of sensitive taxa suggestive degraded conditions at Turkey Creek. Of tributary locations, 40% had fallen outside of 90% confidence of reference sites with differences observed in species richness. The recent delisting of the benthos impairment in Canadian portions of the Detroit River AOC supports that sediment contamination of commonly monitored metal and organic pollutants are unlikely contributing to impacted communities. Macroinvertebrate communities appear to be initially arranged by habitat characteristics but show further differentiation in-line with degraded habitat conditions. This study re-affirms that habitat characteristics predominately influence benthic macroinvertebrate communities in the wetlands more significantly than water quality parameters, but sites show composition differences in-line with degraded habitat conditions (Wood 2004).

On balance, Hypothesis 1 was not supported due to Peche Island communities not showing significant differences to both tributary impacted wetlands. Hypothesis 2 is not supported in

macroinvertebrates despite support of differences between tributary and wetland detected by PERMANOVA. Unexpected in this study was the significant impairment of the upstream portions in Turkey Creek as detected by MRA. Qualitative descriptors of ecosystem health marked by elevated abundances of sensitive taxa did not conform to the underlying assumption of hypothesis 2, that the tributary input is contributing to altered and degraded benthic communities. Instead, the tributary input demonstrated more favourable macroinvertebrate compositions compared to the upstream and downstream strata and which compared more favorably to the sensitive taxa abundances at non-tributary impacted wetlands. Therefore, environmental stressors present at the Turkey Creek wetland do not appear to be attributed to the tributary itself but instead reflect stressors generated by proximate sources upstream of the wetland that may include industrial and municipal wastewater treatment facilities. Thus, future research should attempt to characterize the sources of such stressors and habitat restoration efforts should prioritize remediation of these sources rather than focusing rehabilitation to the tributary itself.

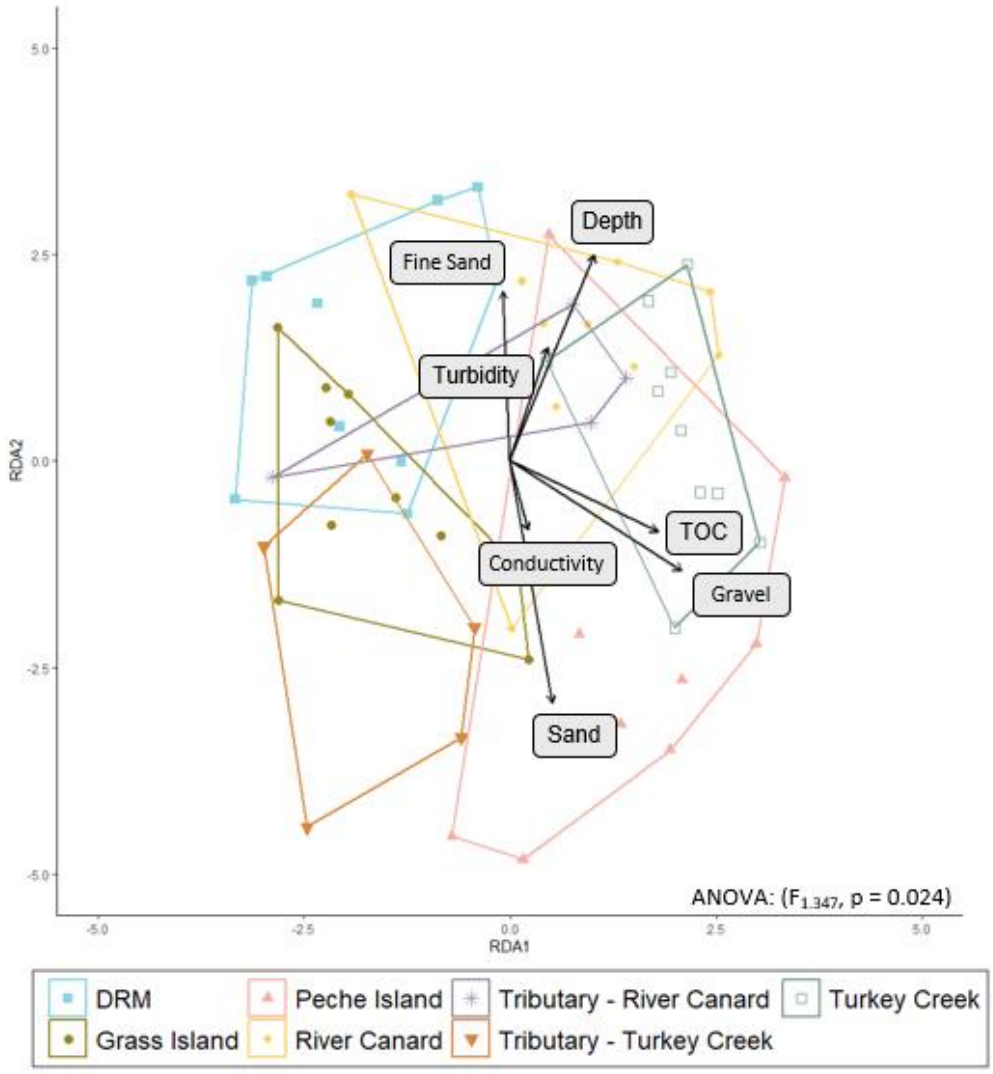


Figure 2.12: RDA of benthic macroinvertebrate community assemblages reveal clustering by sample site and significant associations with key environmental variables. TOC = total organic carbon.

REFERENCES

Alhejoj I, Salameh E, Bandel K (2014) Mayflies (Order Ephemeroptera): An Effective Indicator of Water Bodies Conditions in Jordan. International Journal of Scientific Research in Environmental Sciences 2:361–370. doi: 10.12983/ijsres-2014-p0361-0370

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

Anderson MJ (2006) Distance-Based Tests for Homogeneity of Multivariate Dispersions. Biometrics 62:245–253. doi: 10.1111/j.1541-0420.2005.00440.x

Anderson MJ, Walsh DCI (2013) PERMANOVA, ANOSIM, and the Mantel test in the face of heterogeneous dispersions: What null hypothesis are you testing? Ecological Monographs 83:557–574. doi: 10.1890/12-2010.1

Arimoro FO, Ikomi RB, Iwegbue CMA (2007) Water quality changes in relation to Diptera community patterns and diversity measured at an organic effluent impacted stream in the Niger Delta, Nigeria. Ecological Indicators 7:541–552. doi: 10.1016/j.ecolind.2006.06.002

Bayly IAE (1969) The occurrence of calanoid copepods in athalassic saline waters in relation to salinity and anionic proportions. Internationale Vereinigung für theoretische und angewandte Limnologie: Verhandlungen 17:449–455. doi: 10.1080/03680770.1968.11895872

Bernhardt ES, Palmer MA (2011) The environmental costs of mountaintop mining valley fill operations for aquatic ecosystems of the Central Appalachians. Annals of the New York Academy of Sciences 1223:39–57. doi: 10.1111/j.1749-6632.2011.05986.x

Brunk KM, Vinson MR, Ogle DH, Evrard LM (2014) Burrowing mayfly populations in Chequamegon Bay, Wisconsin: 2002 and 2012. Journal of Freshwater Ecology 29:337–344. doi: 10.1080/02705060.2014.896294

Buhk C, Alt M, Steinbauer MJ, et al (2017) Homogenizing and diversifying effects of intensive agricultural land-use on plant species beta diversity in Central Europe — A call to adapt our conservation measures. Science of The Total Environment 576:225–233. doi: 10.1016/j.scitotenv.2016.10.106

Cao Q, Sun X, Rajesh K, et al (2021) Effects of Rare Microbiome Taxa Filtering on Statistical Analysis. Frontiers in Microbiology 11:607325. doi: 10.3389/fmicb.2020.607325

Carchini G, Bella VD, Solimini AG, Bazzanti M (2009) Relationships between the presence of odonate species and environmental characteristics in lowland ponds of central Italy. Annales de Limnologie - International Journal of Limnology 43:81–87. doi: 10.1051/limn/2007020

Chow-Fraser (2006) Development of the Water Quality Index (WQI) to assess effects of basin-wide land-use alteration on coastal marshes of the Laurentian Great Lakes In Simon, T. P., & P. M. Stewart

(eds), Coastal Wetlands of the Laurentian Great Lakes: Health, Habitat, and indicators. Indiana Biological Survey, Bloomington, Indiana, pp 137–166

Clarke KR (1993) Non-parametric multivariate analyses of changes in community structure. Austral Ecology 18:117–143. doi: 10.1111/j.1442-9993.1993.tb00438.x

Cooper MJ, Uzarski DG, Burton TM (2007) Macroinvertebrate community composition in relation to anthropogenic disturbance, vegetation, and organic sediment depth in four Lake Michigan drowned river-mouth wetlands. Wetlands 27:894–903. doi: 10.1672/0277-5212(2007)27[894:mccirt]2.0.co;2

Corkum LD, Ciborowski JJH, Lazar R (1997) The Distribution and Contaminant Burdens of Adults of the Burrowing Mayfly, *Hexagenia*, in Lake Erie. Journal of Great Lakes Research 23:383–390. doi: 10.1016/s0380-1330(97)70920-1

Cutler J (1994) Benthic Macrofauna Community and Response Characterization, Thin-layer Disposal National Demonstration Project. Mote Environmental Services, Inc., Gulfport, Mississippi

Derecki J (1984) Detroit River Physical and Hydraulic Characteristics. National Oceanic and Atmospheric Administration Great Lakes Environmental Research Laboratory, Ann Arbor, Michigan

Dray S, Bauman D, Blanchet G, et al (2022) adespatial: Multivariate Multiscale Spatial Analysis.

DRCC (2020) Re-designation Report: Assessment of Benthos (BUI #6) in the Detroit River Canadian Area of Concern. Windsor, ON, Canada

Drouillard KG, Ciborowski JJH, Lazar R, Haffner GD (1996) Estimation of the Uptake of Organochlorines by the Mayfly *Hexagenia limbata* (Ephemeroptera: Ephemeridae). Journal of Great Lakes Research 22:26–35. doi: 10.1016/s0380-1330(96)70931-0

Drouillard KG, Tomczak M, Reitsma S, Haffner GD (2006) A River-wide Survey of Polychlorinated Biphenyls (PCBs), Polycylic Aromatic Hydrocarbons (PAHs), and Selected Organochlorine Pesticide Residues in Sediments of the Detroit River1999. Journal of Great Lakes Research 32:209–226. doi: 10.3394/0380-1330(2006)32[209:arsopb]2.0.co;2

Edsall TA, Manny BA, Schloesser DW, et al (1991) Production of *Hexagenia limbata* nymphs in contaminated sediments in the Upper Great Lakes Connecting Channels. Hydrobiologia 219:353–361. doi: 10.1007/bf00024768

Environment Canada (2017) Detroit River Area of Concern: Coastal Wetland Habitat Assessment Report 2016 Update. CWS-ON, Downsview, Ontario

Fallon ME, Horvath FJ (1985) Preliminary Assessment of Contaminants in Soft Sediments of the Detroit River. Journal of Great Lakes Research 11:373–378. doi: 10.1016/s0380-1330(85)71781-9

Gaston KJ, Davies RG, Orme CDL, et al (2007) Spatial turnover in the global avifauna. Proceedings of the Royal Society B: Biological Sciences 274:1567–1574. doi: 10.1098/rspb.2007.0236

Gauch H (1982) Multivariate Analysis in Community Ecology. Cambridge University Press, Cambridge, UK

Goertzen D, Schneider A-K, Eggers TO, Suhling F (2022) Temporal changes of biodiversity in urban running waters – Results of a twelve-year monitoring study. Basic and Applied Ecology 58:74–87. doi: 10.1016/j.baae.2021.11.007

Goral F, Schellenberg J goeveg: Functions for Community Data and Ordinations.

Graf R (2019) A multifaceted analysis of the relationship between daily temperature of river water and air. Acta Geophysica 67:905–920. doi: 10.1007/s11600-019-00285-3

Gray E, Elliott JA, Mackay EB, et al (2019) Modelling lake cyanobacterial blooms: Disentangling the climate-driven impacts of changing mixed depth and water temperature. Freshwater Biology 64:2141–2155. doi: 10.1111/fwb.13402

Hartig JH, Bennion D (2017) Historical Loss and Current Rehabilitation of Shoreline Habitat along an Urban-Industrial River—Detroit River, Michigan, USA. Sustainability 9:828. doi: 10.3390/su9050828

Heathwaite AL, Johnes PJ, Peters NE (1996) Trends in nutrients. Hydrological Processes 10:263–293. doi: 10.1002/(sici)1099-1085(199602)10:2<263::aid-hyp441>3.0.co;2-k

Hieber M, Robinson CT, Uehlinger U, Ward JV (2005) A comparison of benthic macroinvertebrate assemblages among different types of alpine streams. Freshwater Biology 50:2087–2100. doi: 10.1111/j.1365-2427.2005.01460.x

Higgins CL, Willig MR, Strauss RE (2006) The role of stochastic processes in producing nested patterns of species distributions. Oikos 114:159–167. doi: 10.1111/j.2006.0030-1299.14720.x

Hynes HBN (2017) The stream and its valley. SIL Proceedings, 1922-2010 19:1–15. doi: 10.1080/03680770.1974.11896033

Johnson AA, Kleve MG (2000) Strelkovimermis Amphidis N. Sp. From Chironomid Adults Emerging From Lake Itasca and Long Lake, Minnesota. Journal of Parasitology 86:99–102. doi: 10.1645/0022-3395(2000)086[0099:sansfc]2.0.co;2

Jones NE, Schmidt BJ (2018) Influence of tributaries on the longitudinal patterns of benthic invertebrate communities. River Research and Applications 34:165–173. doi: 10.1002/rra.3240

Junk, Bayley, Sparks (1989) The flood pulse concept in river-floodplain systems. Canadian special publication of fisheries and aquatic sciences 1:110–127.

Katano I, Doi H, Houki A, et al (2007) Changes in periphyton abundance and community structure with the dispersal of a caddisfly grazer, Micrasema quadriloba. Limnology 8:219–226. doi: 10.1007/s10201-007-0211-7

Kazancı N, Ekingen P, Dügel M, Türkmen G (2015) Hirudinea (Annelida) species and their ecological preferences in some running waters and lakes. International Journal of Environmental Science and Technology 12:1087–1096. doi: 10.1007/s13762-014-0574-3

Kefford BJ, Buchwalter D, Cañedo-Argüelles M, et al (2016) Salinized rivers: degraded systems or new habitats for salt-tolerant faunas? Biology Letters 12:20151072. doi: 10.1098/rsbl.2015.1072

Kefford BJ, Zalizniak L, Dunlop JE, et al (2010) How are macroinvertebrates of slow flowing lotic systems directly affected by suspended and deposited sediments? Environmental Pollution 158:543–550. doi: 10.1016/j.envpol.2009.08.008

Knop E (2016) Biotic homogenization of three insect groups due to urbanization. Global Change Biology 22:228–236. doi: 10.1111/gcb.13091

Kolde R (2019) pheatmap: Pretty Heatmaps.

Konrad C, Booth D (2005) Hydrologic Changes in Urban Streams and Their Ecological Significance. American Fisheries Society Symposium 47:157–177.

Kovalenko KE, Brady VJ, Brown TN, et al (2014) Congruence of community thresholds in response to anthropogenic stress in Great Lakes coastal wetlands. Freshwater Science 33:958–971. doi: 10.1086/676913

Kovats ZE, Ciborowski JJH (1989) Aquatic Insect Adults as Indicators of Organochlorine Contamination. Journal of Great Lakes Research 15:623–634. doi: 10.1016/s0380-1330(89)71516-1

Krieger KA (1984) Benthic Macroinvertebrates as Indicators of Environmental Degradation in the Southern Nearshore Zone of the Central Basin of Lake Erie. Journal of Great Lakes Research 10:197–209. doi: 10.1016/s0380-1330(84)71826-0

Lamberti GA, Resh VH (1985) Distribution of benthic algae and macroinvertebrates along a thermal stream gradient. Hydrobiologia 128:13–21. doi: 10.1007/bf00008935

Lamberti GA, Resh VH (1983) Stream Periphyton and Insect Herbivores: An Experimental Study of Grazing by a Caddisfly Population. Ecology 64:1124–1135. doi: 10.2307/1937823

Legendre P (2014) Interpreting the replacement and richness difference components of beta diversity. Global Ecology and Biogeography 23:1324–1334. doi: 10.1111/geb.12207

Leprieur F, Tedesco PA, Hugueny B, et al (2011) Partitioning global patterns of freshwater fish beta diversity reveals contrasting signatures of past climate changes. Ecology Letters 14:325–334. doi: 10.1111/j.1461-0248.2011.01589.x

Li J, McPhedran K, Szalińska E, et al (2019) Characterizing polychlorinated biphenyl exposure pathways from sediment and water in aquatic life using a food web bioaccumulation model. Integrated Environmental Assessment and Management 15:398–411. doi: 10.1002/ieam.4130

Lin K-J, Yo S-P (2008) The effect of organic pollution on the abundance and distribution of aquatic oligochaetes in an urban water basin, Taiwan. Hydrobiologia 596:213–223. doi: 10.1007/s10750-007-9098-x

Lombardo P, Cooke GD (2002) Consumption and preference of selected food types by two freshwater gastropod species. Fundamental and Applied Limnology 155:667–685. doi: 10.1127/archiv-hydrobiol/155/2002/667

Maechler M, Rousseeuw P, Struyf A, et al (2022) cluster: Cluster Analysis Basics and Extensions.

Maloney KO, Dodd HR, Butler SE, Wahl DH (2008) Changes in macroinvertebrate and fish assemblages in a medium-sized river following a breach of a low-head dam. Freshwater Biology 53:1055–1068. doi: 10.1111/j.1365-2427.2008.01956.x

Manfrin A, Larsen S, Traversetti L, et al (2013) Longitudinal variation of macroinvertebrate communities in a Mediterranean river subjected to multiple anthropogenic stressors. International Review of Hydrobiology 98:155–164. doi: 10.1002/iroh.201201605

Marchant R (2002) Do rare species have any place in multivariate analysis for bioassessment? Journal of the North American Benthological Society 21:311–313. doi: 10.2307/1468417

Maret TR, Cain DJ, MacCoy DE, Short TM (2003) Response of benthic invertebrate assemblages to metal exposure and bioaccumulation associated with hard-rock mining in northwestern streams, USA. Journal of the North American Benthological Society 22:598–620. doi: 10.2307/1468356

Matlou K, Addo-Bediako A, Jooste A (2017) Benthic Macroinvertebrate Assemblage Along a Pollution Gradient in the Steelpoort River, Olifants River System. African Entomology 25:445–453. doi: 10.4001/003.025.0445

McCabe DJ, Gotelli NJ (2000) Effects of disturbance frequency, intensity, and area on assemblages of stream macroinvertebrates. Oecologia 124:270–279. doi: 10.1007/s004420000369

McKinney ML (2006) Urbanization as a major cause of biotic homogenization. Biological Conservation 127:247–260. doi: 10.1016/j.biocon.2005.09.005

Merritt R, Cummins K, Berg M (2008) Introduction to the Aquatic Insects of North America. Kendall/Hunt Publishing Co, Dubuque, Iowa

Meyer JL, Paul MJ, Taulbee WK (2005) Stream ecosystem function in urbanizing landscapes. Journal of the North American Benthological Society 24:602–612. doi: 10.1899/04-021.1

Milani D, Grapentine LC, Fletcher R (2013) Sediment Contamination in Lyons Creek East, a Tributary of the Niagara River: Part I. Assessment of Benthic Macroinvertebrates. Archives of Environmental Contamination and Toxicology 64:65–86. doi: 10.1007/s00244-012-9817-9

Montgomery F, Reid SM, Mandrak NE (2020) Extinction debt of fishes in Great Lakes coastal wetlands. Biological Conservation 241:108386. doi: 10.1016/j.biocon.2019.108386

Morris T (1994) Quaternary Geology of Essex County, Southwestern Ontario;

Mostertman LJ (1976) Water quality: An overview. Agro-Ecosystems 3:239–251. doi: 10.1016/0304-3746(76)90123-2

Negley TL, Eshleman KN (2006) Comparison of stormflow responses of surface-mined and forested watersheds in the Appalachian Mountains, USA. Hydrological Processes 20:3467–3483. doi: 10.1002/hyp.6148

Oksanen J, Simpson GL, Blanchet FG, et al (2022) vegan: Community Ecology Package.

Orzechowski RM, Steinman AD (2022) Assessment of shoreline restoration using macroinvertebrates in a Great Lakes Area of Concern. Environmental Monitoring and Assessment 194:260. doi: 10.1007/s10661-022-09899-5

Park Y-S, Song M-Y, Park Y-C, et al (2007) Community patterns of benthic macroinvertebrates collected on the national scale in Korea. Ecological Modelling 203:26–33. doi: 10.1016/j.ecolmodel.2006.04.032

Perry JA, Schaeffer DJ (1987) The longitudinal distribution of riverine benthos: A river dis-continuum? Hydrobiologia 148:257–268. doi: 10.1007/bf00017528

Pond GJ, Passmore ME, Borsuk FA, et al (2008) Downstream effects of mountaintop coal mining: comparing biological conditions using family- and genus-level macroinvertebrate bioassessment tools. Journal of the North American Benthological Society 27:717–737. doi: 10.1899/08-015.1

Preston FW (1948) The Commonness, And Rarity, of Species. Ecology 29:254–283. doi: 10.2307/1930989

R Core Team (2023) R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria

R Studio Team (2020) RStudio: Integrated Development for R. Boston, MA

Reise K (1983) Sewage, green algal mats anchored by lugworms, and the effects on Turbellaria and small Polychaeta. Helgoländer Meeresuntersuchungen 36:151–162. doi: 10.1007/bf01983854

Resh VH, Bêche LA, McElravy EP (2005) How common are rare taxa in long-term benthic macroinvertebrate surveys? Journal of the North American Benthological Society 24:976–989. doi: 10.1899/05-026.1

Richardson J (1993) Limits to productivity in streams: evidence from studies of macroinvertebrates. Canadian special publication of fisheries and aquatic sciences 118:9–15.

Sabha I, Hamid A, Bhat SU, Islam ST (2022) Water Quality and Anthropogenic Impact Assessment Using Macroinvertebrates as Bioindicators in a Stream Ecosystem. Water, Air, & Soil Pollution 233:387. doi: 10.1007/s11270-022-05839-8

Scharold J, Corry TD, Yurista PM, Kelly JR (2015) Benthic macroinvertebrate assemblages in the US nearshore zone of Lake Erie, 2009: Status and linkages to landscape-derived stressors. Journal of Great Lakes Research 41:338–347. doi: 10.1016/j.jglr.2015.03.020

Schock NT, Murry BA, Uzarski DG (2014) Impacts of Agricultural Drainage Outlets on Great Lakes Coastal Wetlands. Wetlands 34:297–307. doi: 10.1007/s13157-013-0486-x

Stelzer RS, Lamberti GA (2001) Effects of N: P ratio and total nutrient concentration on stream periphyton community structure, biomass, and elemental composition. Limnology and Oceanography 46:356–367. doi: 10.4319/lo.2001.46.2.0356

Stewart JS, Wang L, Lyons J, et al (2001) Influences of Watershed, Riparian-corridor, and Reach-scale Characteristics on Aquatic Biota in Agricultural Watersheds. JAWRA Journal of the American Water Resources Association 37:1475–1487. doi: 10.1111/j.1752-1688.2001.tb03654.x

Stewart NA, Schriever TA (2023) Local environmental conditions influence species replacement in Great Lakes interdunal wetland macroinvertebrate communities. Freshwater Biology 68:46–60. doi: 10.1111/fwb.14008

Stewart TW, Downing JA (2008) Macroinvertebrate communities and environmental conditions in recently constructed wetlands. Wetlands 28:141–150. doi: 10.1672/06-130.1

Svendsen KM, Renshaw CE, Magilligan FJ, et al (2009) Flow and sediment regimes at tributary junctions on a regulated river: impact on sediment residence time and benthic macroinvertebrate communities. Hydrological Processes 23:284–296. doi: 10.1002/hyp.7144

Szalinska E, Grgicak-Mannion A, Haffner GD, Drouillard KG (2013) Assessment of decadal changes in sediment contamination in a large connecting channel (Detroit River, North America). Chemosphere 93:1773–1781. doi: 10.1016/j.chemosphere.2013.06.009

Taborda J, Soares JAC, Correa MSG, et al (2022) Influences of water and sediment quality on benthic macroinvertebrates in a river with different land use types. Water, Air, & Soil Pollution 233:388. doi: 10.1007/s11270-022-05842-z

Tavzes B, Urbanič G, Toman MJ (2006) Biological and hydromorphological integrity of the small urban stream. Physics and Chemistry of the Earth, Parts A/B/C 31:1062–1074. doi: 10.1016/j.pce.2006.07.009

Thorp J, Rogers D (2016) Keys to nearctic fauna. Volume II, Thorp and Covich's freshwater invertebrates. doi: https://doi.org/10.1016/C2010-0-65589-1

Tindall AR (1964) The skeleton and musculature of the larval thorax of Triaenodes bicolor Curtis (Terichoptera : Limnephilidae). Transactions of the Royal Entomological Society of London 116:151–210. doi: 10.1111/j.1365-2311.1964.tb01085.x

Trauben B, Olive J (1984) Benthic macroinvertebrate assessment of water quality in the Cuyahoga River, Ohio: an update. Ohio Journal of Science (USA) 38:209–212.

UGLCCS (Upper Great Lakes Connecting Channel Study) Management Committee, 1988. Final Report of the Upper Great Lakes Connecting Channels Study Volume 2. U.S. Environmental Protection Agency, Environment Canada, Michigan Department of Natural Resources, Ontario Ministry of the Environment.

USACE (Unite States Army Corps of Engineers)(2023) Great Lakes Hydraulics and Hydrology – Great Lakes Water Level Data Website. https://www.lre.usace.army.mil/Missions/Great-Lakes-Information/Great-Lakes-Information-2/Water-Level-Data/ Accessed: April 26, 2023

Ward JV, Stanford JA (1995a) Ecological connectivity in alluvial river ecosystems and its disruption by flow regulation. Regulated Rivers: Research & Management 11:105–119. doi: 10.1002/rrr.3450110109

Ward JV, Stanford JA (1995b) The serial discontinuity concept: Extending the model to floodplain rivers. Regulated Rivers: Research & Management 10:159–168. doi: 10.1002/rrr.3450100211

Wickham H (2016) ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York

Williams P, Whitfield M, Biggs J, et al (2004) Comparative biodiversity of rivers, streams, ditches and ponds in an agricultural landscape in Southern England. Biological Conservation 115:329–341. doi: 10.1016/s0006-3207(03)00153-8

Wood (2004) The Use of Benthic Macroinvertebrate Community Composition as a Measure of Contaminant Induced Stress in the Sediments of the Detroit RIiver. University of Windsor

Wright DH, Patterson BD, Mikkelson GM, et al (1997) A comparative analysis of nested subset patterns of species composition. Oecologia 113:1–20. doi: 10.1007/s004420050348

Zumberge J, Lee K, Goldstein R (2003) Relation of Periphyton and Benthic Invertebrate Communities to Environmental Factors and Land Use at Selected Sites in Part of the Upper Mississippi River Basin, 1996-98. U.S. Department of the Interior, U.S. Geological Survey, Mounds View, Minnesota

CHAPTER 3

DETROIT RIVER SEDIMENT BACTERIA RESPONSE AT TRIBUTARY JUNCTIONS

3.1: Introduction

Microorganisms are arguably the most abundant life form on Earth and have been observed throughout the biosphere from the atmosphere to subsurface environments (Lighthart 1997; Fujita et al 2000). Microbial communities have long been recognized as important to sediment remediation and the cycling of nutrients and metals (Dittrich et al 2015; Small et al 2016). Microbial macroecology is an emerging subdiscipline that explores microbial diversity and distribution patterns to explore environmental mechanisms. Investigating the mechanisms of macroecology first involve the synthesis of ecological and evolutionary theories, and biogeographical patterns (Xu et al 2020). Microbial biogeography patterns are not as well characterized as for macro-organisms but this a rapidly growing of field of research inquiry.

Microbial communities are shaped by the environment and taxa specific dispersal capabilities over historical conditions as well as under recent conditions (Martiny et al 2006; Martiny et al 2011; Bouzat et al 2013; Fondi et al 2016; Huang et al 2018). In the Mid-Atlantic region of the United States, Hosen et al.(2014; 2017) found patterns in microbial community assembly that were driven by river order and hydrological regime (e.g., temporary vs. permanent streams). Microbial community succession has been shown along continuums of spatial frameworks and physiochemical properties (Read et al 2015; Savio et al 2015). Yannarell and Triplett (2004) studied the biogeography patterns of bacteria communities in 13 temperate lakes of Wisconsin. Communities spatially varied more greatly between lakes (74.8%) than within each lake (17.0%) where similar lake communities associated along primary productivity and dissolved organic carbon gradients and were positively correlated to water temperature and

lake productivity, suggesting temporal variation supported in other studies (Hosen et al 2018; Fraker et al 2021; Shahraki et al 2021).

Local-scale studies suggest microbial communities are strongly affected by both spatial and environmental conditions but also strongly respond to anthropogenic stressors (Rodríguez et al 2021). This implies that microbial community composition can be adapted to biomonitoring practices in similar ways as commonly applied to other taxa including macroinvertebrates, plants, fish and birds (Martiny et al 2011; Yu et al 2017). For example, Simonin et al., (2019) investigated tolerances of 940 bacterial taxa along urbanization gradients and established that many taxa were intolerant to increases in degree of urbanization with highly intolerant taxa eventually becoming lost. However, sediment bacterial communities are not as well researched especially those of riverine systems (Li et al 2021). A study of planktonic and sedimentary bacteria of the Yangtze River had found that sedimentary bacteria had contributed more significantly to biodiversity than planktonic species and were less affected by seasonal changes (Liu et al 2018). This greater degree of temporal stability suggests that sediment microbial communities can serve as time integrated proxies of anthropogenic stressors less affected by hydraulic regimes in flowing systems where convective processes continuously mediate microbial dispersal and transience in the overlying waters. Veach et al., (2021) identified three major habitat types that microbial community compositions vary along; lentic, lotic and wetlands (Zeglin 2015; Monard et al 2016). These researchers echoed that microbial communities found in wetlands and sediment communities to be the least represented in freshwater microbial research. Adapting biomonitoring approaches to sediment microbial communities requires prior characterization and local scale studies to address common patterns and indicator groups at both reference and disturbed habitats.

Metagenomic approaches characterize whole genome sequences present in collected samples and can be applied to both sampled DNA, reflecting taxonomic groups present, and RNA, reflective of active genes being expressed. Whole genome gene expression remains relatively expensive and is still challenging due to the complexity of bacterial strategies like redundancy of functional genes (Allison and Martiny 2008) and dormancy (Jones and Lennon 2010). Alternatively, metataxonomics, identification by 16S ribosomal DNA barcoding is a widely adopted, accurate and a cost-effective method applied to microbial biodiversity measures (Hilton et al 2016). A feasible approach to incorporating bacteria DNA into biomonitoring programs could start by utilizing a multivariate reference approach where microbial assemblages at references sites are contrasted against suspected impacted sites. Such approaches are widely applied in macroinvertebrate composition studies (Reece and Richardson 2000; McDaniel and Pascoe 2017) and do not necessitate pre-assignment of taxa-specific functional qualities (e.g. tolerant vs intolerant species) as required in the formulation of biotic integrity indices (Karr 1981; Capeletti et al 2021). Assessing current limitations of microbial bioassessments, Astudillo-Garcia et al., (2019) identified that there is a lack of studies considering different systems and difficulties related to assigning reference locations. Selection of reference sites must be comparable in environmental and temporal characteristics, and they are assumed to maintain comparable ecosystem functioning as unperturbed habitats (Karr and Dudley 1981). Reference conditions for fish, periphyton and macroinvertebrates are either site-specific, such as sampling around a point-source or regional reference conditions that pair similar ecoregions and habitat types (Hughes et al 1986; Barbour et al 1999). Compared to macroinvertebrates, microbial communities may reflect stronger temporal and spatial variation requiring reference sites be more closely matched over smaller geographic constraints and time. Thus, the incorporation of microbial assemblages into Great Lakes biomonitoring programs

would benefit from understanding the distribution patterns of microbial species (Shupryt and Studinski 2021).

The Laurentian Great Lakes are unique systems for being large, interconnected lakes. Water column communities differ between upper and lower Great Lakes and common groups were of *Proteobacteria, Actinobacteria* and *Bacteroidetes* (Paver et al 2020). Sediment communities in Lakes Huron, Lake Michigan, Lake Ontario, and Lake Superior were predominately *Actinobacteria, Acidobacteria* and *Proteobacteria* (Winters et al 2014); *Proteobacteria* are also common in the sediments of Lake Erie (Small et al., 2016). Connecting channels of the Great Lakes differ in flow, temperature and substrate habitats compared to the lakes, yet as connecting channels, they are also distinct from river systems organized by descending stream order which have substantially greater watershed connectivity and much greater interannual variation in flow. Studies reporting on biomonitoring approaches applied to microbial communities in Great Lakes Connecting channels are scarce. One study by Falk et al. (2019) compared microbial taxa and gene expression at 12 stations in the mid- to lower Detroit River reflecting low, medium and high sediment contamination. The authors observed reduced taxonomic richness, diversity and altered gene expression at stations with high sediment contamination compared to medium and low contamination.

One critical knowledge gap is understanding community assemblages in coastal wetlands of the Detroit River. To explore this further, the present study focuses on bacteria communities across wetlands that represent the transition between Detroit River watersheds and the connecting channel itself. As an initial baseline study for the region, I explore variations in wetland microbial communities with respects to tributary inputs. Coastal wetlands found in the mainstem of the Detroit River are likely more stable than those connected directly with

tributaries. Even though tributaries entering connecting channels such as the Detroit River contribute relatively little flow, they have the potential to influence microbial communities over small spatial scales by altering physiochemical gradients linked to their surrounding land uses (e.g., water quality, sediment, flow) and offering a potential dispersal mechanism (Howell et al 2012; Vilmi et al 2020). The Detroit River has five major submerged wetlands in the Canadian waters, where two are impacted by tributaries. Here we investigate patterns in community composition by exploring two hypotheses:

H1: Bacteria community assemblages will resemble water quality differences between tributary-impacted and unimpacted wetlands.

H2: Bacteria assemblages at tributary-impacted sample sites demonstrate differences with respect to tributary plume position.

As water quality varies across wetlands, I expect to see variations in wetland microbial communities such that sample sites located in tributary impacted wetlands (Turkey Creek and River Canard) will differ from the wetlands without tributary influence (Peche Island, Grass Island and Detroit River Marshes). In hypothesis two, I expect to see within wetland differences by strata such that communities upstream of the tributary input put will more closely resemble the non-impacted wetlands whereas communities present in the tributary and tributary plume will differ. Finally, sites located in strata downstream of the tributary input are expected to have communities intermediate to those observed in the upstream and tributary plume sites as a result of water mass mixing from the two adjacent strata.

3.2 Methodology

3.2.1: Study Area and Site Selection

See 2.2.1: Study Site and Site Selection

3.2.2: Environmental Analysis

See 2.2.2: Environmental Analysis.

3.2.3: Bacteria

At each site microbial communities were collected from sediment using a petite-ponar grab sampler which retrieve up to 15cm of surface sediments. Plant material was not collected in this study, sediment from the surface of the ponar-retrieved sample was transferred into two-cryovials (7.0 mL and 2.5 mL) and frozen in a -80^{o}C dewer containing liquid nitrogen. The samples were transferred to a -80^{o}C freezer upon returning to the lab and inventoried.

Sediment samples were processed for total DNA isolation using Macherey-Nagel NucleoSpin Soil extraction kit as per manufacturer instructions, selecting Buffer SL2. Samples stored in -20 ^{o}C freezer between all stages to sequencing the 16S rRNA genes.

DNA extracts were amplification by Polymerase Chain Reaction (PCR) using the *PCR1* reagents and *PCR1* amplification profile in **Appendix C**. PCR amplified the ~250bp V5-V6 region of the 16S rRNA bacterial gene. V5F primer (5'-ATTAGATACCCNGGTAG-3') and V6R (5'-CGACAGAGCCATGCANCACCT-3') primers were used. DNA amplification was verified by visualization on 2%-agarose TBE gel. DNA was isolated from PCR1 products using Agencourt AMPure XP bead cleaning method as per manufacturer instruction. This process is a solid-phase reversible immobilization paramagnetic bead process that binds DNA fragments greater than 100bp.

The second PCR was for the attachment of Ion Torrent indices (~50bp) for multiplex sequencing. This step was performed using the *PCR2* reagents and *PCR2* amplification profile outlined in **Appendix C**. PCR2 products were visualized on a 2%-agarose TBE gel to determine target band size of 300bp. Band intensity was recorded as proxy for DNA concentration. Samples were combined based on band intensity and excised from 1%-agarose 1X TAE buffer and purified with Qiagen Gel Extraction kit as per manufacturer instructions. Multiplex sequencing was done by Ion Torrent Personal Genome Machine at Environmental Genomics facilities at GLIER, University of Windsor (Falk, 2022).

3.2.4: Statistical Analysis

The approach to data interpretation and statistical analyses is presented as a flow chart in Chapter Two (Figure 2). All statistical analyses were performed using R (R Core Team 2023) in RStudio (R Studio Team 2020) and plots were generated with ggplot2 (Wickham 2016) unless otherwise stated.

3.2.4.1: PCA Water Quality and Habitat

See 2.2.4.1: PCA Water Quality and Habitat

3.2.4.2: Bacteria Community Data

Data were received as a single-ended FASTQ file from Ion Torrent PGM. Demultiplexed FASTQ files were processed with the Quantitative Insights into Microbial Ecology (QIIME) pipeline (Caporaso et al 2010), denoised with DADA2 for removal of singletons, doubletons, low-quality sequences, merge pairs and chimera removal and taxonomy were assigned using and SILVA database (Quast et al 2013). Downstream statistical analyses were performed in R utilizing the packages phyloseq and vegan (McMurdie and Holmes 2013; Oksanen et al 2022). At each sampling site, an average of 151,529 reads were obtained. After trimming and denoising an average of 69,192 reads remained assigned to 2182 taxa; 2140 belonging to Bacteria and the remainder 42 were Archaea. Archaea taxa were excluded from this study. Bacteria counts were log2 transformed and normalized by Cumulative Sum Scaling (CSS) from metagenomeSeq package in R statistical software (Paulson et al 2013) . CSS is a normalization that involves quantile scaling to adjust for differences in sequencing depth between samples which can minimize biases of under sampling.

Alpha diversity metrics (Shannon's H', Pielou J's evenness) were calculated using phyloseq package and base-R. Beta-diversity metrics were calculating using *adespatial* package from R statistical software with Podani family Sorenson-based indices for total beta-diversity, nestedness, and turnover (Legendre 2014; Dray et al 2022). Student T-test was used in testing whether alpha diversity significantly varied between wetlands.

Identifying significantly different taxa abundances between strata were identified by the DESeq2 in R. DESeq2 fits a negative binomial generalized linear model by empirical Bayes shrinkage estimation for dispersions and log-fold change estimations between groups followed by a Wald test, p-values were adjusted by Benjamini and Hochberg method; fit type was set to parametric; alpha was 0.05. Differential abundance by DESeq2 was performed between water quality status groupings, wetlands, tributary-positions, and for sample sites of interest from multivariate reference approach analyses where each test site was run against cluster reference site. Unique taxa were also isolated by status, wetland, and positions.

Heat maps were generated using log-CSS transformed counts for the 40 most abundant phyla using R statistical software, *pheatmap* package (Kolde 2019). Sites were clustered by Ward D method on Bray-Curtis distance matrix.

3.2.4.3: Community Ordination Analysis

Relationships between bacterial communities were visualized with NMDS as per *Chapter 2.2.4.3 Statistical Analysis: Community Ordination* on Bray-Curtis distance matrix. Four NMDS ordinations were made (i) by wetland (k= 3; stress = 0. 0.103) (ii) River Canard by position (k=2; stress=0.100), (iii) Turkey Creek by position (k=2; stress=0.041), Sediment Habitat Group A (k=2; stress = 0.018) and Sediment Habitat Group B (k=2; stress = 0.173).

3.2.4.4: PERMANOVA Procedure

All PERMANOVAs were performed as per *Chapter 2.2.4.4: Statistical Analysis: PERMANOVA Procedure*

3.2.4.5 Multivariate Reference Approach

Sites were clustered by similar habitat characteristics as *per 2.2.4.5: Multivariate Reference Approach* for 56 sample sites*. Centroids are provided in* ***Table 3.1****.* Analysis by PERMANOVA confirms significant habitat differences between groups ($F_{4.879}$, R^2=0.083, p =0.004).

Table 3.1: Habitat group centers (bacteria sample sites).

	Depth (m)	TOC	GRAVEL	SAND	FINE SAND	SILT
Group A (n=26)	1.746	0.862	0.043	0.363	0.439	0.154
Group B (n=30)	0.931	1.146	0.074	0.376	0.366	0.183

3.3 Results

3.3.1: Water Quality and Habitat

See Section 2.3.1 Water Quality and Habitat

3.3.2: Bacteria Community

DNA extraction was performed on all sixty sample sites. However, downstream sequencing processes were successful for only 56 sample sites. The unsuccessful sample sites were DRM-9, DRM-11, DRM-24 and TC-U13 which were excluded from subsequent analysis. The bacterial diversity in this study detected 2140 species where most abundant phyla can be divided into two major groups of common and uncommon taxa. The most abundant phyla were coarsely be broken into two major clusters, one that demonstrates closest correspondence between the phylum *Proteobacteria* (0.577 +- 0.01) and *Acidobacteria* (0.108 +- 0.003) which were the two most abundant phyla. The second major cluster has two further divisions, where one grouping had the next most prevalent phyla, *Verrucomicrobia* (0.05 +- 0.002), *Bacteroidetes* (0.048 +- 0.002) and *Actinobacteria* (0.042 +- 0.003)(**Figure 3.1**). The most abundant class from *Proteobacteria* were *Gamma-*, *Delta-*, and *Alpha-proteobacteria* containing the most abundant orders of *Betaproteobacteriales, Steroidobacterales, Desulfobacterales, Syntrophobacterales, Desulfuromonadales* and *Myxococcales*. Prevalent orders from other dominate phyla include *Pedosphaerales*, *Chitinophagales* and an uncultured prokaryote (*Subgroup 6).* The most abundant families from *Proteobacteria* were *Steroidobacteraceae, Burkholderiaceae, SC.I.84, Rhodocyclaceae,* and *Hydrogenophilaceae.* Abundant families from other phyla were *Pedosphaeraceae (Verrucomicrobia),* and *Chitinophagaceae (Bacteroidetes)*. Of the most abundant genera, three were from the order *Betaproteobacteriales – Thiobacillus, Cupriavidus*,

and *uncultured Burkholderia species* (family *SC.I.84*); two from the class *Deltaproteobacteria* – *Geobacter* and *SVA0081* sediment group. The genera *Spirochaeta* (*Spirochaetes*), *Dinghuibacter* (*Bacteroidetes*) and *Arenimonas* (*Proteobacteria*) were also prevalent across the river wetlands.

The mean species richness at each sample site was 286.232 ± 22.561. Overall, bacterial community assemblages across the Detroit River can be described as highly even with Pielou J's evenness of 0.859 ± 0.003, beta-diversity being principally driven by nestedness of 0.751 (richness difference/beta-diversity total) and overall turnover of 0.249 (replenishment/beta-diversity total)

Figure 3.1: Heat map with cluster analysis of Detroit River and tributaries: forty most abundant bacteria phyla and associated water quality status and location - relative abundance (CSS-transformed).

3.3.2: Hypothesis 1 - Wetland Community Contrasts

Analysis of dissimilarity between the five wetland groupings using PERMANOVA detected differences between wetland groups ($F_{1.929}$, $R^2 = 0.162$, p=0.001). Turkey Creek was significantly different than all other wetlands. Similarities were seen between all other wetland pairings by PERMANOVA with Grass Island and Detroit River Marshes showing the greatest similarity (p = 1.00), as were Peche Island and River Canard (p=1.00). NMDS ordination showed the most similarity between Peche Island to River Canard and Turkey Creek (**Figure 3.2**).

Differential abundance analysis between wetlands indicated three taxa which were significantly different (**Table 3.2**) belonging to the genus (family), CL500-29 marine group (*Ilumatobacteraceae)*, *Terrimicrobium* (*Terrimicrobiaceae)* and *Sediminibacterium* (*Chitinophagaceae)*, showing highest prevalence in Peche Island. At Turkey Creek, the above taxa were not detected (**Figure 3.3**). Additional analysis by tributary-impact status isolated the four mentioned above and *Sphingorhabdus* (*Sphingomonadaceae)* and one additional, CL500-29 marine group *(Ilumatobacteraceae).* These six taxa were more abundant in unimpacted wetlands than the tributary-impacted wetlands.

Table 3.3 summarizes community metrics related to species diversity, richness, evenness. **Table 3.4** summarizes beta-diversity calculations. Full tables of unique species, statistical tables of top phyla, T-Test results are provided in Appendix C. They are briefly described below.

At the unimpacted wetland, Detroit River Marshes (DRM), the average species richness was 260.571 ± 47.131 and Pielou J's evenness of 0.864 ± 0.005. Nestedness was 0.713 and the amount of beta-diversity attributed to turnover was 0.287. DRM had 84 unique species detected

which include the orders of *Coriobacteriales* (*Actinobacteria*) and *Streptosporangiales* (*Actinobacteria*); some exclusive families include Streptosporangiaceae, Parvularculaceae. Select species detected only at DRM included *Luteolibacter pohnpeiensis, Amaricoccus veronensis, Plasticicumulans lactativorans* and *Thermoflavimicrobium dichotomicum*. Grass Island had an average richness of 202.400 ± 24.687 and Pielou J's evenness of 0.867 ± 0.004. Nestedness represented 0.635 of the measured beta-diversity and the remaining 0.365 was species turnover. There were 71 unique taxa to Grass Island, Rhodomicrobiaceae is a family only found at Grass Island, along with the species *Formivibrio citricus, Devosia humi, Ferruginibacter yonginensis.* An iron-reducing bacterium had also been identified belonging to the family *Christensenellaceae* (*Firmicutes*), iron-reducing bacterium enrichment culture clone HN117.

Peche Island sample sites had a Pielou J's evenness of 0.856 ± 0.007 and average species richness of 471.7 ± 46.6. In a paired t-test of alpha-diversity between wetlands, Peche Island sites had significantly higher richness, Pielou J evenness, and Shannon H' than DRM (p=0.014), Grass Island (p=0.002) and to River Canard, with exception to Pielou J evenness at River Canard. As for beta-diversity, nestedness (0.747) was the more dominant component of beta-diversity and turnover represents 0.253. There were 290 unique taxon groups identified at Peche Island including a phylum, *Dadabacteria*. Unique classes were *Dadabacteria* and *Negativicutes*. Two Cyanobacteria families were also only detected at Peche Island, *Cyanobacteriaceae* and *Microcystaceae*. Other unique families include *Cyclobacteriaceae, Rhodospirillaceae, Chlamydiaceae, Magnetospirillaceae* and *Veillonellaceae*. Unique species include *Sphingomonas naphthae.*

The tributary-impacted wetland, River Canard samples had a species richness of 251.7 ± 37.3 and evenness of 0.867 ± 0.006. River Canard's beta-diversity were mostly nested

communities (0.720) with lower species turnover between sites (0.280). There were 111 unique taxa found including the class *Fischerbacteria (Acidobacteria)* , the order *Elsterales (Alphaproteobacteria)*, the families *Dysgonomonadaceae* and *Pasteuriaceae*. Few of the species only detected at River Canard are *Desulforhopalus singaporensis, Clostridium cylindrosporum, Methyloglobulus morosus* KoM1.

Lastly, Turkey Creek wetland samples had a mean richness of 240.55 ± 43.29 and evenness was 0.849 ± 0.010. Beta-diversity had more nestedness (0.796) than species turnover (0.204). Candidatus *Firestonebacteria* (phylum) was one of 68 unique taxa identified at Turkey Creek. Some orders include, *Immundisolibacterales, Streptomycetales, Synechococcales*; families include *Streptomycetaceae, Paenibacillaceae, Cyanobiaceae*; and species *Streptomyces guanduensis, Paenibacillus pectinilyticus, Clostridium algidicarnis*.

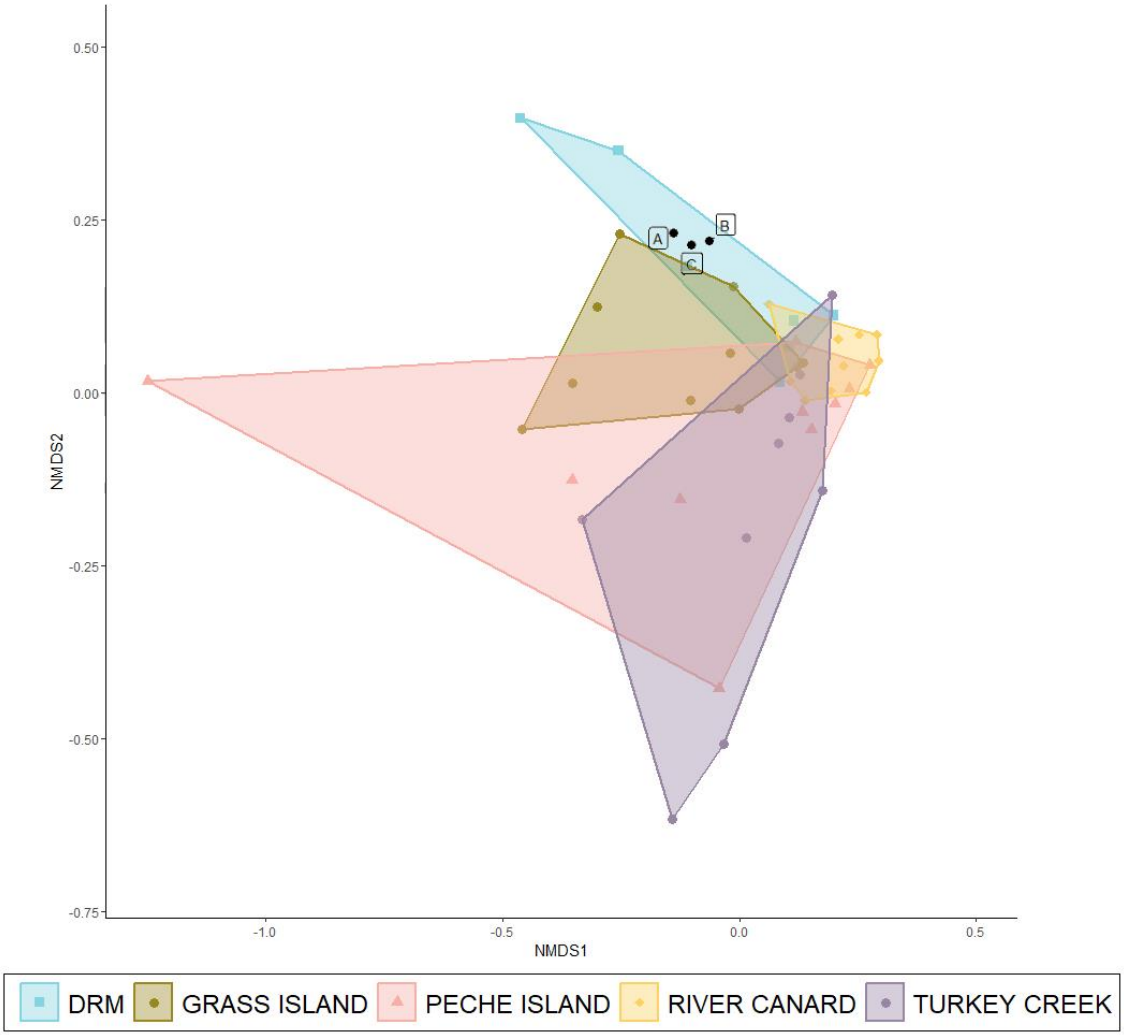


Figure 3.2: NMDS ordination: bacteria communities by wetland

Table 3.2: Significantly different taxa by wetland (DESeq2)

	Phylum	Class	Order	Family	Genus	Species
A	Actinobacteria	Acidimicrobiia	Microtrichales	Ilumatobacteraceae	CL500-29 marine group	marine metagenome
B	Verrucomicrobia	Verrucomicrobiae	Chthoniobacterales	Terrimicrobiaceae	Terrimicrobium	uncultured bacterium
D	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	uncultured Bacteroidetes bacterium

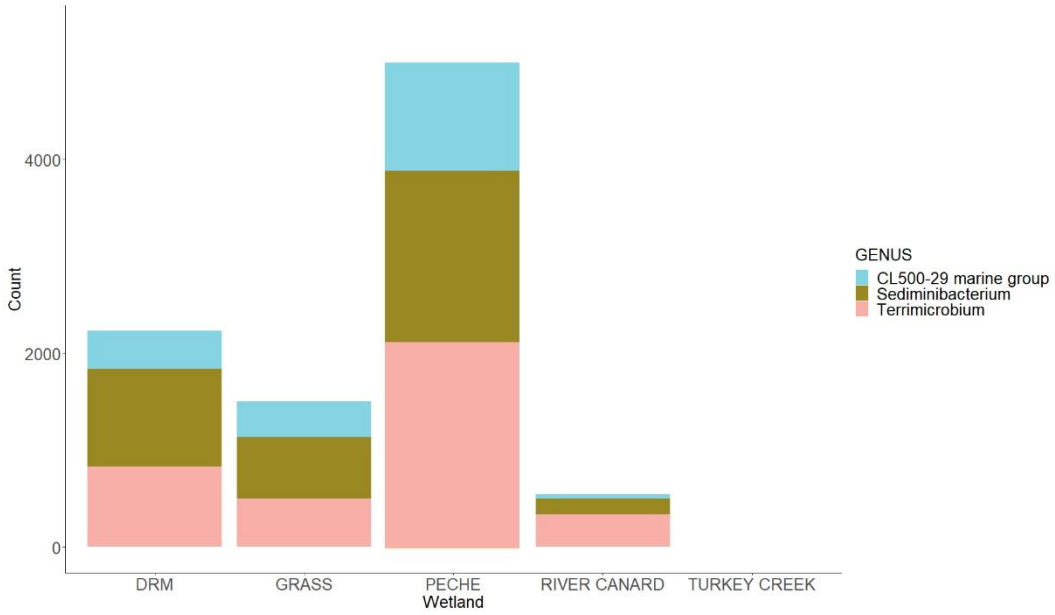


Figure 3.3: Significantly different taxa by wetland (DESeq2)

Table 3.3: Alpha Diversity Means by (a) Detroit River (b) wetland and (c) position.

	Shannon H'	Richness (n)	Pielou J
a) Detroit River			
Wetlands (n=46)	4.733 ± 0.073	286.232 ± 22.561	0.859 ± 0.003
b) Wetland			
Detroit River Marshes (DRM)	4.723 ± 0.166	260.571 ± 47.131	0.864 ± 0.005
Grass Island (GI)	4.544 ± 0.119	202.400 ± 24.687	0.867 ± 0.004
Peche Island (PI)	5.170 ± 0.091	471.700 ± 46.646	0.856 ± 0.007
River Canard (RC)	4.634 ± 0.127	251.700 ± 37.309	0.867 ± 0.006
Turkey Creek (TC)	4.649 ± 0.235	240.556 ± 43.286	0.849 ± 0.010
c) Position			
RC - Tributary	4.386 ± 0.231	218 ± 61.179	0.838 ± 0.009
RC – Downstream	4.631 ± 0.165	233.8 ± 32.788	0.866 ± 0.008
RC - Upstream	4.638 ± 0.212	269.6 ± 70.912	0.868 ± 0.009
TC – Tributary	4.946 ± 0.184	338.4 ± 68.355	0.864 ± 0.008
TC – Downstream	4.970 ± 0.165	305 ± 32.788	0.846 ± 0.008
TC – Upstream	4.247 ± 0.212	160 ± 70.912	0.853 ± 0.009

Table 3.4: Beta-diversity Metrics (Sorenson) - Means (a) Detroit River (b) wetland and (c) position

	β-diversity Total (BDtotal)	Replenishment (Repl)	Richness Difference (RichDif)	Turnover (proportion) Repl/BDtotal	Nestedness (proportion) RichDif/BDtotal
a) Detroit River					
Wetlands (n=46)	0.302	0.075	0.227	0.249	0.751
b) Wetlands					
DRM	0.292	0.084	0.208	0.287	0.713
Grass Island	0.269	0.098	0.171	0.365	0.635
Peche Island	0.237	0.060	0.177	0.253	0.747
River Canard (RC)	0.292	0.082	0.210	0.280	0.720
Turkey Creek (TC)	0.304	0.062	0.242	0.204	0.796
c) Position					
RC - Tributary	0.341	0.112	0.229	0.328	0.672
RC - Downstream	0.250	0.102	0.148	0.408	0.592
RC - Upstream	0.325	0.065	0.261	0.199	0.801
TC - Tributary	0.301	0.066	0.234	0.220	0.780
TC - Downstream	0.274	0.053	0.221	0.193	0.807
TC - Upstream	0.320	0.079	0.241	0.247	0.753

3.3.4: Hypothesis 2a - - Community Contrast by Tributary Position

Summaries of community diversity can be found in **Table 3.5** related to species diversity, richness, evenness. **Table 3.6** summarizes beta-diversity calculations. Full tables of unique species, statistical tables of top phyla, T-Test results are provided in Appendix C. They are also briefly described below.

3.3.4.1 River Canard

River Canard sample sites were grouped as tributary plume or by position of riverbed sites, downstream and upstream strata from the tributary. The dissimilarity between upstream, downstream, and tributary locations was explored using PERMANOVA and NMDS ordination. NMDS ordination showed the downstream section as intermediates of upstream and tributary such that the tributary varies from the upstream (**Figure 3.4**). Yet, PERMANOVA indicated these differences were not significantly between strata ($F_{1.187}$, R^2=0.165, p=0.122). Significantly different species were determined to be of the genera *Methylocaldum*, bacterium TG161, and an uncultured Chloroflexi bacterium (*Anaerolineae*/SJA-15), such that these taxa were not detected at tributary plume sites (**Table 3.5**). Most phyla are *Proteobacteria, Acidobacteria* and *Actinobacteria* (**Figure 3.5**).

The downstream portion of River Canard had a species richness of 233.8 ± 32.7 and evenness of 0.866 ± 0.008. Contributions to beta-diversity were almost even between nestedness (0.592) and species turnover (0.408). Unique classes found in RC downstream were *Fischerbacteria* and *Berkelbacteria*; two unique orders of *Proteobacteria*, *Beggiatoales* and *Arenicellales*; families include *Chromobacteriaceae*, *Nannocystaceae*, *Pseudomonadaceae*, *Rubritaleaceae, Midichloriaceae, Sphingobacteriaceae, Christensenellaceae*.

The tributary plume of River Canard had a Pielou J's evenness of 0.866 and species richness 218 ± 61.2. Beta-diversity was slightly more nested (0.672) than species turned over (0.328). Unique taxa were detected in the tributary positions; phyla, *Deinococcus-Thermus* and *Atribacteria;* families *Fodinicurvataceae, Nocardioidaceae Roseiflexaceae, Tepidisphaeraceae, Armatimonadaceae;* genera*: Thermasporomyces, Kocuria, Desulforegula, Methylomicrobium, Rhodoferax, Candidatus Megaira* and species *Geobacter hephaestius* and *Treponema stenostreptum.*

The upstream position of River Canard had a species richness of 269.6 ± 71.0 and evenness of 0.868 (Pielou J). Variation is predominately nestedness (0.801); turnover represented 0.199 of the beta-diversity. The phylum *Acetothermia* was absent from the downstream and tributary; unique orders were *Thermomicrobiales, Bacillales, Esterales*; families *Pasteuriaceae* and genera *Hyphomonas, Devosia,* and *Brevifollis*.

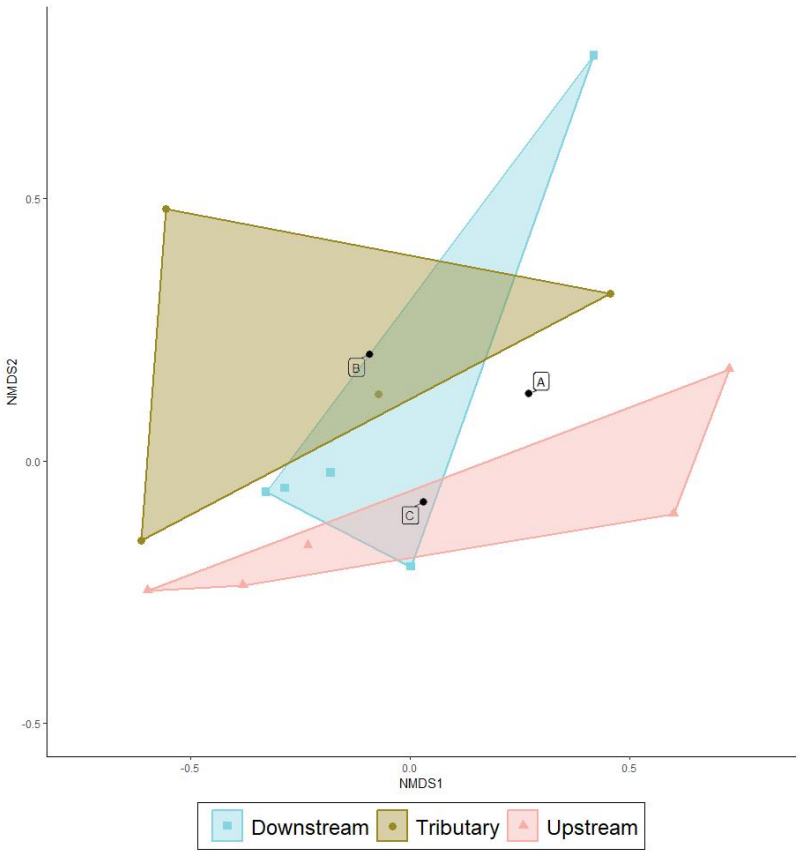


Figure 3.4: NMDS ordination: bacteria communities at River Canard by tributary position.

Table 3.5: Significantly different taxa at River Canard by position (DESeq2).

	Phylum	Class	Order	Family	Genus	Species
A	Proteobacteria	Gammaproteobacteria	Methylococcales	Methylococcaceae	Methylocaldum	uncultured bacterium
B	Chloroflexi	Anaerolineae	SJA-15	uncultured Chloroflexi bacterium	uncultured Chloroflexi bacterium	uncultured Chloroflexi bacterium
C	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	TRA3-20	bacterium TG161	bacterium TG161

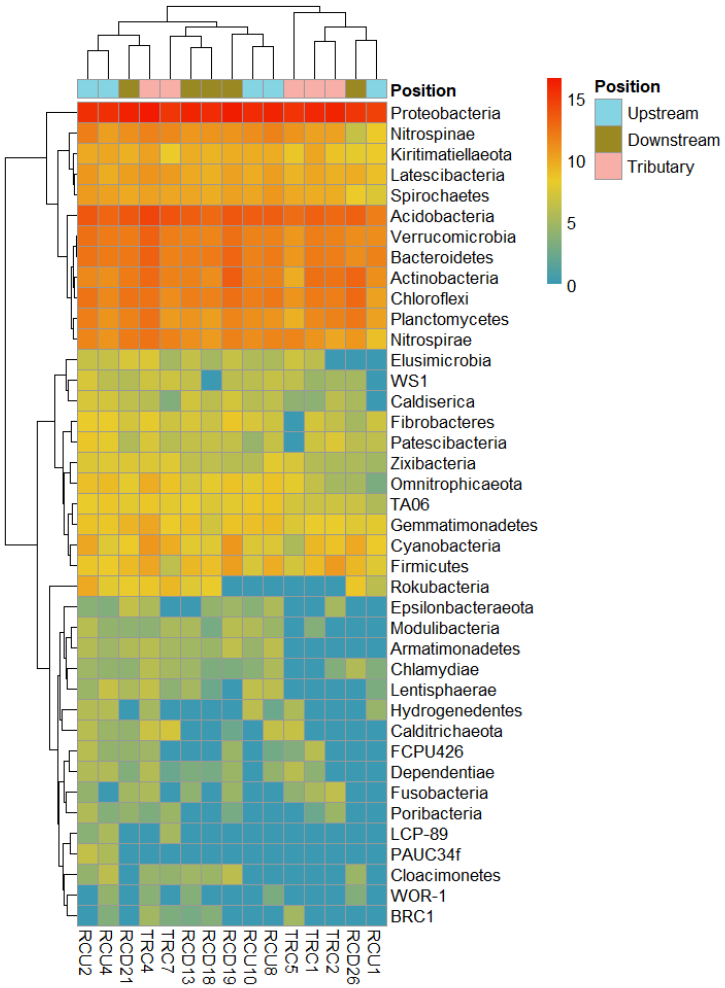


Figure 3.5: Heat map with cluster analysis of River Canard: forty most abundant bacteria phyla and position - relative abundance (CSS-transformed).

3.3.4.2 Turkey Creek

The dissimilarity between Turkey Creek plume, downstream and upstream of tributary input was assessed using a PERMANOVA and NMDS ordination. NMDS ordination depicts a clear distinction between river wetland strata (upstream and downstream) from the tributary plume. The downstream position is an intermediate towards upstream conditions. (**Figure 3.6**). PERMANOVA indicated significant differences between positions ($F_{2.167}$, R^2=0.283, p = 0.002). Pairwise PERMANOVA indicates that the tributary plume differed from both the upstream (p = 0.021) and downstream (p = 0.021) wetland sites.

Differential expression analysis via DESeq2 isolated 6 taxa which significantly differed between groupings; they are uncultured species belonging to the orders, *Betaproteobacteriales, Desulfobacterales*, and *Spirochaetales. Betaproteobacteriales,* species were of the genera *Sulfuricella, Ferritrophicum* and an uncultured *Rhodocyclaceae* (**Table 3.6**). These were only present at tributary plume sites. Also exclusive to the plume was the uncultured Spirochaetacea. Absent from the tributary plume were taxa of the genera *Candidatus Electronema (Desulfobacterales)*.

Diversity at the tributary plume recorded the highest species richness 338.4 ± 68.355 and were considered even with a Pielou J's of 0.864 ± 0.008. Beta-diversity at the tributary strata can be described as mostly nested (0.780) with moderate turnover (0.220). The most detected families were *Burkholderiaceae, Steroidobacteraceae, Hydrogenophilaceae, Rhodocyclaceae*. Unique taxa were found within the tributary are the families, *Defluviitaleaceae, Brachyspirales, Cryomorphaceae, Heliobacteriaceae,* and *Mycoplasma* and the species *Prosthecobacter flubiatilis.*

The downstream strata of Turkey Creek samples had an average species richness of 305 ± 32.788 and an evenness of 0.846 ± 0.008. Variance between sample sites, the beta-

diversity, was significantly nested (0.807) with moderate turnover (0.193). The most abundant families at downstream strata were *Steroidobacteraceae*, *Burkholderiaceae*, SC.I.84, *Pedosphaeraceae*. Phyla only detected in the downstream portion were *Atribacteria*, *Epsilonbacteraeota*, GAL15 and WS2. Unique orders to this stratum were *Bacilli* and *Campylobacterales*. Some unique genera identified were *Paenibacillus* (*Paenibacillaceae*) and *Sulfuricurvum* (*Thiovulaceae*).

Species richness in the upstream portion of Turkey Creek was the lowest at 160 ± 70.9 with a Pielou J's evenness of 0.853 ± 0.009. The beta-diversity was mostly nested (0.753) with some turnover (0.247). The most abundant families were *Steroidobacteraceae*, SC.I.84, *Burkholderiaceae, Pedosphaeraceae*. Unique taxa found are *Bacillales*, *Methylacidiphilales*, *Saprospira and* Ochromonas sp (CCMP1393).

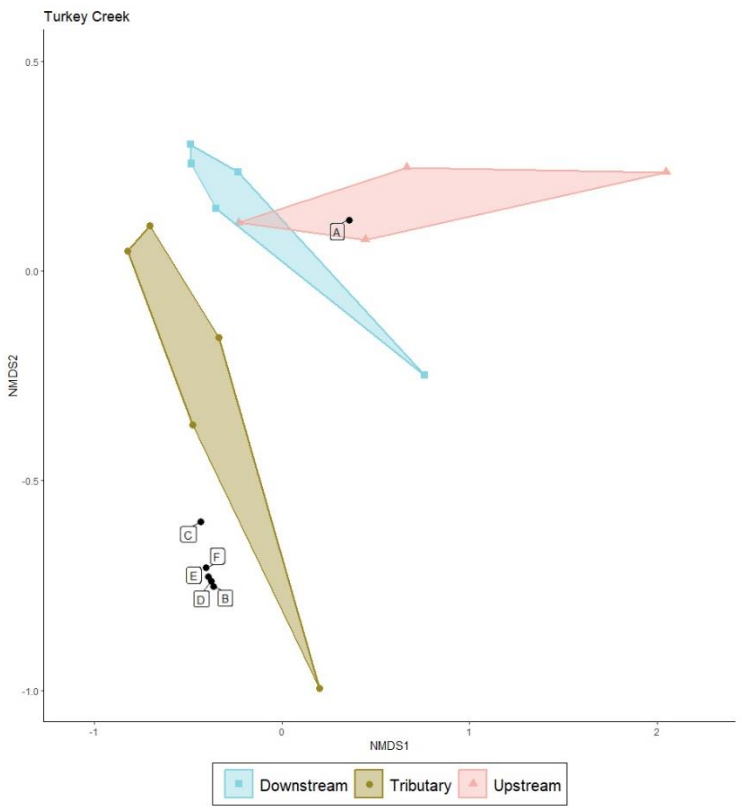


Figure 3.6: NMDS ordination: bacteria communities at Turkey Creek by tributary position.

Table 3.6: Significantly different taxa at Turkey Creek by position (DESeq2).

	Phylum	Class	Order	Family	Genus	Species
A	Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobulbaceae	Candidatus Electronema	uncultured soil bacterium
B	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Sulfuricellaceae	Sulfuricella	Ambiguous taxa
C	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Sulfuricellaceae	Ferritrophicum	Ambiguous taxa
D	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Rhodocyclaceae	uncultured	uncultured gamma proteo-bacterium
E	Spirochaetes	Spirochaetia	Spirochaetales	Spirochaetaceae	uncultured	uncultured soil bacterium
F	Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobacteraceae	Desulfatirhabdium	Ambiguous taxa

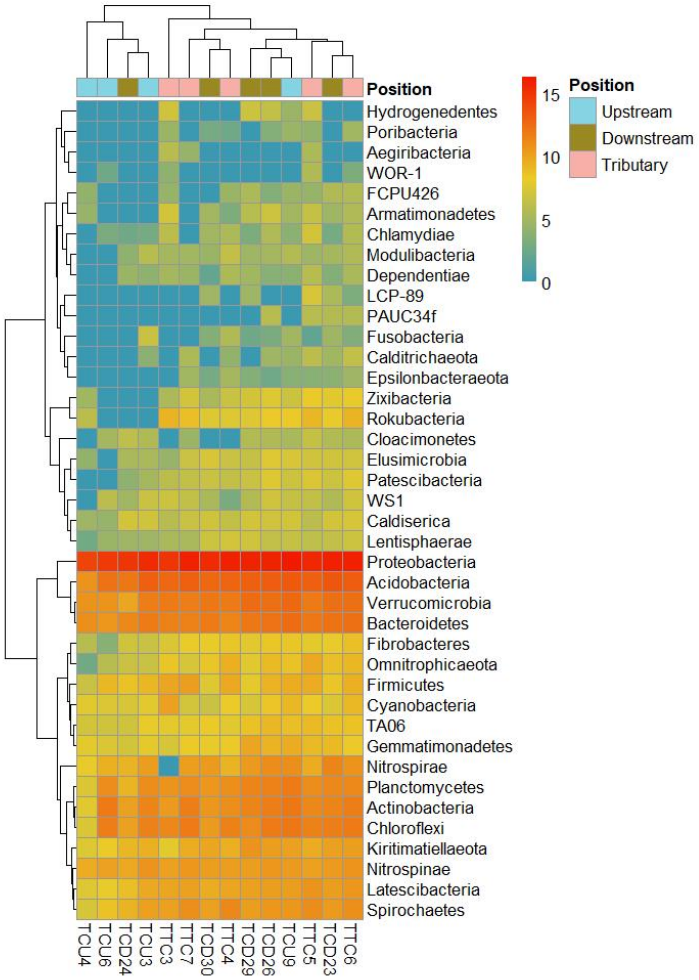


Figure 3.7: Heat map with cluster analysis of Turkey Creek: forty most abundant bacteria phyla and position - relative abundance (CSS-transformed).

3.3.4: Hypothesis 2b - Multivariate Reference Approach: Site-specific Differences at Tributary Impacted Wetlands

Group A sediment habitat is characterized by deeper water, lower TOC, and larger percentages of sands. A total of 26 sample sites displayed these attributes. Among these, 9 were assigned as unimpacted (non-tributary influenced wetlands) locations and 17 potentially impacted sites due to their proximity of tributary influences. Eight of the potentially impacted locations were from River Canard (2 upstream, 5 downstream and 1 tributary). The remaining 9 potentially impacted locations were from Turkey Creek (4 upstream, 5 downstream, 0 tributaries) (**Figure 3.8**).

Group B sediment habitat grouping was composed of shallower sample sites with higher TOC and slightly coarser grain-sizes. There were 30 sites assigned to cluster B with 12 reflecting potentially impacted sites and 18 assigned as unimpacted sites. Seven of the potentially impacted sites were from River Canard (3 upstream and 4 tributary locations) and the other seven were from Turkey Creek (2 upstream locations and 5 tributary sites) (**Figure 3.9**).

The collective of River Canard sites, a total of 9/15 sites exceeded 90% of microbial community reference condition; 2/5 downstream RC-D18, RC-D26; 3/5 upstream RCU1, RCU8 RCU10; 4/5 tributary locations TRC1, TRC2, TRC5, TRC7.

From Turkey Creek sample sites, 10/14 sample sites exceeded 90% of the reference communities comprised of all 5 tributary sample sites (TT6, TTC7 TTC3, TTC4, TTC5), 2/5 downstream (TCD24 and TCD30) and 3/4 upstream (TCU3, TCU4, TCU6)

Results by DESeq2 for significantly different taxa can be found in Appendix C for all test sites greater than 90% with exception to TTC6, TTC7, TTC2, TTC5; as none were found.

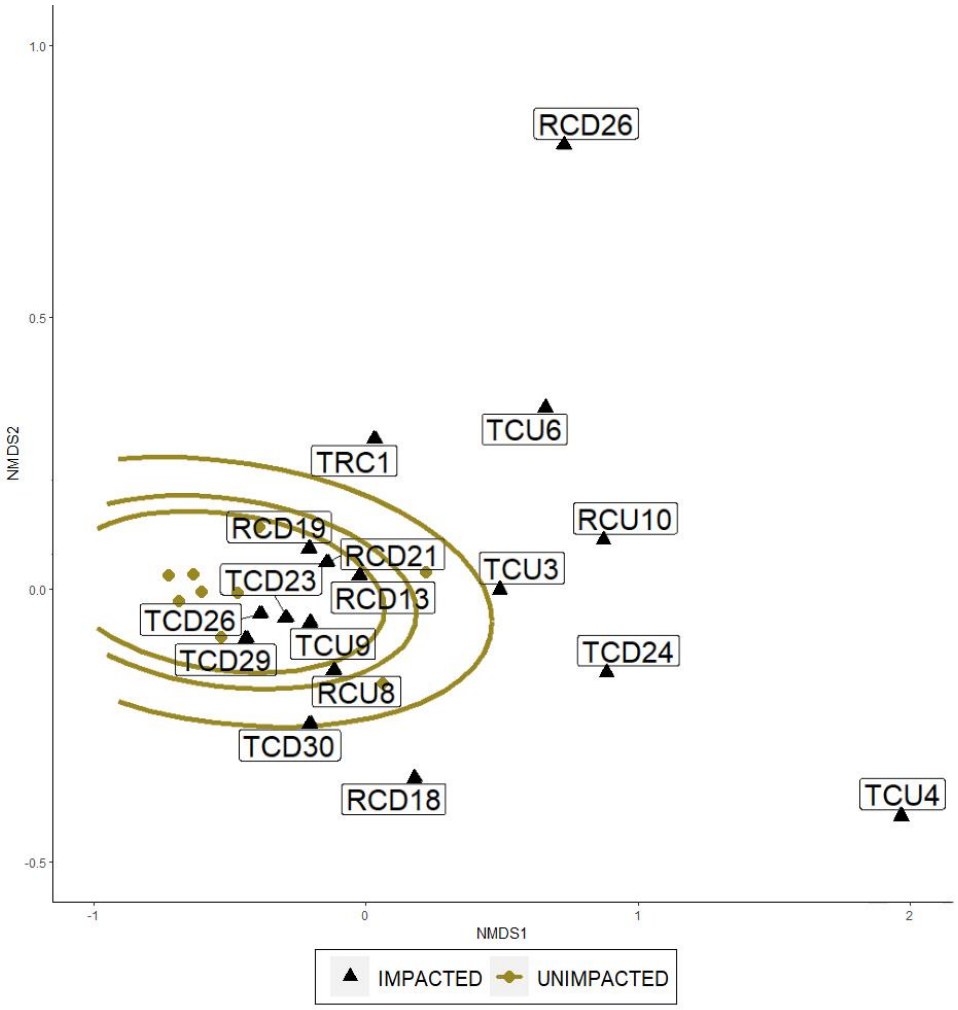


Figure 3.8: NMDS ordination: bacteria communities – MRA sediment habitat group A with unimpacted confidence ellipses from inner representing 90, 95, 99%

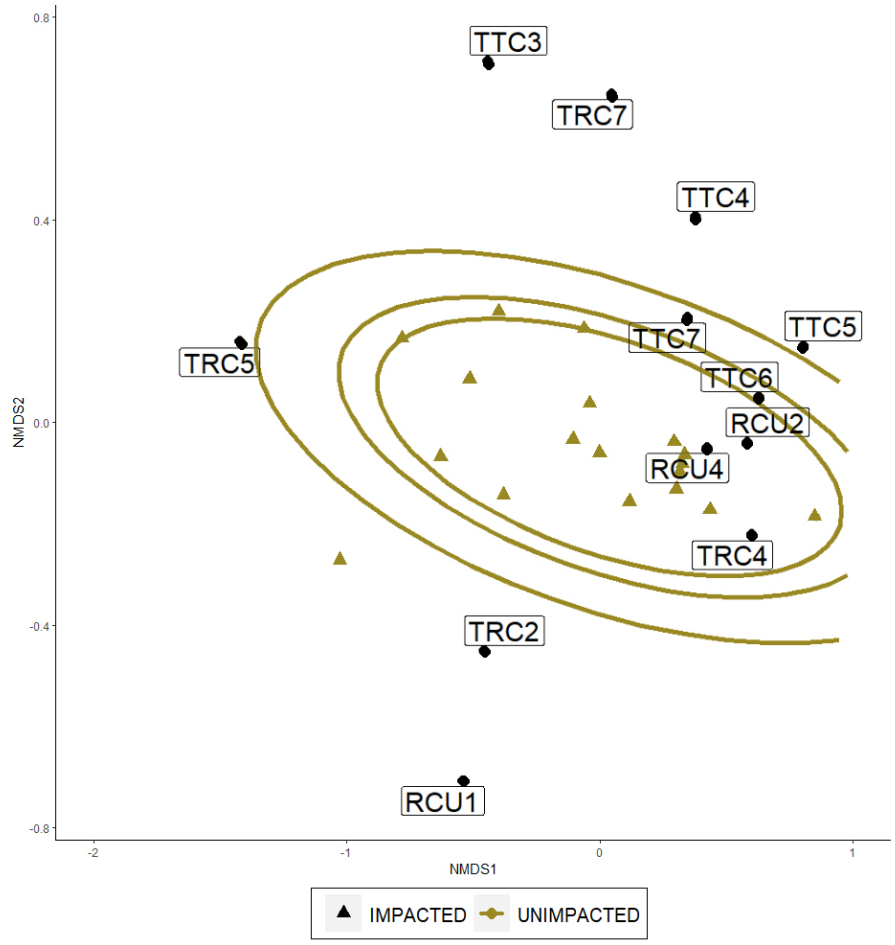


Figure 3.9: NMDS ordination: bacteria communities – MRA sediment habitat group B with unimpacted confidence ellipses from inner representing 90, 95, 99%

3.4 Discussion:

Community differences were found between unimpacted and impacted wetlands of sediment bacteria found in Detroit River wetlands. Community assemblages were dominate in *Proteobacteria*, following with *Acidobacteria, Bacteroidetes, Verrucomicrobia* consistent with several river sediment studies (Gibbons et al 2014; Xie et al 2016; Zhang and Wang 2022). Species richness was significantly higher at unimpacted wetlands compared to tributary-impacted wetlands. Although many components of diversity should be considered in assessing ecosystem function, reductions in species richness are often interpreted as a loss to ecosystem function (Bell et al 2005; Cardinale et al 2012; Venail and Vives 2013). Beta-diversity across the river and at wetlands was predominately nested which may be attributed to bacteria dispersal not being greatly inhibited across the river coupled to the capacity of bacteria to enter dormant states. Meta-transcriptomic techniques may be better suited to determine active species and confirm beta-diversity trends (Falk et al., 2019).

Five species were identified by differential expression analysis as significantly different between tributary-impacted and unimpacted sites, each were present at nearly all unimpacted wetland sample sites and were seldomly detected at tributary influenced wetlands. These included: *Sphingomonadaceae*, *CL500-29 marine group, Terrimicrobium (uncultured), Sediminibacterium*.

Sphingomonadaceae are defined by a ubiquinone-Q10 respiratory system and sphingoglycolipid 1 (SGL-1) in lipids (Naka et al 2000) and are predominately chemoorganotrophs (Glaeser and Kämpfer 2014) involved in carbon cycling. In mesocosms, the genus *CL500-29 marine group* (*Actinobacteria*) is a carbon generalist (Gómez-Consarnau et al 2012; Lindh et al 2015). The genus *Terrimicrobium* has one described species, *Terrimicrobium*

saccharіphilum classified as anaerobic bacterium utilizing carbohydrate fermentation (Qiu et al 2014). Anthropogenic stress can alter carbon cycling processes in wetlands and detection would require measuring total sequences of functional genes for Calvin cycling; *cbbL* and *cbbM* genes (Wang et al 2021). The genus *Sediminibacterium* was first isolated by Qu and Yuan., (2008) from sediments and eight species have since been identified. Much of the genus remains poorly characterized but appear to have utility in bioremediation by participating in the breakdown of vinyl chloride (Wilson et al 2016) and are reported in activated sludges (Ayarza et al 2014).

Rarity in microbial assemblages can be resultant of species traits, abiotic and biotic interactions with the environment, niche breadth or can be due to stochastic drifts in abundances between equally competitive species (Ai et al 2013). High specialization can impart a competitive advantage but can also limit distributions to select habitat types. Rare species can have slow growth rates limiting their densities and distributions until environmental perturbations create otherwise stressful conditions into a competitive advantage for select species (Gudelj et al 2010). Despite low overall abundances, rare species contain functional diversity that can provide ecosystems with stability (Elshahed et al 2008). Two unique candidate phyla were found, Firestonebacteria at the impacted Turkey Creek and Dadabacteria at the unimpacted, Peche Island wetlands. Research into the candidate phylum Dadabacteria is limited, investigations into marine species found the phylum can be divided into two clades on the capability of photoheterotrophy and potentially are involved in the degradation of microbially-derived dissolved organic matter, peptidoglycan, and phospholipids (Graham and Tully 2021).

The candidate phylum, Firestonebacteria appears to be capable of fermentation (Anantharaman et al 2016), the methylation of inorganic mercury (Hg) into methylmercury

(MeHg) (Doud et al 2020) and can likely mitigate cellular damage caused by reactive oxygen species. Reactive oxygen is formed as an intermediate during O_2 reduction in aerobic metabolism. Johnson & Hug (2019) found that Firestonebacteria have genes to produce catalase enzymes that convert hydrogen peroxide to water and oxygen ($2H_2O_2 \rightarrow 2H_2O + O_2$). They found that catalase genes are not an overly prevalent adaption to reactive oxygen, only found in roughly 60% of phylum investigated. Firestonebacteria appear to be capable of sulfur reduction ($S \rightarrow H_2S$), H_2 production and oxidation, arsenic reduction and can produce Group 3b (NiFe), Group 4 (NiFe) and FeFe hydrogenases.

A unique order to Turkey Creek, *Immundisolibacterales* (*Gammaproteobacteria*) was identified to the genus *Immundisolibacter.* Only one species has been described, *Immundisolibacter cernigliae,* as a high-molecular weight polycyclic aromatic hydrocarbon degrader (Corteselli et al 2017) recommended in bioremediation of petroleum contaminated soils (Li et al 2020; Lee et al 2022). Unique bacteria species associated with petroleum-based products are not exclusive to tributary-impacted wetlands. At Peche Island, *Sphingomonas naphthae* had been detected at site PI-16, isolated from oil-contaminated soils by Chaudhary et al., (2017) show the ability to degrade aliphatic compounds and is closely related (>98%), to other *Sphingomonas* species found associated to oil contaminated soils, *Sphingobium olei* (Young et al 2007), *Sphingobium abikonense* (Kumari et al 2009). The order *Streptomycetales* and species *Streptomyces guanduensis,* were unique taxa found at TC-D26. *S. guanduensis* is an aerobic, neutrotolerant, acidophile that grows within pH values 4.5-7.0 with growth optimum at pH 5-5.5 (Xu et al 2006). *Streptomyces* can reduce diseases in plants as they compete with other microorganisms through the production of secondary metabolites; antibiotics, antifungals, antivirals (Kinkel et al 2012; Procópio et al 2012). The soils at Turkey Creek may be slightly acidic, as a second unique taxa was detected with tolerances to acidic soils, *Paenibacillus pectinilyticus*.

(Wen et al 2020). The sediment communities at Turkey Creek were significantly different from every wetland tested, it is likely that sediment and overlaying water quality at Turkey Creek vary sufficiently to exhibit distinct communities. When grouping by similar habitat characteristics in the multivariate reference approach, four of the Turkey Creek riverbed sites were significantly different from the unimpacted (reference) wetlands; two in the upstream section and two downstream sample sites. The upstream sites and TC-D24 had reduced species richness from the river average. Taxa found to be significantly different at test sites than reference locations were predominately associated with sulfur and nitrogen cycles. This is not unusual as biogeochemical processing of sulfur and nitrogen cycling is expected in wetlands. The tributary may be a natural source of sulfur with sedimentary-rock origin. The geology surrounding the Canadian shoreline and into both tributaries was reported to be limestone, stromatolitic calcarenite and anhydrite, anhydrite is also known as calcium sulphate ($CaSO_4$) (Morris 1994). Large natural contributors of nitrogen in wetlands come from sediment dependent upon plant production and decomposition processes (Bowden 1987). Potential anthropogenic sources of excess sulfur and nitrogen mustn't be overlooked given that the tributary of Turkey Creek passes through urbanized areas and the wetland's upstream strata is downstream of a municipal waste-water treatment plant. In addition to wastewater stress, the upstream is directly adjacent to an underground rock salt mining facility. Salt mines have negative impacts on land-use and water quality (Naseem et al 2020) and the mine may be an intermittent source of chloride entering into the downstream wetland as complete containment is likely not feasible. NaCl alters microbial community compositions by shifting abundance toward tolerant species and their effects are more pronounced in mixtures of inorganic nutrients, metals and organic contaminants (Fournier et al 2022). Effluent from the upstream wastewater treatment plant uses UV for sanitization before release into the river but may still a source of additional

inorganic nutrients, metals, and other contaminants (VanDrecht et al 2009; Zamora-Ledezma et al 2021).

Nutrients, ammonium, and nitrate are associated with the use of fertilizers in agricultural production and urban centers are also a source through point sources such as wastewater and non-point sources such as storm run-off. The input of nutrients shifts bacteria community abundances by supplementing limiting resources. Excess nutrients can settle to benthic habitats where sediments shift between being sources and sinks. This study did not specifically characterized communities by metabolic processes, and it is suggested further analyses explore nitrogen and sulfur cycling at Turkey Creek, including community compositional changes due to salinity (Shen et al 2022). Increases in sulfate can promote the release of phosphorus from sediments, and the release is upregulated under anoxic conditions, this can lead to the promotion of harmful algal blooms and eutrophication (Caraco et al 1993). In a study examining microbial community responses in the Great Lakes coastal wetlands, Horton et al., (2016) found that microbial diversity responded to anthropogenic nutrient enrichment with altered diversity, such that diversity responses at Lake Erie was most dissimilar in the region. They had hypothesized that nutrients were driving microbial community structure in coastal wetlands, which is also hypothesized in this thesis as an explanation for significant community differences found at Turkey Creek .

All tributary sample sites with exception of one River Canard location were significantly different from reference wetlands of similar habitat characteristics. Conductivity was higher in both tributaries compared to their respective wetland. Water chemistry of tributaries and nearby mixing zones vary from connected waterbodies and plume intensity is influenced by discharge intensity and volume (Goldman et al 1974; Wiseman and Garvine 1994). River Canard

tributary sites were not significantly different than the river wetland sites but community assemblages did differ between Turkey Creek wetland to the tributary. There were four unique tributary genera determined to also be significantly different to the riverbed communities; *Sulfuricella*, *Ferritrophicum*, uncultured Rhodocyclaceae, uncultured Spirochaetacea. *Sulfuricella* are facultative anaerobes that utilize sulfur oxidation in growth and can reduce nitrate in anaerobic conditions with preferences for low salt concentrations in media (Watanabe and Kojima 2020). Only one member of *Ferritrophicum* has currently been described. Weiss et al., (2007) described them as a neutrophilic, iron-reducing bacterium, isolated from the rhizosphere of wetland plants. The effects of both agricultural and urban tributaries are pronounced in pulse events associated with high degrees of precipitation, typically in late winter-spring and summer when discharge and precipitation is highest in the river (Lemieux et al 1995; ECCC 2023). Monitoring River Canard communities before and after flooding events would be beneficial in assessing the resilience of community structure and possible successions after perturbation (Ding et al 2017).

At time of the study, differences were found in the urbanized tributary of Turkey Creek. While no significant differences were observed in alpha diversity at Turkey Creek positions, the tributary supported the highest richness whereas the upstream supported the least. The downstream portion, a tributary-river mixing zone, had intermediate richness. Bacterial distributions are limited by dispersal capabilities along tributaries and conditioned by environmental gradients (Chen et al 2020). Conditions at the tributary of Turkey Creek supporting more diversity could be due to regimes of varying physiochemical conditions creating habitat heterogeneity both spatially and temporally, more connectivity to bacteria from the adjoining watershed, or simply that conditions in the tributary are more favourable than the river community as evidence by the upstream portion demonstrating lowest diversity.

Researchers, Kiffney et al., (2006) investigated tributary-river junctions of 13 tributaries spanning three basins investigating whether tributaries altered habitat heterogeneity, resources, and biological responses. They found tributaries create environmental gradients that alter substrate, nutrients, and dependent biological communities. These gradients are modulated by timing and intensity of disturbance regimes that may cause redistributions of chemicals and species. Similar to their findings, our study supports that tributaries support rich biodiversity.

The downstream portions of River Canard and upstream position of Turkey Creek had increased turnover than respective counterpart positions. The downstream River Canard receiving inflow from an agricultural tributary may be creating diverse benthic habitat through sedimentation or changes to water quality via nutrients and other pollutants. Fine sand was found in higher proportions in downstream River Canard and agricultural tributaries are sources of finer particles and elevated nutrients (Crosbie and Chow-Fraser 1999) which could be providing more habitat complexity thereby increasing turnover (Wang et al 2012). In addition to altered habitat, chemical stress, such as nutrient enrichment increases turnover as excess nitrogen enrichment can cause soils to be more acidic, promoting the dominance of tolerant species and loss of sensitive species (Wang et al 2012; Liu et al 2022; Zhou et al 2022). As previously addressed with Turkey Creek, two unique species were detected with tolerances for acidic soils. It is hypothesized that sediments at Turkey Creek may be slightly more acidic than other wetlands with the upstream displaying the most significant impairments to diversity.

The bacteria communities of the Detroit River show diverse biogeochemical cycling capabilities of organic and inorganic compounds and demonstrate some variability, particularly at Turkey Creek which varied significantly from all other wetlands. As such, there is partial

support for hypothesis one as Turkey Creek demonstrates significant differences to all other wetlands, but ultimately the hypothesis is rejected due to Peche Island and River Canard bearing similarity despite vast differences in water quality status; Peche Island has the highest year-to-year average for water quality in the river and River Canard has the poorest water quality.

Hypothesis two investigated whether communities had demonstrated variations with respects to the tributary plume, such that downstream locations are intermediates of upstream and tributary plume sites. This hypothesis is rejected for River Canard as PERMANOVA and the MRA did not support differences. The NMDS had offered partial support for community variations with respects to tributary plume communities.

There were distinct differences detected at Turkey Creek, such that the river strata had varied significantly from tributary communities by PERMANOVA but pairwise analysis showed these differences were between the tributary to river, thus not supportive at Turkey Creek. NMDS was mostly supportive of intermediate downstream communities. Through the multivariate reference approach, a total of ten of the 14 sample sites demonstrated alteration from the Detroit River wetland reference condition. All five tributary locations were isolated as potentially altered from reference conditions, yet the tributary plume supports more diverse communities than the non-tributary influenced river wetlands. In fact, reduced diversity in seen the upmost margin of Turkey Creek (3/4) indicating more significant pressures from the upstream waters than the lateral connectivity to the tributary. Overall, when grouping the tributary-impacted zones – tributary and downstream, 75% show deviation from reference Detroit River wetlands and isn't supportive of hypothesis two at Turkey Creek.

In addressing potential limitations of this study, we do not expect bacteria communities to demonstrate significant variation in their community assemblages across the 9 day sampling

period because they do not show significant temporal differences as seen in planktonic communities (Liu et al., 2018). One issue that had arose was not strongly controlling for depth in bacteria community sampling. Bacteria communities can show variation with respects to depth in the sediment (Horton et al., 2016) and ponar grabs are not a controlled method of ensuring that we are sampling from the same depth. While this study had attempted to sample upper layer of retrieve sample material, it is possible that some sites are representative of communities at depths up to 15cm. Future studies should collect sediment cores for more standardized depth collection.

REFERENCES

Ai D, Chu C, Ellwood MDF, et al (2013) Migration and niche partitioning simultaneously increase species richness and rarity. Ecological Modelling 258:33–39. doi: 10.1016/j.ecolmodel.2013.03.001

Allison SD, Martiny JBH (2008) Colloquium paper: resistance, resilience, and redundancy in microbial communities. Proceedings of the National Academy of Sciences 105 Suppl 1:11512–9. doi: 10.1073/pnas.0801925105

Anantharaman K, Brown CT, Hug LA, et al (2016) Thousands of microbial genomes shed light on interconnected biogeochemical processes in an aquifer system. Nature Communications 7:13219. doi: 10.1038/ncomms13219

Astudillo-García C, Hermans SM, Stevenson B, et al (2019) Microbial assemblages and bioindicators as proxies for ecosystem health status: potential and limitations. Applied Microbiology and Biotechnology 103:6407–6421. doi: 10.1007/s00253-019-09963-0

Ayarza JM, Figuerola ELM, Erijman L (2014) Draft Genome Sequences of Type Strain *Sediminibacterium salmoneum* NJ-44 and *Sediminibacterium* sp. Strain C3, a Novel Strain Isolated from Activated Sludge. Genome Announcements 2:e01073-13. doi: 10.1128/genomea.01073-13

Barbour M, Gerritsen J, Snyder B, Stribling J (1999) Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, Second Edition.

Bell T, Newman JA, Silverman BW, et al (2005) The contribution of species richness and composition to bacterial services. Nature 436:1157–1160. doi: 10.1038/nature03891

Bowden WB (1987) The biogeochemistry of nitrogen in freshwater wetlands. Biogeochemistry 4:313–348. doi: 10.1007/bf02187373

Bouzat JL, Hoostal MJ, Looft T (2013) Spatial patterns of bacterial community composition within Lake Erie sediments. Journal of Great Lakes Research 39:344–351. doi: 10.1016/j.jglr.2013.03.003

Capeletti J, Marchese MR, Zilli FL (2021) Evaluating macroinvertebrate metrics for ecological assessment of large saline rivers (Argentina). Environmental Science and Pollution Research 28:66464–66476. doi: 10.1007/s11356-021-16559-7

Caporaso JG, Kuczynski J, Stombaugh J, et al (2010) QIIME allows analysis of high-throughput community sequencing data. Nature Methods 7:335–336. doi: 10.1038/nmeth.f.303

Caraco NF, Cole JJ, Likens GE (1993) Sulfate control of phosphorus availability in lakes. Hydrobiologia 253:275–280. doi: 10.1007/bf00050748

Cardinale BJ, Duffy JE, Gonzalez A, et al (2012) Biodiversity loss and its impact on humanity. Nature 486:59–67. doi: 10.1038/nature11148

Chaudhary DK, Jeong S-W, Kim J (2017) *Sphingobium naphthae* sp. nov., with the ability to degrade aliphatic hydrocarbons, isolated from oil-contaminated soil. International Journal of Systematic and Evolutionary Microbiology 67:2986–2993. doi: 10.1099/ijsem.0.002064

Chen L, Liu S, Chen Q, et al (2020) Dispersal limitation drives biogeographical patterns of *anammox* bacterial communities across the Yangtze River. Applied Microbiology and Biotechnology 104:5535–5546. doi: 10.1007/s00253-020-10511-4

Corteselli EM, Aitken MD, Singleton DR (2017) Description of *Immundisolibacter cernigliae* gen. nov., sp. nov., a high-molecular-weight polycyclic aromatic hydrocarbon-degrading bacterium within the class *Gammaproteobacteria*, and proposal of *Immundisolibacterales* ord. nov. and *Immundisolibacteraceae* fam. nov. International Journal of Systematic and Evolutionary Microbiology 67:925–931. doi: 10.1099/ijsem.0.001714

Crosbie B, Chow-Fraser P (1999) Percentage land use in the watershed determines the water and sediment quality of 22 marshes in the Great Lakes basin. Canadian Journal of Fisheries and Aquatic Sciences 56:1781–1791. doi: 10.1139/f99-109

Ding L-J, Su J-Q, Li H, et al (2017) Bacterial succession along a long-term chronosequence of paddy soil in the Yangtze River Delta, China. Soil Biology and Biochemistry 104:59–67. doi: 10.1016/j.soilbio.2016.10.013

Dittrich M, Moreau L, Gordon J, et al (2015) Geomicrobiology of Iron Layers in the Sediment of Lake Superior. Aquatic Geochemistry 21:123–140. doi: 10.1007/s10498-015-9258-y

Doud DFR, Bowers RM, Schulz F, et al (2020) Function-driven single-cell genomics uncovers cellulose-degrading bacteria from the rare biosphere. The ISME Journal 14:659–675. doi: 10.1038/s41396-019-0557-y

Dray S, Bauman D, Blanchet G, et al (2022) adespatial: Multivariate Multiscale Spatial Analysis.

ECCC (2023) Environment and Climate Change Canada Historical Climate Data web site. https://climate.weather.gc.ca/index_e.html.

Elshahed MS, Youssef NH, Spain AM, et al (2008) Novelty and Uniqueness Patterns of Rare Members of the Soil Biosphere. Applied and Environmental Microbiology 74:5422–5428. doi: 10.1128/aem.00410-08

Falk N, Reid T, Skoyles A, et al (2019) Microbial metatranscriptomic investigations across contaminant gradients of the Detroit River. Science of The Total Environment 690:121–131. doi: 10.1016/j.scitotenv.2019.06.451

Falk N, Droppo IG, Drouillard KG, et al (2022) Integrated microbial DNA community analyses into time-integrated suspended sediment sampling methods. Journal of Soils and Sediments. doi: 10.1007/s11368-022-03293-x

Fondi M, Bosi E, Presta L, et al (2016) Modelling microbial metabolic rewiring during growth in a complex medium. BMC Genomics 17:970. doi: 10.1186/s12864-016-3311-0

Fournier IB, Lovejoy C, Vincent WF (2022) Road Salt versus Urban Snow Effects on Lake Microbial Communities. Microorganisms 10:803. doi: 10.3390/microorganisms10040803

Fraker ME, Shrestha A, Marshall L, et al (2021) Seasonal variation in light penetration and subsurface chlorophyll-α in southern Lake Michigan observed by a glider. Journal of Great Lakes Research 47:1228–1234. doi: 10.1016/j.jglr.2021.04.007

Fujita Y, Ferris FG, Lawson RD, et al (2000) Subscribed Content Calcium Carbonate Precipitation by Ureolytic Subsurface Bacteria. Geomicrobiology Journal 17:305–318. doi: 10.1080/782198884

Gibbons SM, Jones E, Bearquiver A, et al (2014) Human and Environmental Impacts on River Sediment Microbial Communities. PLoS ONE 9:e97435. doi: 10.1371/journal.pone.0097435

Glaeser SP, Kämpfer P (2014) The Prokaryotes, *Alphaproteobacteria* and *Betaproteobacteria*. 641–707. doi: 10.1007/978-3-642-30197-1_302

Goldman CR, Richards RC, Paerl HW, et al (1974) Limnological studies and remote sensing of the Upper Truckee River sediment plume in Lake Tahoe, California-Nevada. Remote Sensing of Environment 3:49–67. doi: 10.1016/0034-4257(74)90038-8

Gómez-Consarnau L, Lindh MV, Gasol JM, Pinhassi J (2012) Structuring of bacterioplankton communities by specific dissolved organic carbon compounds. Environmental Microbiology 14:2361–2378. doi: 10.1111/j.1462-2920.2012.02804.x

Graham ED, Tully BJ (2021) Marine *Dadabacteria* exhibit genome streamlining and phototrophy-driven niche partitioning. The ISME Journal 15:1248–1256. doi: 10.1038/s41396-020-00834-5

Gudelj I, Weitz JS, Ferenci T, et al (2010) An integrative approach to understanding microbial diversity: from intracellular mechanisms to community structure. Ecology Letters 13:1073–1084. doi: 10.1111/j.1461-0248.2010.01507.x

Hilton SK, Castro-Nallar E, Pérez-Losada M, et al (2016) Metataxonomic and Metagenomic Approaches vs. Culture-Based Techniques for Clinical Pathology. Frontiers in Microbiology 7:484. doi: 10.3389/fmicb.2016.00484

Horton DJ, Theis KR, Uzarski DG, et al (2019) Microbial community structure and microbial networks correspond to nutrient gradients within coastal wetlands of the Laurentian Great Lakes. FEMS Microbiology Ecology 95:1-17.

Hosen J, McDonough O, Febria C, Palmer M (2014) Dissolved Organic Matter Quality and Bioavailability Changes Across an Urbanization Gradient in Headwater Streams. Environmental Science & Technology 48:7817–7824.

Hosen JD, Armstrong AW, Palmer MA (2018) Dissolved organic matter variations in coastal plain wetland watersheds: The integrated role of hydrological connectivity, land use, and seasonality. Hydrological Processes 32:1664–1681. doi: 10.1002/hyp.11519

Hosen JD, Febria CM, Crump BC, Palmer MA (2017) Watershed Urbanization Linked to Differences in Stream Bacterial Community Composition. Frontiers in Microbiology 8:1452. doi: 10.3389/fmicb.2017.01452

Howell ET, Chomicki KM, Kaltenecker G (2012) Tributary discharge, lake circulation and lake biology as drivers of water quality in the Canadian Nearshore of Lake Ontario. Journal of Great Lakes Research 38:47–61. doi: 10.1016/j.jglr.2012.03.008

Huang S, Chen C, Jaffé PR (2018) Seasonal distribution of nitrifiers and denitrifiers in urban river sediments affected by agricultural activities. Science of The Total Environment 642:1282–1291. doi: 10.1016/j.scitotenv.2018.06.116

Hughes RM, Larsen DP, Omernik JM (1986) Regional reference sites: a method for assessing stream potentials. Environmental Management 10:629–635. doi: 10.1007/bf01866767

Johnson LA, Hug LA (2019) Distribution of reactive oxygen species defense mechanisms across domain bacteria. Free Radical Biology and Medicine 140:93–102. doi: 10.1016/j.freeradbiomed.2019.03.032

Jones SE, Lennon JT (2010) Dormancy contributes to the maintenance of microbial diversity. Proceedings of the National Academy of Sciences 107:5881–5886. doi: 10.1073/pnas.0912765107

Karr J, Dudley D (1981) Ecological Perspective on Water Quality Goals. Environmental Management 5:55–68.

Karr JR (1981) Assessment of Biotic Integrity Using Fish Communities. Fisheries 6:21–27. doi: 10.1577/1548-8446(1981)006<0021:aobiuf>2.0.co;2

Kiffney PM, Greene CM, Hall JE, Davies JR (2006) Tributary streams create spatial discontinuities in habitat, biological productivity, and diversity in mainstem rivers. Canadian Journal of Fisheries and Aquatic Sciences 63:2518–2530. doi: 10.1139/f06-138

Kinkel LL, Schlatter DC, Bakker MG, Arenz BE (2012) *Streptomyces* competition and co-evolution in relation to plant disease suppression. Research in Microbiology 163:490–499. doi: 10.1016/j.resmic.2012.07.005

Kolde R (2019) pheatmap: Pretty Heatmaps.

Kumari H, Gupta SK, Jindal S, et al (2009) *Sphingobium lactosutens* sp. nov., isolated from a hexachlorocyclohexane dump site and *Sphingobium abikonense* sp. nov., isolated from oil-contaminated soil. International Journal of Systematic and Evolutionary Microbiology 59:2291–2296. doi: 10.1099/ijs.0.004739-0

Lee Y-Y, Lee SY, Lee SD, Cho K-S (2022) Seasonal Dynamics of Bacterial Community Structure in Diesel Oil-Contaminated Soil Cultivated with Tall Fescue (Festuca arundinacea). International Journal of Environmental Research and Public Health 19:4629. doi: 10.3390/ijerph19084629

Legendre P (2014) Interpreting the replacement and richness difference components of beta diversity. Global Ecology and Biogeography 23:1324–1334. doi: 10.1111/geb.12207

Lemieux C, Quémerais B, Lum KR (1995) Seasonal patterns of atrazine loading for the St Lawrence River (Canada) and its tributaries. Water Research 29:1491–1504. doi: 10.1016/0043-1354(94)00307-s

Li K, Hu J, Li T, et al (2021) Microbial abundance and diversity investigations along rivers: Current knowledge and future directions. Wiley Interdisciplinary Reviews: Water. doi: 10.1002/wat2.1547

Lighthart B (1997) The ecology of bacteria in the alfresco atmosphere. FEMS Microbiology Ecology 23:263–274. doi: 10.1111/j.1574-6941.1997.tb00408.x

Lindh MV, Lefébure R, Degerman R, et al (2015) Consequences of increased terrestrial dissolved organic matter and temperature on bacterioplankton community composition during a Baltic Sea mesocosm experiment. AMBIO 44:402–412. doi: 10.1007/s13280-015-0659-3

Liu T, Zhang AN, Wang J, et al (2018) Integrated biogeography of planktonic and sedimentary bacterial communities in the Yangtze River. Microbiome 6:16. doi: 10.1186/s40168-017-0388-x

Liu W, Yang X, Jiang L, et al (2022) Partitioning of beta-diversity reveals distinct assembly mechanisms of plant and soil microbial communities in response to nitrogen enrichment. Ecology and Evolution 12:e9016. doi: 10.1002/ece3.9016

Martiny JBH, Bohannan BJM, Brown JH, et al (2006) Microbial biogeography: putting microorganisms on the map. Nature Reviews Microbiology 4:102–112. doi: 10.1038/nrmicro1341

Martiny JBH, Eisen JA, Penn K, et al (2011) Drivers of bacterial β-diversity depend on spatial scale. Proceedings of the National Academy of Sciences 108:7850–7854. doi: 10.1073/pnas.1016308108

McDaniel T, Pascoe T (2017) Applying the reference condition approach to Lake of the Woods: sediment and benthic invertebrate community assessment for lake-wide management. Lake and Reservoir Management 33:452–471. doi: 10.1080/10402381.2017.1379573

McMurdie PJ, Holmes S (2013) phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. PLoS ONE 4:e61217.

Monard C, Gantner S, Bertilsson S, et al (2016) Habitat generalists and specialists in microbial communities across a terrestrial-freshwater gradient. Scientific Reports 6:37719. doi: 10.1038/srep37719

Morris T (1994) Quaternary Geology of Essex County, Southwestern Ontario; Ontario Geological Survey: Open File Report 5886

Naka T, Fujiwara N, Yabuuchi E, et al (2000) A Novel Sphingoglycolipid Containing Galacturonic Acid and 2-Hydroxy Fatty Acid in Cellular Lipids of *Sphingomonas yanoikuyae*. Journal of Bacteriology 182:2660–2663. doi: 10.1128/jb.182.9.2660-2663.2000

Naseem S, Fu GL, Mohsin M, et al (2020) Semi-Quantitative Environmental Impact Assessment of Khewra Salt Mine of Pakistan: an Application of Mathematical Approach of Environmental Sustainability. Mining, Metallurgy & Exploration 37:1185–1196. doi: 10.1007/s42461-020-00214-9

Oksanen J, Simpson GL, Blanchet FG, et al (2022) vegan: Community Ecology Package.

Paulson J, Olson N, Braccia D, et al (2013) metagenomeSeq: Statistical analysis for sparse high-throughput sequncing.

Paver SF, Newton RJ, Coleman ML (2020) Microbial communities of the Laurentian Great Lakes reflect connectivity and local biogeochemistry. Environmental Microbiology 22:433–446. doi: 10.1111/1462-2920.14862

Procópio RE de L, Silva IR da, Martins MK, et al (2012) Antibiotics produced by *Streptomyces*. The Brazilian Journal of Infectious Diseases 16:466–471. doi: 10.1016/j.bjid.2012.08.014

Quast C, Pruesse E, Yilmaz P, et al (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Research 41:D590–D596. doi: 10.1093/nar/gks1219

Qiu Y-L, Kuang X, Shi X, et al (2014) *Terrimicrobium sacchariphilum* gen. nov., sp. nov., an anaerobic bacterium of the class '*Spartobacteria*' in the phylum *Verrucomicrobia*, isolated from a rice paddy field. International Journal of Systematic and Evolutionary Microbiology 64:1718–1723. doi: 10.1099/ijs.0.060244-0

Qu X-D, Yuan, H-L (2008*) Sediminibacterium salmoneum* gen. nov., sp. nov.,a member of the phylum *Bacteroidetes* isolated from sediment of a eutrophic reservoir. International Journal of Systematic and Evolutionary Microbiology 58: 2191-2194

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

Reece, Richardson (2000) Biomonitoring with the Reference Condition Approach for the Detection of Aquatic Ecosystems at Risk. Proceedings of the biology and management of species and habitats at risk. Vancouver: British Columbia, pp 15–19

Rodríguez J, Gallampois CMJ, Haglund P, et al (2021) Bacterial communities as indicators of environmental pollution by POPs in marine sediments. Environmental Pollution 268:115690. doi: 10.1016/j.envpol.2020.115690

Savio D, Sinclair L, Ijaz UZ, et al (2015) River bacterioplankton diversity. Environmental Microbiology 17:4994–5007. doi: 10.1111/1462-2920.12886

Shahraki AH, Chaganti SR, Heath D (2021) Spatio-temporal dynamics of bacterial communities in the shoreline of Laurentian great Lake Erie and Lake St. Clair's large freshwater ecosystems. BMC Microbiology 21:253. doi: 10.1186/s12866-021-02306-y

Shen M-C, Zhang Y-Z, Bo G-D, et al (2022) Microbial Responses to the Reduction of Chemical Fertilizers in the Rhizosphere Soil of Flue-Cured Tobacco. Frontiers in Bioengineering and Biotechnology 9:812316. doi: 10.3389/fbioe.2021.812316

Shupryt MP, Studinski JM (2021) Spatial correlation of macroinvertebrate assemblages in streams and the implications for bioassessment programs. Environmental Monitoring and Assessment 193:322. doi: 10.1007/s10661-021-09101-2

Simonin M, Voss KA, Hassett BA, et al (2019) In search of microbial indicator taxa: shifts in stream bacterial communities along an urbanization gradient. Environmental Microbiology 21:3653–3668. doi: 10.1111/1462-2920.14694

Small GE, Finlay JC, McKay RML, et al (2016) Large differences in potential denitrification and sediment microbial communities across the Laurentian great lakes. Biogeochemistry 128:353–368. doi: 10.1007/s10533-016-0212-x

Vannote RL, Minshall GW, Cummins KW, et al (1980) The River Continuum Concept. Canadian Journal of Fisheries and Aquatic Sciences 37:130–137. doi: 10.1139/f80-017

Veach AM, Troia MJ, Cregger MA (2021) Assessing biogeographic survey gaps in bacterial diversity knowledge: A global synthesis of freshwaters. Freshwater Biology 66:1595–1605. doi: 10.1111/fwb.13777

VanDrecht G, Bouwman AF, Harrison J, Knoop JM (2009) Global nitrogen and phosphate in urban wastewater for the period 1970 to 2050. Global Biogeochemical Cycles 23:n/a-n/a. doi: 10.1029/2009gb003458

Venail PA, Vives MJ (2013) Phylogenetic distance and species richness interactively affect the productivity of bacterial communities. Ecology 94:2529–2536. doi: 10.1890/12-2002.1

Vilmi A, Zhao W, Picazo F, et al (2020) Ecological processes underlying community assembly of aquatic bacteria and macroinvertebrates under contrasting climates on the Tibetan Plateau. Science of The Total Environment 702:134974. doi: 10.1016/j.scitotenv.2019.134974

Wang J, Soininen J, Zhang Y, et al (2012) Patterns of elevational beta diversity in micro- and macroorganisms. Global Ecology and Biogeography 21:743–750. doi: 10.1111/j.1466-8238.2011.00718.x

Wang X, Li W, Xiao Y, et al (2021) Abundance and diversity of carbon-fixing bacterial communities in karst wetland soil ecosystems. CATENA 204:105418. doi: 10.1016/j.catena.2021.105418

Watanabe T, Kojima H (2020) Bergey's Manual of Systematics of Archaea and Bacteria. 1–6. doi: 10.1002/9781118960608.gbm01704

Weiss JV, Rentz JA, Plaia T, et al (2007) Characterization of Neutrophilic Fe(II)-Oxidizing Bacteria Isolated from the Rhizosphere of Wetland Plants and Description of Ferritrophicum radicicola gen. nov. sp. nov., and Sideroxydans paludicola sp. nov. Geomicrobiology Journal 24:559–570. doi: 10.1080/01490450701670152

Wen Z-L, Yang M-K, Fazal A, et al (2020) Differential Impacts on Bacterial Composition and Abundance in Rhizosphere Compartments between Al-Tolerant and Al-Sensitive Soybean Genotypes in Acidic Soil. Journal of Microbiology and Biotechnology 30:1169–1179. doi: 10.4014/jmb.2003.03018

Wilson FP, Liu X, Mattes TE, Cupples AM (2016) *Nocardioides, Sediminibacterium, Aquabacterium, Variovorax*, and *Pseudomonas* linked to carbon uptake during aerobic vinyl chloride biodegradation. Environmental Science and Pollution Research 23:19062–19070. doi: 10.1007/s11356-016-7099-x

Winters AD, Marsh TL, Brenden TO, Faisal M (2014) Molecular characterization of bacterial communities associated with sediments in the Laurentian Great Lakes. Journal of Great Lakes Research 40:640–645. doi: 10.1016/j.jglr.2014.04.008

Wiseman WJ, Garvine RW (1994) Plumes and coastal currents near large river mouths. Estuaries 18:509. doi: 10.2307/1352368

Xie Y, Wang J, Wu Y, et al (2016) Using in situ bacterial communities to monitor contaminants in river sediments. Environmental Pollution 212:348–357. doi: 10.1016/j.envpol.2016.01.031

Xu C, Wang L, Cui Q, et al (2006) Neutrotolerant acidophilic *Streptomyces* species isolated from acidic soils in China: *Streptomyces guanduensis* sp. nov., *Streptomyces paucisporeus* sp. nov., *Streptomyces rubidus* sp. nov. and *Streptomyces yanglinensis* sp. nov. International Journal of Systematic and Evolutionary Microbiology 56:1109–1115. doi: 10.1099/ijs.0.63959-0

Xu X, Wang N, Lipson D, et al (2020) Microbial macroecology: In search of mechanisms governing microbial biogeographic patterns. Global Ecology and Biogeography 29:1870–1886. doi: 10.1111/geb.13162

Yannarell AC, Triplett EW (2004) Within- and between-Lake Variability in the Composition of Bacterioplankton Communities: Investigations Using Multiple Spatial Scales. Applied and Environmental Microbiology 70:214–223. doi: 10.1128/aem.70.1.214-223.2004

Young CC, Ho M-J, Arun AB, et al (2007) *Sphingobium* olei sp. nov., isolated from oil-contaminated soil. International Journal of Systematic and Evolutionary Microbiology 57:2613–2617. doi: 10.1099/ijs.0.65187-0

Yu D, Lu N, Fu B (2017) Establishment of a comprehensive indicator system for the assessment of biodiversity and ecosystem services. Landscape Ecology 32:1563–1579. doi: 10.1007/s10980-017-0549-0

Zamora-Ledezma C, Negrete-Bolagay D, Figueroa F, et al (2021) Heavy metal water pollution: A fresh look about hazards, novel and conventional remediation methods. Environmental Technology & Innovation 22:101504. doi: 10.1016/j.eti.2021.101504

Zhang T, Wang P (2022) The response of bacterial communities to organic matter in the surface sediment of the Pearl River estuary. Plankton and Benthos Research 17:45–56. doi: 10.3800/pbr.17.45

Zhou Z, Zheng M, Xia J, Wang C (2022) Nitrogen addition promotes soil microbial beta diversity and the stochastic assembly. Science of The Total Environment 806:150569. doi: 10.1016/j.scitotenv.2021.150569

Zeglin LH (2015) Stream microbial diversity in response to environmental changes: review and synthesis of existing research. Frontiers in Microbiology 6:454. doi: 10.3389/fmicb.2015.00454

CHAPTER 4

SYNTHESIS OF BENTHIC MACROINVERTEBRATE AND BACTERIA COMMUNITIES IN DETROIT RIVER WETLANDS

This thesis investigated community assemblages in each taxonomic group, benthic macroinvertebrates, and bacteria, to independently test the hypothesis that tributaries draining into Canadian waters of the Detroit River impact biological communities of coastal wetlands. This thesis was organized into two main objectives, the first examining inter-wetland differences by contrasting community assemblages in reference wetlands compared to tributary-impacted wetlands. The second objective examined intra-wetland differences within the tributary-impacted wetlands to identify if 1) community assemblages differed based on wetland position (strata) with respect to the tributary drainage point and 2) to identify individual stations from tributary-impacted wetland falling outside the reference wetland community condition determined by a multivariate reference approach.

The first objective and associated hypothesis was informed by previous assessments completed by government agencies who concluded that the water quality of wetlands receiving tributary inputs draining into the Detroit River are degraded (Environment Canada, 2017). Based on this information, I predicted that macroinvertebrate and bacteria assemblages would respond to these water quality differences such that unimpaired wetlands (DRM, Grass Island and Peche Island) would be similar to one another and differ from impaired wetlands - River Canard and Turkey Creek. Furthermore, River Canard was designated as the most degraded according to the Water Quality Index (WQI) and was therefore expected to differ the most from the unimpaired wetlands.

The second thesis objective was informed by the fact that the wetlands associated with River Canard and Turkey Creek have continuous macrophyte beds that extend both upstream and downstream of the tributary drainage point. If the tributaries were the major cause of community assemblage differences, I hypothesized that macroinvertebrate and bacteria composition at bed positions upstream of the tributary drainage point would differ from community assemblages present within the tributary plume and potentially downstream of the tributary plume. Two approaches were used to uncover community assemblage differences in Chapters 2 and 3. The first approach adopted various diversity indices along with multivariate ordination (NMDS) and multivariate hypothesis testing (PERMANOVA) to directly compare community composition differences at each wetland. Both wetland position in the community ordination space and pairwise comparisons of between wetland community composition differences were examined to determine if unimpaired wetlands (Peche Island, DRM and Grass Islands) bore similarities to one another and were distinct from the impaired wetlands associated with River Canard and Turkey Creek. This approach was repeated as part of hypothesis 2 testing to examine for differences in community composition between upstream, within plume and downstream strata at each of the two tributary-impacted wetlands. The first approach assumes that habitats within coastal wetlands of the Detroit River are relatively homogenous apart from water quality differences used to designate wetlands into reference and impacted groups.

The second approach adopted a multivariate reference approach which accounts for habitat diversity among the sampling sites tested. Sites were first subdivided into subsets of sites sharing similar habitat characteristics defined by site depth and sediment characteristics (sediment organic carbon content and grain sizes). Sites from reference wetlands of a given habitat type were combined and used to generate confidence intervals of the reference

community ordination space to which impaired sample sites were contrasted against and the proportion of sites falling outside of the reference was determined and their locations with respect to tributary position were considered. This approach considered within-wetland habitat heterogeneity by standardizing for habitat type prior to making community contrasts. This approach assumed that each reference wetland of a given habitat type are similar in community composition to one another. This assumption was necessary to establish sufficient statistical power to define the reference community condition.

In assessing the first objective and hypothesis 1, macroinvertebrate communities did not track wetland water quality status as predicted. Peche Island, which had the highest year-to-year average WQI ranking, differed in its macroinvertebrate community to the other reference wetlands of DRM and Grass Island, implying community differences across the unimpaired wetlands. Furthermore, Peche Island macroinvertebrate communities resembled the assemblages at the impaired wetlands of River Canard and Turkey Creek. For bacteria, the unimpaired wetlands had similar community compositions to one another, but qualitative differences were noted in the ordination position of Peche Island which bore greater overlap with Turkey Creek and River Canard. River Canard had similar bacterial community composition as the reference wetlands while Turkey Creek showed significant deviation. Overall, the first hypothesis is rejected for macroinvertebrate communities but partially supported for bacteria. In the case of bacteria, only 1 of 2 tributary impacted wetlands showed deviation from the reference. However, it is noted that Turkey Creek had better water quality scores compared to River Canard and therefore environmental stressors shaping macroinvertebrate and bacteria communities at Turkey Creek cannot be directly tied to differences in the water quality index reported at this location.

The second hypothesis predicted benthic macroinvertebrates and bacteria assemblages within tributary impacted wetlands would differ between strata such that assemblages upstream of the tributary input would differ from sites located within the tributary plume and potentially downstream of the plume. When adopting the first methodological approach to detect community differences between River Canard strata, no significant differences in community composition were detected for macroinvertebrates assemblages by PERMANOVA. Macroinvertebrate community ordination also showed no separation in community composition between strata at this wetland indicating a lack of support for hypothesis 2 for macroinvertebrate taxa. When the multivariate reference approach was applied at River Canard, 5 of 15 stations were identified as being altered in macroinvertebrate composition. Among these sites with altered macroinvertebrate communities, two were located within the River Canard tributary strata and 3 was downstream of the plume. Although the above trend supported the expected pattern, there were too few observations of sites with altered macroinvertebrate communities at River Canard tributary to substantiate a conclusion about hypothesis 2. The weight of evidence suggests that hypothesis 2 is rejected at River Canard for macroinvertebrates.

A slightly more nuanced but similar relationship emerged for bacteria in the first methodological approach. PERMANOVA indicated no significant differences in bacteria assemblages between strata at River Canard. However, bacteria community ordinations were consistent with expectations from hypothesis 2. The NMDS ordination of bacterial communities demonstrated differences in position of ordination between the upstream and tributary plume and intermediate positioning of the sites located downstream of the tributary plume. When the multivariate reference approach was applied at River Canard, 9 of 15 stations were identified as altered in bacteria composition compared to reference. The locations of altered bacteria sites

were distributed across the strata with 3/5 upstream strata, 4/5 in the tributary plume and 2/5 in the downstream strata. The tributary differs most significantly from reference wetlands, yet the upstream portion had poorly reflected river communities compared with the downstream. Thus, despite the partial support for hypothesis 2 arising from the NMDS ordination used in approach 1, neither PERMANOVA nor multivariate reference analysis supported hypothesis 2 for bacteria. Therefore hypothesis 2 is also rejected at River Canard for bacteria assemblages.

At Turkey Creek, PERMANOVA indicated that benthic macroinvertebrate and bacteria assemblages were similar in the upstream and downstream strata but significantly differed from the overall assemblage measured within tributary plume sites. Community ordinations reinforced this with similar community composition in the upstream and downstream strata that were distinct from sites located in the tributary plume, however bacteria had shown downstream communities in an intermediate position. According to the multivariate reference approach, macroinvertebrates differed at 6/15 stations from the reference condition. These stations were equally distributed in upstream, downstream and plume strata (2 stations each per strata) and thus not generally supportive of hypothesis 2. For bacteria, 10/14 stations had altered bacteria assemblages relative to the reference, with the majority of these (7/10) observed in sites within the plume strata. The remaining three in the upstream had reduced diversity metrics that suggest alteration from the reference conditions. All the tributary locations differed from reference wetlands, the upstream appears more altered compared to reference (3/4) than the downstream (2/5). Hypothesis two is not accepted through the MRA approach. Overall, evidence lines related to macroinvertebrates (PERMANOVA and multivariate ordinations) were mostly not supportive of hypothesis 2 at Turkey Creek. Bacteria was not supported in pairwise PERMANOVA, but supported by NMDS, and MRA approach. Therefore

hypothesis 2 is rejected for bacteria assemblages at the Turkey Creek wetland reinforcing the altered community composition concluded in hypothesis 1.

Across evidence lines examined, bacteria appeared to be more sensitive indicators of tributary influences altering biological structure in the coastal wetlands of the Detroit River. There was partial support for altered bacteria composition at Turkey Creek tested in hypothesis 1 and a larger number of evidence lines supporting altered bacteria community composition differences between Turkey Creek strata compared to macroinvertebrates. In addition, the multivariate reference approach identified a larger number of stations with altered bacteria compared to reference at both Turkey Creek and River Canard wetlands.

In both communities, Peche Island communities resembled one or both impaired wetlands. In the habitat characterization, Peche Island resembled the impaired wetlands in terms of grain-size distributions. Grain-size distributions were a prominent influence on macroinvertebrate assemblages along with depth in an RDA ordination. Lake St. Pierre, a lake within the St. Lawrence River was sampled for benthic macroinvertebrates across three years to test how hydrological regimes, landscape effects, and modifying local environments contribute to variations seen in benthic assemblages by Tall et al. (2016). Researchers selected diverse emergent vegetation habitats with considerations similar to features of the Detroit River, they considered flow and erosion differences around islands and anthropogenic nutrient sources in tributaries, particularly agriculture. They found that sites become more stable from wave energy as depths increase over a meter, which many Detroit River sites exceeded. They found water depth, water level change, and sediment characteristics (silts, nitrogen) to be significant influences on community compositions in the lake. In a second, multi-year study into coastal wetlands of Lake Huron, researchers also supported that macroinvertebrate communities are

primarily shaped by flooding regimes of changing elevation and depth and these factors were more significant than wetland-vegetation type (Gathman and Burton 2011). While habitat characteristics are influential on community differences, analyses of biodiversity metrics show some impairment at Peche Island (reduced EPT) and impaired wetlands had significantly different species richness, evenness, Shannon diversity and EPT% taxa, indicating impaired biodiversity at these wetlands. On the basis of alpha-diversity and EPT taxa at Turkey Creek strata, the tributary is providing more suitable conditions in supporting biodiversity than the river wetland and compared against upstream and downstream strata surrounding the tributary plume. The upstream portions of Turkey Creek receive waters from an upstream wastewater treatment plant and salt mine, details regarding potential impairments from these activities were discussed in Chapter 2 and 3 and the impact of such stressors on macroinvertebrate communities are further described in McDaniel and Pascoe (2017) and Pinter et al. (2022). Both upstream and downstream strata showed similarities to one another, distinct from the tributary plume, suggesting that upstream and downstream flow linkages generate connectivity in impaired community composition. At Turkey Creek, the tributary itself may not be the cause of stress given its sensitive indicator species presence, whereas activities at the upstream location subject to mining and wastewater treatment effluent inputs may be of relevant and contributing to environmental stress.

Bacteria communities at River Canard showed more variation than macroinvertebrates as per the MRA analyses. In agricultural tributaries, such as River Canard, tributaries introduce excess nutrients and organic matter into adjoining waterbodies that are more pronounced in flooding events (Junk et al 1989; Amoros and Bornette 2002). Agricultural activities are well established to introduce excess nutrients such as nitrogen and phosphorus to aquatic systems, and the magnitude of nutrients is dependent on timing of agricultural activities and high flow

periods where nutrients settle and are retained in sediments (DeBruyn et al 2004; Uwimana et al 2018; Rixon et al 2020). Legacy nutrients retained in sediments effects water quality for decades which can make bacteria communities indicators of long-term water and sediment quality (Hamilton 2012; Zhang et al 2020).

For this discussion, an RDA of habitat influences on bacteria community compositions was explored to demonstrate how bacteria comparatively respond to water quality and sediment characteristics (*as per 2.2.4.3: Community Ordinations*). **Figure 4.1** provides a triplot of the RDA for bacteria assemblages across the wetland strata. Based on **Figure 4.1**, depth, conductivity, sediment TOC and turbidity were much more influential compared to grain-size distributions. Bacteria community compositions associated more robustly with chemical parameters compared to benthic macroinvertebrate communities which classically associate strongly with grain size (**Figure 2.12**). As indicated in the description of the study site and sample period, water levels in the Great Lakes were higher than what is considered typical in the region. Consider that both macroinvertebrates and bacteria communities show significant correspondence to depth, careful consideration should be made when comparing community compositions in this study to other surveys. While species distributions were likely affected by changes associated with depth, the preclusion of species by depth changes is expected to be mostly uniform across the river given the uniform slope of the river provides consistent flow rates whose depths are dependent on water levels between Lake Huron and Lake Erie. Rising water levels in the Great Lakes are expected to increase under climatic changes (Seglenieks and Temgoua, 2022) and this study can provide utility to community composition comparisons to lower water level years.

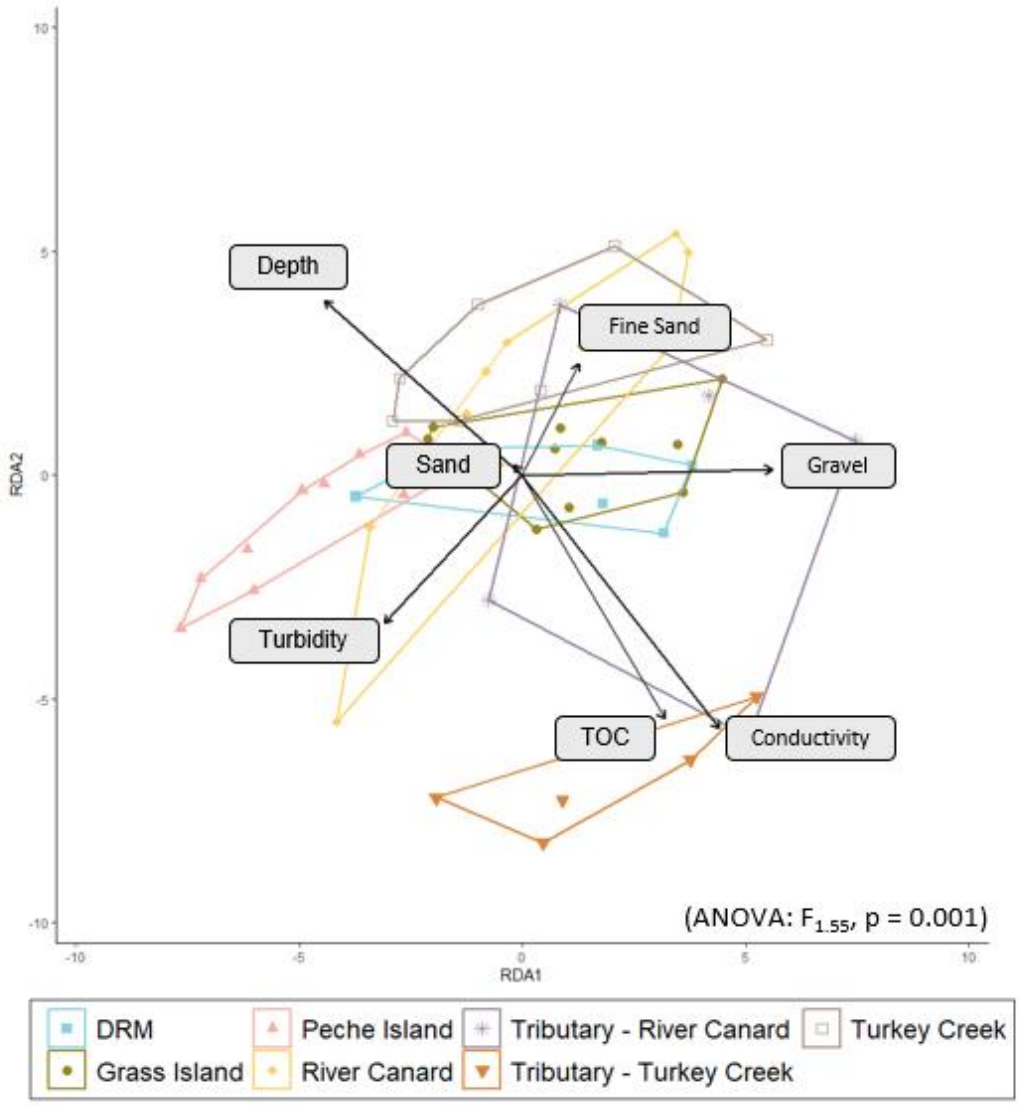


Figure 4.1: RDA of bacteria community assemblages reveal clustering by sample site and significant associations with key environmental variables. TOC = total organic carbon.

Peche Island is the furthest upstream wetland and receives water coming directly from Lake St. Clair, which is in a heavily agricultural watershed of crops and pastures (Metcalfe et al 2019). Long-term datasets on Peche Island show a propensity for ammonia and phosphorus concentrations that exceed concentrations present at the unimpaired wetlands (DRM and Grass Island) and are more comparable to River Canard (Environment Canada 2017). Bacteria communities at Peche Island tended to resemble River Canard and may be indicative of nutrient impacts on bacteria community compositions at Peche. Despite the higher nutrients at Peche, turbidity and conductivity, key determinants of the water quality score, suggest minimal impairment. Together these observations suggest that the water quality score is not indicative of a stressor to either macroinvertebrates or bacteria communities. The WQI employed by ECCC utilizes limited components of water quality, pH, Temperature, conductivity and turbidity calibrated to the Great Lakes region (Chow-Fraser, 2006). The divergence of both benthic communities from qualitative descriptions of water quality suggests that water quality is not representative of community responses. ECCC takes surveys of biota in the Detroit River AOC that may be sufficient toward their broad conservation objectives. However, if there was interest in inferring biological responses to water quality, the WQI may benefit from expanding into parameters that directly affect food webs such as nutrients. The similarities of Peche and River Canard communities in community compositions and nutrient concentrations may support utility in incorporating nutrients or generating an index focused on nutrients. Providing water quality assessments that incorporate nutrients are applied in other water quality assessments that are concerned with public health measures as excess nutrients can affect drinking water sources (Trebitz et al., 2007; TSRSPC, 2020).

Turkey Creek wetland communities were distinct from all wetlands and four impacted sample sites were isolated as being significantly different than reference wetlands. Significantly different taxa associated with these impacted sample sites belong to the phyla *Proteobacteria, Bacteroidetes and Verrucomicrobia* and are predominately involved in nitrogen and sulfur cycling. *Bacteroidetes* was the fourth most abundant phyla at Turkey Creek. The literature supports the observation that rivers undergoing rapid urbanization generate bacteria community compositions increases in *Bacteroidetes* abundances along urbanization gradients (Li et al 2019). The significantly different *Bacteroidetes* in this study was of the family *Chitinophagaceae*, a heterotrophic nitrifier of ammonia. Heterotrophic nitrification does not appear to contribute to cell growth, but it remains important to nitrogen cycling in soils, particularly acidic soils that inhibit competition from autotrophic nitrifiers (Hayatsu et al 2008).

Turkey Creek had shown indication in community impairment and had significantly varied from every wetland for bacteria communities and similarly for macroinvertebrate communities having only resembled Peche Island. Given these divergences of community patterns with respects to water quality, particularly evidenced by Peche Island communities, I suggest that future research should examine sediment chemistry with respects to nutrient inputs coupled to analysis of gene expression by transcriptomic analysis to determine whether significantly different functioning is occurring at Turkey Creek wetland. In exploration of transcription in the Detroit River by level of sediment contamination, Falk et al., (2019) found that genes associated with nitrate reduction and methanogenesis were significantly expressed by differential abundance methods. They created sediment contaminant profiles of metals, PAHs, PCB congeners, and organochlorine pesticides in the lower reach of the Detroit River, which didn't include sample sites in the Turkey Creek river wetland. However, sites included in this study are within DRM and Grass Island, located downstream of Turkey Creek. Highlighted in

this study is a significantly expressed rate of nitrate reduction and methanogens that may be associated with the breakdown of hydrocarbons as total nitrogen in sediments was low in the area based on the findings of Colbourne et al. (2019). However, Colbourne et al., found that isotopic nitrate steadily declined from the upper reach to lower reach but was greatest at transects up to Turkey Creek; not excluding that excess total nitrogen in Turkey Creek altering community assemblages.

Between wetlands, macroinvertebrate and bacteria communities had shown differences in community patterns between wetlands. For macroinvertebrates, more wetland communities had significantly varied and beta-diversity was mostly represented by turnover. The opposite observations were made for bacteria communities, whose communities were mostly nested and mostly similar across the wetlands. Community compositions can be explained by the relative importance of two major processes used to explain variation in community compositions; deterministic processes, based on niche-partitioning and responses to environmental constraints and biological interactions or stochastic processes that are independent of ecological niches and consider neutral, random events such as chance colonization, dispersal capacity, ecological drift (Hubbell 2001; Clark 2009; Vellend 2010). Determining the degree to which stochastic events are explanatory to community composition remains unclear, while deterministic factors can be manipulated and measured, the challenge in assigning relative importance of stochastic and determinate factors is identifying all the abiotic and biotic factors (Vellend et al 2014; Zhang et al 2019). Currently, evidence supports that both processes explain variances in community diversity (Dini-Andreote et al 2015) that can vary by spatial scale, ecosystem type, and taxonomic group (Logue et al 2011; He et al 2020). Vilmi et al., (2020), studying community patterns between bacteria and macroinvertebrates found that in harsh environments, both communities are more shaped by deterministic processes, whereas in more

benign habitats, stochastic processes tend to dominate. The degree of stochastic to deterministic processes in wetlands is dependent on whether the wetland is permanent in the landscape or ephemeral in nature, deterministic processes are more prevalent in the former and cause significant constraints on community assemblages (Daniel et al 2019). Body size is one contributing factor to predicting deterministic and stochastic influences on community assembly, macroinvertebrate communities are less influenced by stochastic events than smaller-bodied organisms such as zooplankton and microbes (Farjalla et al 2012). Microorganisms are regulated by stochastic processes but associate more strongly with climate and water quality than larger bodied organisms such as macroinvertebrates (Soininen et al 2013). However, analysis into drivers of community composition at finer scales show stochastic events having a more predominate role in larger bodied organisms (Zinger et al 2019). The degree of stochastic to deterministic processes in community arrangements were not monitored in this study. However, they can confound the effectiveness of restoration efforts in AOCs.

This study had made two important observations stemming from results gathered at Turkey Creek. Tributary communities were significantly different from the river wetland communities and that wetland communities appear to be impaired whereas tributary communities resemble non-impaired wetland conditions. A major distinction in the protocol of this thesis is sample site selection. ECCC and other government monitoring programs in the Great Lakes region adapt an approach based on vegetation type and often return to similar locations (Environment Canada, 2017; GLCWMP, 2018). Our study used a randomization process that included areas with varying vegetation, including submerged aquatic beds. In our inclusion of the submerged wetlands, we were able to detect impairment in portions of the Turkey Creek wetland that are excluded in the government monitoring program. It is suggested that protocols employ a random stratified sampling procedure to select additional wetland locations

and use ponar collections in the event a site is non-wadable. It should be noted that ECCC's sampling protocol of the Turkey Creek tributary is effective in post-restoration monitoring as upstream portions of the Turkey Creek tributary had contaminated sediments removed in 2008. This effort was targeted to reduce sediment contamination and the risk of mobilization of contaminated sediments into the Detroit River during flood events. Our study supports that remediation may have been effective considering the differences in community assemblages and their quality between the tributary and wetland. Validating the effectiveness of the sediment removal on benthic macroinvertebrate communities near the river would require data on community compositions prior to 2008 and whether the Turkey Creek-Grand Marias drain was contributing to downstream stress at the river junction, which was not the scope of our study. Since it is hypothesized that the upstream wastewater treatment plant may be responsible for the impairment of benthic community diversity, wetland restoration with vegetation near the WWTP may improve conditions in the downstream waters while mitigating the risks of flooding events and can improve water quality (Mitsch and Wang, 2000). The WWTP effluent passes through a small stream before entering the Detroit River, wetland plants can retain nutrients before entering the river system (Kao et al., 2003).

The findings of thesis also suggest that benthic macroinvertebrates and sediment bacteria communities can be jointly interpreted as indicators of environmental stressors in aquatic systems. The findings suggest that between the two taxa, bacteria assemblages appear to be more sensitive indicators of chemical stressors since they were able to detect many of the macroinvertebrate MRA sites and a few more additional sites at the River Canard wetland-tributary system but had resembled macroinvertebrate sample sites at Turkey Creek, suggesting that bacteria sensitivity may vary by differences in stressor. The greater sensitivity may be due to the much larger difference in the magnitude of species richness of bacteria compared to

macroinvertebrates. In addition, bacteria appear to have a much higher degree of interspecific interactions involved nutrient and carbon cycling. Unique species in the wetlands were primarily involved in carbon, nitrogen and sulfur cycling. This greater degree of between taxa connectivity may make the assemblage more responsive to a wide array of individual stressors. Monitoring programs would benefit from the addition of bacteria community analyses.

In New Zealand, Lau et al., (2015) developed a bacterial community index (BCI) to assess the conditions of streams in different catchment land-use areas across several regions and had contrasted their BCI model to a macroinvertebrate-based index. The model was generated with regression methods against the well-utilized macroinvertebrate model. They had found that macroinvertebrates were better predictors of surrounding land-use and that regions had more of an effect on bacteria community compositions, but the BCI was still able detect habitat quality, but refinement was needed to distinguish sites of intermediate stress. The generation of this model shows a promising pathway toward the application of bacteria communities into biomonitoring programs and the authors suggest building indices independent of the macroinvertebrate model based on environmental data. As acknowledged by researchers, the development of such a model requires long-term data sets and a more complete understanding of ecological contributions of bacteria taxa.

A model that factors species contributions and tolerances would be the most ideal tool in using bacteria communities in aquatic ecosystems due to the validation processes embedded in growing researching and mathematical models. However, a multivariate reference approach, sampling reference conditions alongside potentially impaired locations is more readily applicable to bacteria communities which show significant geographical patterns and can differ within watersheds from connectivity to landscape communities (Besemer et al 2012; Sun et al

2021). Such an approach is less contingent on extensive background research for the region in question compared to biotic integrity indices. A disadvantage is the robustness of the interpretation will require supplementary information and can only indicate potential sites of impairment. It would benefit from validation from a second taxonomic group, such as macroinvertebrates. An advantage to bacteria communities is that they can be early indicators of stress before cascading into higher trophic levels (Sarmento et al 2010; Cronin-O'Reilly et al 2018).

The Great Lakes provide a unique global habitat for freshwater research where the GLWQA allows for a cohesive management between Canada and the United States and could provide a working framework for region-based strategies in implementing bacteria communities to biomonitoring. Two key commitments of Annex 7 of the GLWQA, is to "increase awareness of native species and habitat and the methods to protect, conserve, maintain, restore and enhance their resilience" and "Conduct research and monitoring as needed to implement prevention measures that consider the impacts of climate change and other stressors and improve the resilience of native species and habitat" (GLWQA 2012). Currently, the GLWQA does not consider microbial communities within its monitoring mandates. However, considering their importance to ecosystem functioning (Lynch et al 2023) understanding these communities is critical to maintaining biodiversity in the Great Lakes. Despite current limitations into the interpretation of assemblages, a database can help aid future development of bacteria indices in the Great Lakes region and understand baseline biogeography patterns in the area. This research presents a step in the direction of forming such a database.

This thesis focused on the community composition of two taxonomic groups in the coastal wetlands of a connecting channel. While aspects of the Detroit River such as short

residency time of water and lack of influence to the watershed are features that separate it from other aquatic systems, there are broad applications to other systems. Macroinvertebrate and sediment bacteria communities live within the similar space in benthic habitats, under similar chemical and physical pressures, yet they had shown opposing beta-diversity metrics and primary influences on community compositions such that interactions with the physical environment are more influential for macroinvertebrates, likely due to differences in selection pressures from body morphology. This study is the first characterization of coastal wetland bacteria communities in the Detroit River. Coastal wetlands are unique habitats that transition terrestrial and aquatic systems supporting biodiversity and biogeochemical cycles. The Detroit River coastal wetlands are a highly fragmented system due to dredged shipping channels and heavily modified shorelines, leaving each wetland disconnected with varying physical and chemical characteristics. We had observed that macroinvertebrate communities varying most significantly across the river yet bacteria communities were mostly consistent, illustrating connectivity of bacteria communities in the river despite fragmentation of habitats. Additionally, we had examined both of these communities at tributary junctions, where fluvial processes involving sediment depositions and chemical gradients are likely to affect benthic communities yet do not get represented in biomonitoring by government agency. While we had not found a gradient in communities from the tributaries into the Detroit River, we had found different responses to tributary inputs at both wetlands such that river wetland and tributary communities can be homogenized or less-fragmented, as seen at River Canard or they can show significant dissimilarities as seen in Turkey Creek. These findings are critical to show that both community types can show abrupt changes along these junctions.

REFERENCES

Amoros C, Bornette G (2002) Connectivity and biocomplexity in waterbodies of riverine floodplains. Freshwater Biology 47:761–776. doi: 10.1046/j.1365-2427.2002.00905.x

Chow-Fraser (2006) Development of the Water Quality Index (WQI) to assess effects of basin-wide land-use alteration on coastal marshes of the Laurentian Great Lakes In Simon, T. P., & P. M. Stewart (eds), Coastal Wetlands of the Laurentian Great Lakes: Health, Habitat, and indicators. Indiana Biological Survey, Bloomington, Indiana, pp 137–166

Clark JS (2009) Beyond neutral science. Trends in Ecology & Evolution 24:8–15. doi: 10.1016/j.tree.2008.09.004

Colborne SF, Maguire TJ, Mayer B, et al. (2019) Water and Sediment as sources of phosphate in aquatic ecosystems: The Detroit River and its role in the Laurentian Great Lakes. Science of the Total Environment 647:1594-1603.

Cronin-O'Reilly S, Taylor JD, Jermyn I, et al (2018) Limited congruence exhibited across microbial, meiofaunal and macrofaunal benthic assemblages in a heterogeneous coastal environment. Scientific Reports 8:15500. doi: 10.1038/s41598-018-33799-9

Daniel J, Gleason JE, Cottenie K, Rooney RC (2019) Stochastic and deterministic processes drive wetland community assembly across a gradient of environmental filtering. Oikos 128:1158–1169. doi: 10.1111/oik.05987

DeBruyn JM, Leigh-Bell JA, McKay RML, et al (2004) Microbial Distributions and the Impact of Phosphorus on Bacterial Activity in Lake Erie. Journal of Great Lakes Research 30:166–183. doi: 10.1016/s0380-1330(04)70338-x

Dini-Andreote F, Stegen JC, Elsas JD van, Salles JF (2015) Disentangling mechanisms that mediate the balance between stochastic and deterministic processes in microbial succession. Proceedings of the National Academy of Sciences 112:E1326–E1332. doi: 10.1073/pnas.1414261112

Environment Canada (2017) Detroit River Area of Concern: Coastal Wetland Habitat Assessment Report 2016 Update. CWS-ON, Downsview, Ontario

Falk N, Reid T, Skoyles A, et al (2019) Microbial metatranscriptomic investigations across contaminant gradients of the Detroit River. Science of The Total Environment 690:121–131. doi: 10.1016/j.scitotenv.2019.06.451

Farjalla VF, Srivastava DS, Marino NAC, et al (2012) Ecological determinism increases with organism size. Ecology 93:1752–1759. doi: 10.1890/11-1144.1

Gathman JP, Burton TM (2011) A Great Lakes Coastal Wetland Invertebrate Community Gradient: Relative Influence of Flooding Regime and Vegetation Zonation. Wetlands 31:329–341. doi: 10.1007/s13157-010-0140-9

GLCWMP (Great Lakes Coastal Wetland Monitoring Program) (2018) Standard operating procedure: macroinvertebrate sampling and laboratory processing.

GLWQA (2012) Great Lakes Water Quality Agreement: Protocol Amending the Agreement Between Canada and the United States of America on Great Lakes Water Quality, 1978, as Amended on October 16, 1983, and on November 18, 1987.

Hamilton SK (2012) Biogeochemical time lags may delay responses of streams to ecological restoration. Freshwater Biology 57:43–57. doi: 10.1111/j.1365-2427.2011.02685.x

Hayatsu M, Tago K, Saito M (2008) Various players in the nitrogen cycle: Diversity and functions of the microorganisms involved in nitrification and denitrification. Soil Science & Plant Nutrition 54:33–45. doi: 10.1111/j.1747-0765.2007.00195.x

He S, Soininen J, Chen K, Wang B (2020) Environmental Factors Override Dispersal-Related Factors in Shaping Diatom and Macroinvertebrate Communities Within Stream Networks in China. Frontiers in Ecology and Evolution 8:141. doi: 10.3389/fevo.2020.00141

Hubbell S (2001) The unified neutral theory of biodiversity and biogeography. Princeton University Press, Princeton, NJ

Junk, Bayley, Sparks (1989) The flood pulse concept in river-floodplain systems. Canadian special publication of fisheries and aquatic sciences 1:110–127.

Kao J, Titus J, Zhu W-X (2003) Differential nitrogen and phosphorus retention by five wetland plant species. Wetlands 23(4):979-987.

Lau KEM, Washington VJ, Fan V, et al (2015) A novel bacterial community index to assess stream ecological health. Freshwater Biology 60:1988–2002. doi: 10.1111/fwb.12625

Li Y, Fan L, Zhang W, et al (2019) How did the bacterial community respond to the level of urbanization along the Yangtze River? Environmental Science: Processes & Impacts 22:161–172. doi: 10.1039/c9em00399a

Logue JB, Mouquet N, Peter H, et al (2011) Empirical approaches to metacommunities: a review and comparison with theory. Trends in Ecology & Evolution 26:482–491. doi: 10.1016/j.tree.2011.04.009

Lynch AJ, Cooke SJ, Arthington AH, et al (2023) People need freshwater biodiversity. Wiley Interdisciplinary Reviews: Water. doi: 10.1002/wat2.1633

McDaniel T, Pascoe T (2017) Applying the reference condition approach to Lake of the Woods: sediment and benthic invertebrate community assessment for lake-wide management. Lake and Reservoir Management 33:452–471. doi: 10.1080/10402381.2017.1379573

Metcalfe CD, Helm P, Paterson G, et al (2019) Pesticides related to land use in watersheds of the Great Lakes basin. Science of The Total Environment 648:681–692. doi: 10.1016/j.scitotenv.2018.08.169

Mitsch WJ, Wang N (2002) Large-scale coastal wetland restoration on the Laurentian Great Lakes: determining the potential for water quality improvement. Ecological Engineering 15(3-4):267-282. doi: 10.1016/S0925-8574(00)00081-1

Pinter J, Jones BS, Vriens B (2022) Loads and elimination of trace elements in wastewater in the Great Lakes basin. Water Research 209:117949. doi: 10.1016/j.watres.2021.117949

Rixon S, Levison J, Binns A, Persaud E (2020) Spatiotemporal variations of nitrogen and phosphorus in a clay plain hydrological system in the Great Lakes Basin. Science of The Total Environment 714:136328. doi: 10.1016/j.scitotenv.2019.136328

Sarmento H, Montoya JM, Vázquez-Domínguez E, et al (2010) Warming effects on marine microbial food web processes: how far can we go when it comes to predictions? Philosophical Transactions of the Royal Society B: Biological Sciences 365:2137–2149. doi: 10.1098/rstb.2010.0045

Seglenieks F, Temgoua A (2022) Future water levels of the Great Lakes under 1.5°C to 3°C warmer climates. Journal of Great Lakes Research. 48(4):865-875.

Soininen J, Korhonen JJ, Luoto M (2013) Stochastic species distributions are driven by organism size. Ecology 94:660–670. doi: 10.1890/12-0777.1

Tall L, Armellin A, Pinel-Alloul B, et al (2016) Effects of hydrological regime, landscape features, and environment on macroinvertebrates in St. Lawrence River wetlands. Hydrobiologia 778:221–241. doi: 10.1007/s10750-015-2531-7

Trebitz A, Brazner J, Cotter A, et al (2007) Water quality in Great Lakes coastal wetlands: basin-wide patterns and responses to an anthropogenic disturbance gradient. Journal of Great Lakes Research 33(sp3):67-85

TSRSPC (Thames - Sydenham and Region Source Protection Committee) (2020) Lower Thames Valley Source Protection Area: Updated Assessment Report. Version 2.5 Amendments initiated under s36 of the CWA.

Uwimana A, Dam AA van, Gettel GM, Irvine K (2018) Effects of agricultural land use on sediment and nutrient retention in valley-bottom wetlands of Migina catchment, southern Rwanda. Journal of Environmental Management 219:103–114. doi: 10.1016/j.jenvman.2018.04.094

Vellend M (2010) Conceptual Synthesis in Community Ecology. The Quarterly Review of Biology 85:183–206. doi: 10.1086/652373

Vellend M, Srivastava DS, Anderson KM, et al (2014) Assessing the relative importance of neutral stochasticity in ecological communities. Oikos 123:1420–1430. doi: 10.1111/oik.01493

Vilmi A, Zhao W, Picazo F, et al (2020) Ecological processes underlying community assembly of aquatic bacteria and macroinvertebrates under contrasting climates on the Tibetan Plateau. Science of The Total Environment 702:134974. doi: 10.1016/j.scitotenv.2019.134974

Zhang W, Lei M, Li Y, et al (2019) Determination of vertical and horizontal assemblage drivers of bacterial community in a heavily polluted urban river. Water Research 161:98–107. doi: 10.1016/j.watres.2019.05.107

Zhang F, Zhang H, Yuan Y, et al (2020) Different response of bacterial community to the changes of nutrients and pollutants in sediments from an urban river network. Frontiers of Environmental Science & Engineering 14:28. doi: 10.1007/s11783-019-1207-3

Zinger L, Taberlet P, Schimann H, et al (2019) Body size determines soil community assembly in a tropical forest. Molecular Ecology 28:528–543. doi: 10.1111/mec.14919

APPENDICES

Appendix A

Appendix A-1: Habitat PCA loadings, eigenvalues, and percent variance

	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7	PC 8	PC 9	PC 10
Conductivity	0.449	-0.132	0.200	0.097	0.100	-0.441	0.391	0.312	0.527	0.000
Temperature	0.518	0.040	0.132	0.087	-0.039	0.154	0.355	0.171	-0.725	0.000
pH	0.403	-0.051	-0.103	-0.021	0.186	0.770	0.041	-0.209	0.388	0.000
Turbidity	-0.161	-0.076	0.679	0.056	0.683	0.033	-0.115	-0.113	-0.101	0.000
Depth	0.034	-0.205	-0.610	0.498	0.523	-0.187	-0.005	-0.100	-0.132	0.000
GRAVEL	0.241	0.445	0.048	0.329	0.006	0.038	-0.607	0.428	0.056	0.280
SAND	0.129	0.541	-0.109	-0.299	0.197	-0.231	0.199	-0.434	0.001	0.519
FINE SAND	0.088	-0.610	0.124	0.090	-0.239	-0.023	-0.161	-0.169	-0.047	0.694
SILT	-0.473	0.044	-0.104	0.001	0.150	0.303	0.431	0.539	0.040	0.413
TOC	-0.187	0.255	0.239	0.726	-0.307	0.078	0.295	-0.343	0.107	0.000
Eigen-value	3.144	2.359	1.062	0.958	0.857	0.696	0.477	0.371	0.075	0.000
% variance	31.441	23.586	10.616	9.584	8.569	6.964	4.775	3.714	0.752	0.000

Appendix A-2: Pairwise PERMANOVA of habitat characteristics by wetlands

PECHE		0.1545	0.0298	0.5321	0.2095
DRM	0.1545		0.3949	0.1904	0.8987
GRASS	0.0298	0.3949		0.0756	0.4902
RIVER CANARD	0.5321	0.1904	0.0756		0.2054
TURKEY CREEK	0.2095	0.8987	0.4902	0.2054	

Appendix A-3: Raw data: habitat characteristics and cluster assignments

	Depth	TOC	GRAVEL	SAND	SAND (FINE)	SILT/CLAY	CLUSTER (Macroinvertebrate – Bacteria)
PI-02	1.369	1.241	0.040	0.459	0.277	0.225	B – B
PI-06	1.463	0.778	0.000	0.253	0.414	0.332	A – A
PI-07	1.245	1.011	0.011	0.294	0.466	0.229	B – B
PI-08	1.413	1.072	0.007	0.357	0.337	0.299	A – A
PI-09	1.362	0.720	0.015	0.487	0.381	0.117	A – A
PI-12	1.279	1.073	0.008	0.342	0.330	0.320	B – B
PI-15	1.522	0.923	0.002	0.343	0.272	0.383	A – A
PI-16	1.008	0.850	0.003	0.257	0.282	0.458	B– B
PI-17	1.218	0.751	0.002	0.276	0.300	0.422	B – B
PI-18	3.193	1.239	0.002	0.387	0.265	0.346	A – A
DRM-02	1.266	0.973	0.306	0.475	0.131	0.089	B– B
DRM-03	1.430	0.787	0.165	0.564	0.163	0.109	A – A
DRM-04	0.868	0.391	0.002	0.403	0.549	0.047	B – B
DRM-06	1.566	-0.043	0.000	0.465	0.387	0.149	A – A
DRM-09	1.681	0.845	0.210	0.379	0.284	0.126	A – N/A
DRM-11	0.781	0.639	0.049	0.619	0.229	0.103	B – N/A
DRM-13	1.083	0.837	0.006	0.386	0.376	0.231	B – B
DRM-19	0.849	0.935	0.131	0.591	0.252	0.026	B – B
DRM-21	0.769	0.931	0.007	0.205	0.575	0.213	B – B
DRM-24	1.024	0.867	0.002	0.101	0.714	0.184	B – N/A
GI-01	0.718	2.435	0.127	0.421	0.293	0.160	B – B
GI-03	0.707	0.915	0.101	0.452	0.307	0.139	B – B
GI-04	0.792	0.989	0.057	0.549	0.250	0.143	B – B
GI-06	0.758	1.277	0.052	0.488	0.259	0.202	B – B
GI-09	1.135	1.552	0.186	0.403	0.228	0.183	B – B
GI-11	1.258	0.922	0.069	0.521	0.233	0.178	B – B
GI-13	1.053	0.798	0.017	0.539	0.215	0.229	B – B
GI-19	1.014	0.891	0.078	0.557	0.204	0.162	B– B
GI-21	1.410	0.977	0.131	0.567	0.174	0.128	A – A
GI-24	1.502	0.942	0.078	0.536	0.220	0.166	A – A
RC-01	0.601	0.430	0.000	0.161	0.813	0.026	B– B
RC-02	1.031	1.643	0.000	0.219	0.435	0.345	B – B
RC-04	1.214	0.903	0.030	0.444	0.307	0.220	B – B
RC-08	1.807	1.155	0.110	0.487	0.224	0.179	A – A
RC-10	1.805	1.021	0.024	0.273	0.592	0.110	A – A
RC-13	1.625	0.950	0.003	0.356	0.505	0.137	A – A
RC-18	1.906	1.033	0.020	0.252	0.625	0.103	A – A

	Depth	TOC	GRAVEL	SAND	SAND (FINE)	SILT/CLAY	CLUSTER (Macroinvertebrate – Bacteria)
RC-19	1.780	0.825	0.018	0.332	0.559	0.091	A – A
RC-21	1.896	0.878	0.001	0.156	0.724	0.119	A – A
RC-26	2.145	0.685	0.004	0.207	0.713	0.075	A – A
TRC-01	1.446	0.844	0.007	0.236	0.743	0.015	A – A
TRC-02	0.823	0.394	0.021	0.222	0.716	0.041	B – B
TRC-04	1.442	1.730	0.026	0.387	0.475	0.112	B – B
TRC-05	0.456	1.714	0.517	0.350	0.095	0.038	B – B
TRC-07	0.700	1.828	0.314	0.457	0.168	0.061	B – B
TC-03	1.801	0.777	0.091	0.422	0.334	0.153	A – A
TC-04	1.596	1.045	0.071	0.349	0.408	0.172	A – A
TC-06	1.776	0.825	0.038	0.375	0.461	0.126	A – A
TC-09	1.500	0.850	0.063	0.345	0.477	0.116	A – A
TC-13	1.160	0.944	0.034	0.347	0.502	0.117	B – N/A
TC-23	1.847	0.762	0.006	0.345	0.532	0.117	A – A
TC-24	1.916	0.774	0.089	0.316	0.465	0.129	A – A
TC-26	2.000	1.060	0.013	0.439	0.402	0.147	A – A
TC-29	1.789	0.709	0.163	0.299	0.455	0.083	A – A
TC-30	1.887	0.823	0.021	0.295	0.587	0.097	A – A
TTC-03	0.354	1.417	0.056	0.586	0.280	0.078	B – B
TTC-04	0.899	1.390	0.003	0.171	0.672	0.154	B – B
TTC-05	1.097	1.324	0.024	0.286	0.575	0.115	B – B
TTC-06	0.442	1.212	0.002	0.179	0.538	0.280	B – B
TTC-07	0.503	1.628	0.012	0.220	0.401	0.367	B – B

Appendix A-4: In-situ water parameters statistical table by strata

	Mean	Std.Dev.	Std.Error	Median	Minimum	Maximum
DRM (n=10)						
Conductivity	0.185	0.001	0	0.185	0.184	0.188
Turbidity	20.043	10.585	3.347	17.426	6.675	33.741
Temperature	17.415	0.116	0.037	17.378	17.277	17.607
pH	8.693	0.056	0.018	8.685	8.587	8.798
Depth	1.132	0.336	0.106	1.053	0.769	1.681
TOC	0.716	0.318	0.1	0.841	-0.043	0.973
Gravel	0.088	0.109	0.035	0.028	0	0.306
Sand	0.419	0.165	0.052	0.434	0.101	0.619
Fine Sand	0.366	0.193	0.061	0.33	0.131	0.714
Silt	0.128	0.068	0.021	0.117	0.026	0.231
GRASS (n=10)						
Conductivity	0.199	0.004	0.001	0.198	0.195	0.205
Turbidity	16.009	10.258	3.244	11.764	5.247	39.69
Temperature	19.627	0.162	0.051	19.669	19.334	19.819
pH	8.542	0.231	0.073	8.611	8.109	8.891
Depth	1.035	0.291	0.092	1.034	0.707	1.502
TOC	1.17	0.497	0.157	0.959	0.798	2.435
Gravel	0.09	0.048	0.015	0.078	0.017	0.186
Sand	0.503	0.059	0.019	0.528	0.403	0.567
Fine Sand	0.238	0.04	0.013	0.23	0.174	0.307
Silt	0.169	0.03	0.01	0.164	0.128	0.229
PECHE (n=10)						
Conductivity	0.193	0.006	0.002	0.195	0.177	0.197
Turbidity	26.555	10.295	3.256	25.392	9.32	48.452
Temperature	19.279	0.241	0.076	19.318	18.937	19.816
pH	8.385	0.096	0.03	8.393	8.242	8.585
Depth	1.507	0.61	0.193	1.365	1.008	3.193
TOC	0.966	0.192	0.061	0.967	0.72	1.241
Gravel	0.009	0.012	0.004	0.005	0	0.04
Sand	0.345	0.081	0.025	0.342	0.253	0.487
Fine Sand	0.332	0.068	0.022	0.315	0.265	0.466
Silt	0.313	0.101	0.032	0.326	0.117	0.458
RIVER CANARD (n=10)						
Conductivity	0.204	0.001	0	0.205	0.202	0.205
Turbidity	31.935	25.054	7.923	21.504	6.38	73.754
Temperature	20.034	0.112	0.037	20.01	19.876	20.2
pH	8.664	0.151	0.05	8.693	8.336	8.817
Depth	1.581	0.479	0.151	1.792	0.601	2.145

	Mean	Std.Dev.	Std.Error	Median	Minimum	Maximum
TOC	0.858	0.128	0.043	0.823	0.709	1.06
Gravel	0.059	0.05	0.017	0.038	0.006	0.163
Sand	0.354	0.051	0.017	0.347	0.295	0.439
Fine Sand	0.461	0.075	0.025	0.461	0.334	0.587
Silt	0.127	0.028	0.009	0.126	0.083	0.172
TC (n=9)						
Conductivity	0.198	0.002	0	0.197	0.196	0.2
Turbidity	20.976	14.233	4.744	19.695	8.376	53.003
Temperature	18.615	0.09	0.029	18.62	18.481	18.745
pH	8.489	0.147	0.046	8.56	8.253	8.671
Depth	1.581	0.479	0.151	1.792	0.601	2.145
TOC	0.952	0.316	0.1	0.926	0.43	1.643
Gravel	0.021	0.033	0.011	0.011	0	0.11
Sand	0.289	0.114	0.036	0.263	0.156	0.487
Fine Sand	0.55	0.187	0.059	0.575	0.224	0.813
Silt	0.141	0.09	0.028	0.115	0.026	0.345
RC – UPSTREAM (n=5)						
Conductivity	0.199	0.001	0	0.199	0.198	0.2
Turbidity	43.08	29.934	13.387	43.159	12.344	73.754
Temperature	18.541	0.047	0.021	18.539	18.481	18.605
pH	8.379	0.125	0.056	8.347	8.253	8.567
Depth	1.292	0.52	0.232	1.214	0.601	1.807
TOC	1.03	0.438	0.196	1.021	0.43	1.643
Gravel	0.033	0.045	0.02	0.024	0	0.11
Sand	0.317	0.142	0.064	0.273	0.161	0.487
Fine Sand	0.474	0.235	0.105	0.435	0.224	0.813
Silt	0.176	0.12	0.054	0.179	0.026	0.345
RC – DOWNSTREAM (n=5)						
Conductivity	0.196	0.001	0	0.196	0.196	0.197
Turbidity	20.789	14.346	6.416	19.872	6.38	43.481
Temperature	18.689	0.049	0.022	18.697	18.635	18.745
pH	8.599	0.049	0.022	8.574	8.554	8.671
Depth	1.871	0.191	0.085	1.896	1.625	2.145
TOC	0.874	0.132	0.059	0.878	0.685	1.033
Gravel	0.009	0.009	0.004	0.004	0.001	0.02
Sand	0.26	0.084	0.037	0.252	0.156	0.356
Fine Sand	0.625	0.095	0.043	0.625	0.505	0.724
Silt	0.105	0.024	0.011	0.103	0.075	0.137
RC – TRIBUTARY (n=5)						
Conductivity	0.268	0.053	0.024	0.266	0.2	0.349

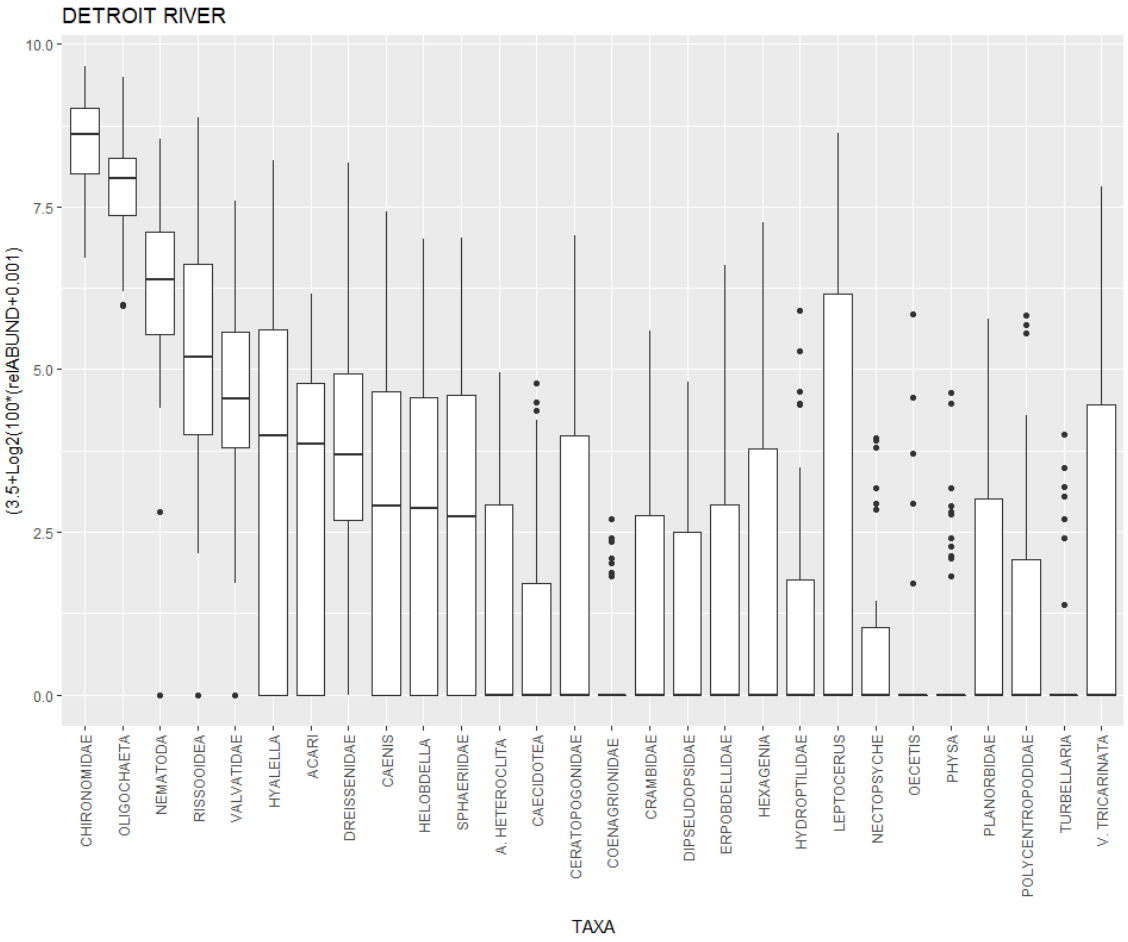
	Mean	Std.Dev.	Std.Error	Median	Minimum	Maximum
Turbidity	19.657	10.532	4.71	19.141	9.451	33.015
Temperature	19.218	0.218	0.098	19.151	18.965	19.513
pH	8.453	0.132	0.059	8.468	8.264	8.596
Depth	0.973	0.45	0.201	0.823	0.456	1.446
TOC	1.302	0.645	0.288	1.714	0.394	1.828
Gravel	0.177	0.229	0.103	0.026	0.007	0.517
Sand	0.33	0.101	0.045	0.35	0.222	0.457
Fine Sand	0.439	0.301	0.135	0.475	0.095	0.743
Silt	0.053	0.037	0.016	0.041	0.015	0.112
TC – UPSTREAM (n=5)						
Conductivity	0.205	0	0	0.205	0.205	0.205
Turbidity	13.493	6.602	3.301	11.453	8.376	22.688
Temperature	19.953	0.08	0.04	19.935	19.876	20.064
pH	8.585	0.193	0.097	8.604	8.336	8.797
Depth	1.163	0.499	0.25	1.122	0.601	1.805
TOC	0.898	0.121	0.06	0.885	0.777	1.045
Gravel	0.058	0.027	0.014	0.055	0.034	0.091
Sand	0.373	0.035	0.018	0.362	0.347	0.422
Fine Sand	0.426	0.073	0.036	0.435	0.334	0.502
Silt	0.142	0.025	0.013	0.139	0.117	0.172
TC – DOWNSTREAM (n=5)						
Conductivity	0.204	0.001	0	0.204	0.202	0.205
Turbidity	26.962	16.482	7.371	21.731	9.313	53.003
Temperature	20.099	0.092	0.041	20.138	19.994	20.2
pH	8.727	0.08	0.036	8.749	8.608	8.817
Depth	1.871	0.191	0.085	1.896	1.625	2.145
TOC	0.825	0.137	0.061	0.774	0.709	1.06
Gravel	0.059	0.067	0.03	0.021	0.006	0.163
Sand	0.339	0.059	0.026	0.316	0.295	0.439
Fine Sand	0.488	0.072	0.032	0.465	0.402	0.587
Silt	0.115	0.025	0.011	0.117	0.083	0.147
TC – TRIBUTARY (n=5)						
Conductivity	0.257	0.017	0.008	0.261	0.238	0.279
Turbidity	20.506	0.322	0.144	1.39	0.002	0.586
Temperature	20.506	0.279	0.125	20.358	20.26	20.824
pH	8.759	0.322	0.144	8.608	8.416	9.152
Depth	0.659	0.322	0.144	0.503	0.354	1.097
TOC	1.394	0.153	0.068	1.39	1.212	1.628
Gravel	0.019	0.022	0.01	0.012	0.002	0.056
Sand	0.288	0.172	0.077	0.22	0.171	0.586

	Mean	Std.Dev.	Std.Error	Median	Minimum	Maximum
Fine Sand	0.493	0.154	0.069	0.538	0.28	0.672
Silt	0.199	0.121	0.054	0.154	0.078	0.367

Appendix B

Appendix B-1: Boxplots of transformed abundances and statistical tables of relative abundance by strata

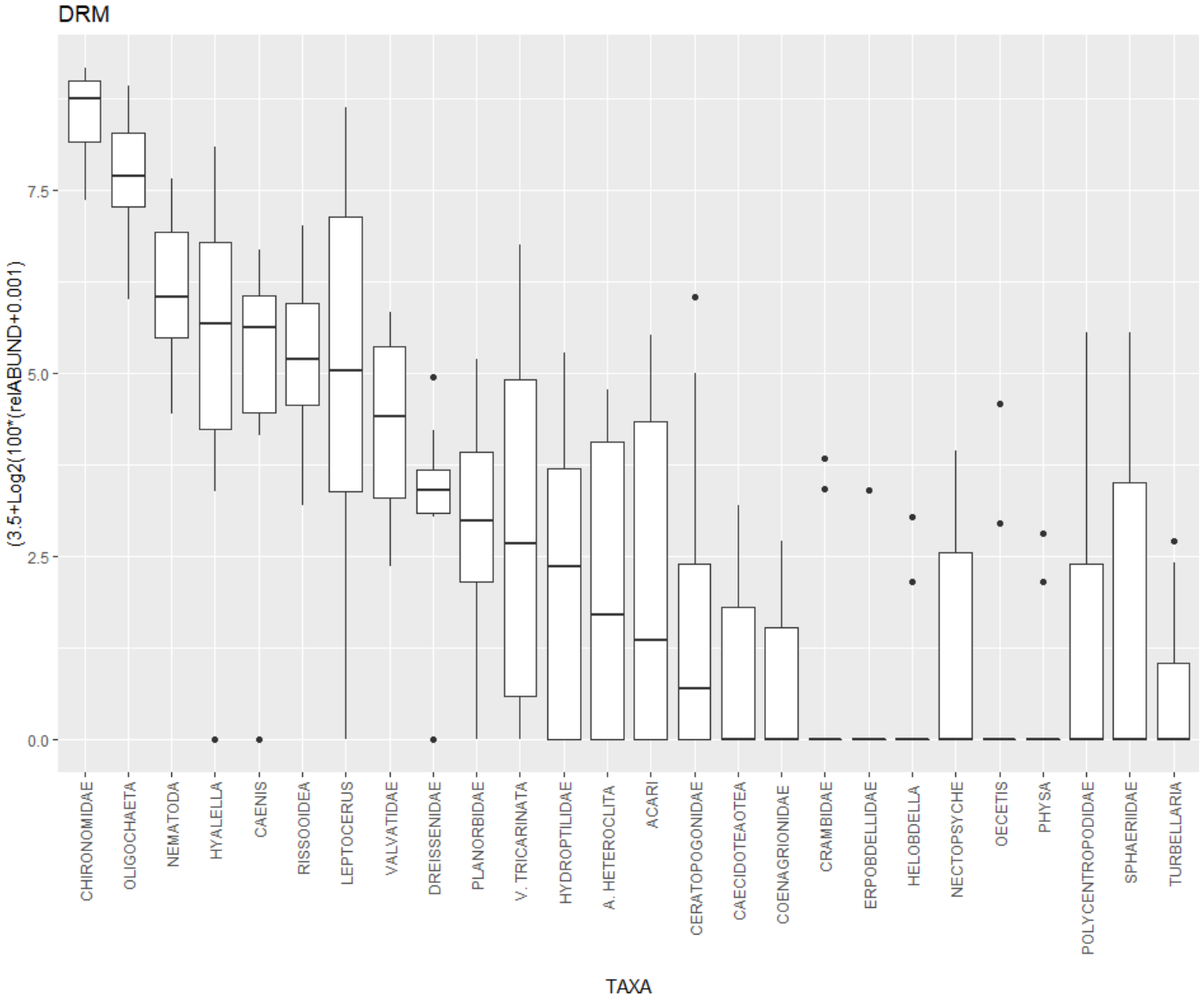
Detroit River Boxplot



Statistics of taxon relative abundance of Detroit River wetland sites (n=50)

	Mean	Std. Dev.	Std. Error	Medians	Minimum	Maximum	Lower Quantile	Upper Quantile	Interquartile Range
CHIRONOMIDAE	0.356	0.121	0.038	0.382	0.146	0.507	0.007	0.000	0.193
OLIGOCHAETA	0.204	0.118	0.037	0.187	0.056	0.433	0.008	0.012	0.140
VALVATIDAE	0.022	0.016	0.005	0.018	0.004	0.050	0.010	0.000	0.028
NEMATODA	0.078	0.056	0.018	0.058	0.018	0.179	0.026	0.000	0.069
RISSOOIDEA	0.042	0.033	0.011	0.031	0.007	0.113	0.000	0.000	0.035
DREISSENIDAE	0.009	0.008	0.002	0.008	0.000	0.026	0.000	0.005	0.004
ACARI	0.011	0.014	0.005	0.002	0.000	0.040	0.002	0.041	0.017
SPHAERIIDAE	0.008	0.014	0.004	0.000	0.000	0.041	0.017	0.000	0.010
CAENIS	0.043	0.030	0.010	0.044	0.000	0.090	0.040	0.000	0.039
CERATOPOGONIDAE	0.009	0.019	0.006	0.001	0.000	0.057	0.000	0.000	0.004
HYALELLA	0.070	0.077	0.024	0.044	0.000	0.241	0.000	0.000	0.083
LEPTOCERUS	0.082	0.110	0.035	0.041	0.000	0.351	0.000	0.008	0.116
HELOBDELLA	0.001	0.002	0.001	0.000	0.000	0.006	0.010	0.000	0.000
PLANORBIDAE	0.010	0.011	0.003	0.006	0.000	0.032	0.041	0.000	0.010
V.TRICARINATA	0.020	0.030	0.010	0.005	0.000	0.095	0.000	0.004	0.025
CRAMBIDAE	0.002	0.004	0.001	0.000	0.000	0.012	0.019	0.015	0.000
POLYCENTROPODIDAE	0.006	0.013	0.004	0.000	0.000	0.041	0.044	0.033	0.005
ERPOBDELLIDAE	0.001	0.003	0.001	0.000	0.000	0.008	0.058	0.000	0.000
HYDROPTILIDAE	0.008	0.011	0.004	0.004	0.000	0.033	0.090	0.000	0.015
A.HETEROCLITA	0.008	0.009	0.003	0.004	0.000	0.023	0.000	0.004	0.014
CAECIDOTEA	0.002	0.003	0.001	0.000	0.000	0.007	0.000	0.014	0.003
PHYSA	0.001	0.002	0.001	0.000	0.000	0.005	0.001	0.023	0.000
COENAGRIONIDAE	0.001	0.002	0.001	0.000	0.000	0.005	0.004	0.000	0.002
NECTOPSYCHE	0.003	0.004	0.001	0.000	0.000	0.013	0.057	0.000	0.005
OECETIS	0.003	0.006	0.002	0.000	0.000	0.020	0.000	0.000	0.000
TURBELLARIA	0.001	0.002	0.001	0.000	0.000	0.005	0.016	0.003	0.001

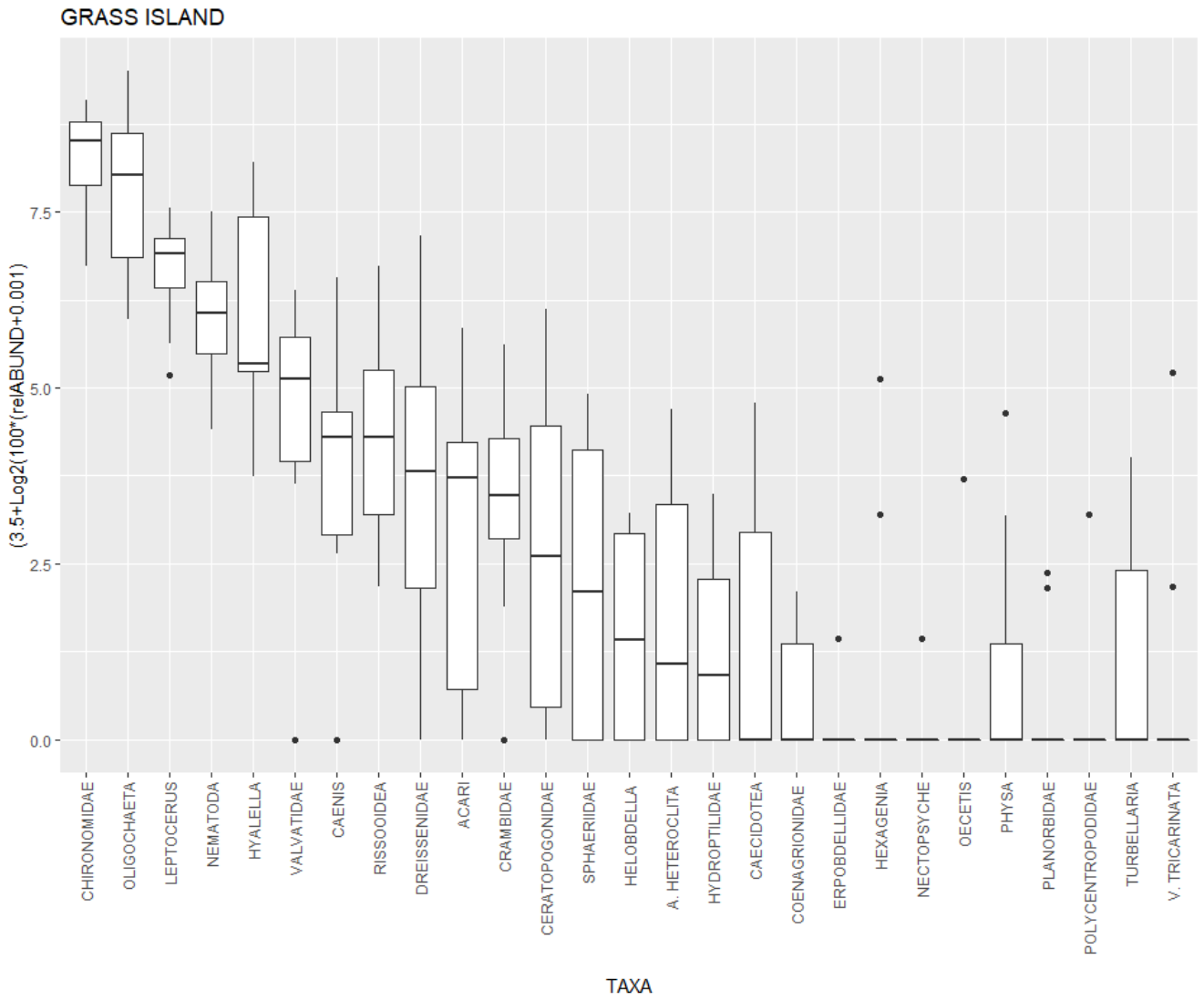
DRM sites boxplot



Statistics of taxon relative abundance of DRM wetland sites

	Mean	Std. Dev.	Std. Error	Medians	Minimum	Maximum	Lower Quantile	Upper Quantile	Interquartile Range
CHIRONOMIDAE	0.356	0.121	0.038	0.382	0.146	0.507	0.007	0.000	0.193
OLIGOCHAETA	0.204	0.118	0.037	0.187	0.056	0.433	0.008	0.012	0.140
VALVATIDAE	0.022	0.016	0.005	0.018	0.004	0.050	0.010	0.000	0.028
NEMATODA	0.078	0.056	0.018	0.058	0.018	0.179	0.026	0.000	0.069
RISSOOIDEA	0.042	0.033	0.011	0.031	0.007	0.113	0.000	0.000	0.035
DREISSENIDAE	0.009	0.008	0.002	0.008	0.000	0.026	0.000	0.005	0.004
ACARI	0.011	0.014	0.005	0.002	0.000	0.040	0.002	0.041	0.017
SPHAERIIDAE	0.008	0.014	0.004	0.000	0.000	0.041	0.017	0.000	0.010
CAENIS	0.043	0.030	0.010	0.044	0.000	0.090	0.040	0.000	0.039
CERATOPOGONIDAE	0.009	0.019	0.006	0.001	0.000	0.057	0.000	0.000	0.004
HYALELLA	0.070	0.077	0.024	0.044	0.000	0.241	0.000	0.000	0.083
LEPTOCERUS	0.082	0.110	0.035	0.041	0.000	0.351	0.000	0.008	0.116
HELOBDELLA	0.001	0.002	0.001	0.000	0.000	0.006	0.010	0.000	0.000
PLANORBIDAE	0.010	0.011	0.003	0.006	0.000	0.032	0.041	0.000	0.010
V. TRICARINATA	0.020	0.030	0.010	0.005	0.000	0.095	0.000	0.004	0.025
CRAMBIDAE	0.002	0.004	0.001	0.000	0.000	0.012	0.019	0.015	0.000
POLYCENTROPODIDAE	0.006	0.013	0.004	0.000	0.000	0.041	0.044	0.033	0.005
ERPOBDELLIDAE	0.001	0.003	0.001	0.000	0.000	0.008	0.058	0.000	0.000
HYDROPTILIDAE	0.008	0.011	0.004	0.004	0.000	0.033	0.090	0.000	0.015
A. HETEROCLITA	0.008	0.009	0.003	0.004	0.000	0.023	0.000	0.004	0.014
CAECIDOTEA	0.002	0.003	0.001	0.000	0.000	0.007	0.000	0.014	0.003
PHYSA	0.001	0.002	0.001	0.000	0.000	0.005	0.001	0.023	0.000
COENAGRIONIDAE	0.001	0.002	0.001	0.000	0.000	0.005	0.004	0.000	0.002
NECTOPSYCHE	0.003	0.004	0.001	0.000	0.000	0.013	0.057	0.000	0.005
OECETIS	0.003	0.006	0.002	0.000	0.000	0.020	0.000	0.000	0.000
TURBELLARIA	0.001	0.002	0.001	0.000	0.000	0.005	0.016	0.003	0.001

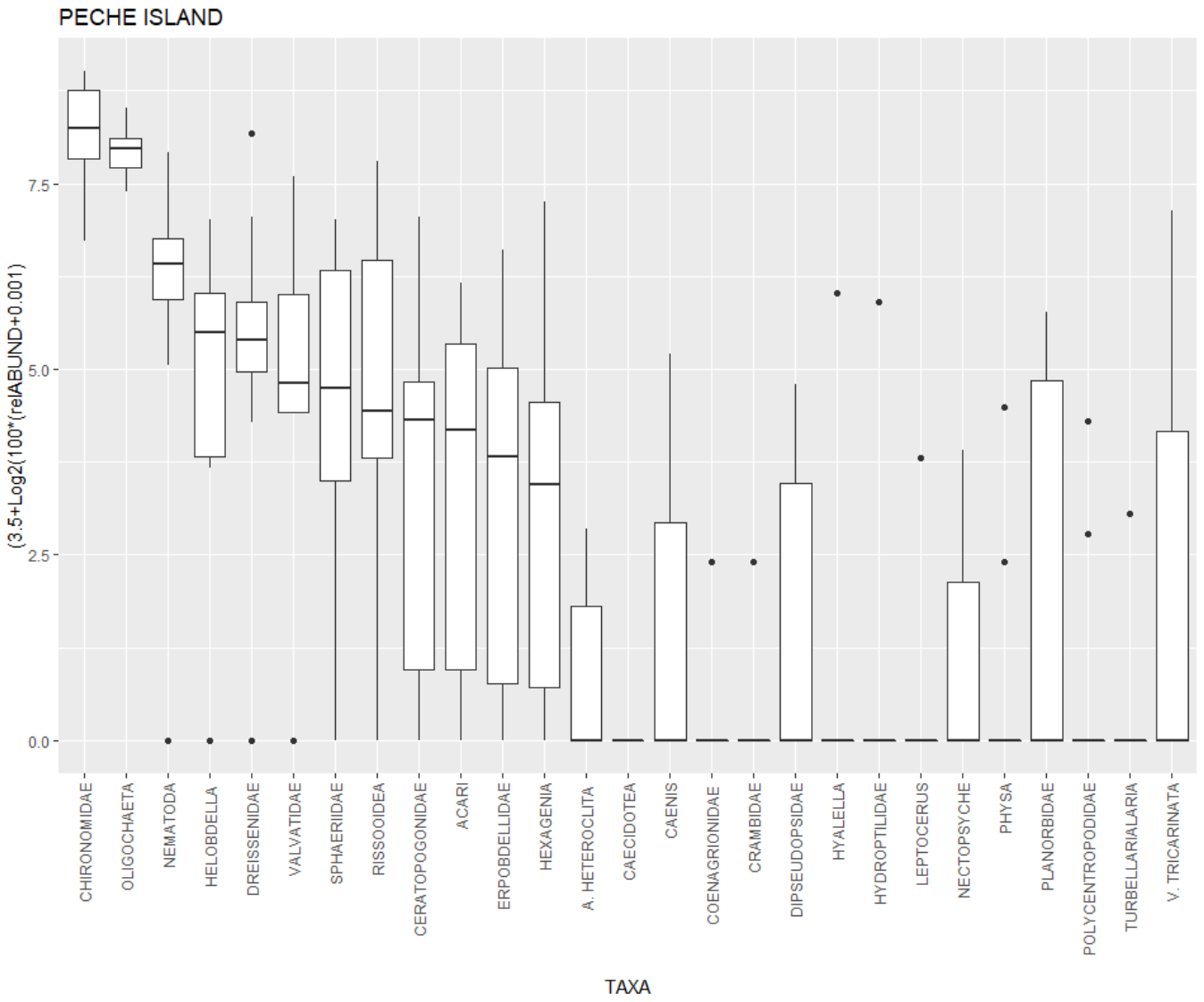
Grass Island sites boxplot



Statistics of taxon relative abundance of Grass Island wetland sites

	Mean	Std. Dev.	Std. Error	Medians	Minimum	Maximum	Lower Quantile	Upper Quantile	Interquartile Range
CHIRONOMIDAE	0.301	0.121	0.038	0.320	0.093	0.479	0.012	0.005	0.182
OLIGOCHAETA	0.256	0.185	0.058	0.228	0.054	0.635	0.028	0.009	0.246
VALVATIDAE	0.032	0.024	0.008	0.030	0.000	0.073	0.125	0.016	0.032
NEMATODA	0.067	0.042	0.013	0.060	0.018	0.159	0.000	0.042	0.041
RISSOOIDEA	0.025	0.027	0.009	0.018	0.003	0.093	0.001	0.000	0.025
DREISSENIDAE	0.030	0.043	0.014	0.012	0.000	0.125	0.011	0.000	0.025
ACARI	0.012	0.015	0.005	0.011	0.000	0.050	0.015	0.000	0.014
SPHAERIIDAE	0.008	0.010	0.003	0.003	0.000	0.026	0.050	0.000	0.015
CAENIS	0.020	0.024	0.008	0.016	0.000	0.082	0.000	0.007	0.015
CERATOPOGONIDAE	0.014	0.019	0.006	0.004	0.000	0.061	0.000	0.000	0.018
HYALELLA	0.089	0.087	0.027	0.035	0.011	0.261	0.003	0.000	0.118
LEPTOCERUS	0.100	0.045	0.014	0.105	0.031	0.164	0.015	0.000	0.047
HELOBDELLA	0.003	0.003	0.001	0.003	0.000	0.007	0.026	0.000	0.006
PLANORBIDAE	0.001	0.001	0.000	0.000	0.000	0.004	0.000	0.001	0.000
V.TRICARINATA	0.003	0.010	0.003	0.000	0.000	0.032	0.006	0.000	0.000
HEXAGENIA	0.004	0.009	0.003	0.000	0.000	0.030	0.016	0.000	0.000
CRAMBIDAE	0.013	0.012	0.004	0.009	0.000	0.042	0.021	0.001	0.011
POLYCENTROPODIDAE	0.001	0.002	0.001	0.000	0.000	0.007	0.082	0.003	0.000
ERPOBDELLIDAE	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.009	0.000
HYDROPTILIDAE	0.002	0.003	0.001	0.001	0.000	0.009	0.001	0.000	0.003
A. HETEROCLITA	0.006	0.008	0.003	0.001	0.000	0.022	0.004	0.000	0.008
CAECIDOTEA	0.004	0.008	0.002	0.000	0.000	0.023	0.019	0.001	0.006
PHYSA	0.003	0.007	0.002	0.000	0.000	0.021	0.061	0.008	0.002
COENAGRIONIDAE	0.001	0.001	0.000	0.000	0.000	0.003	0.011	0.022	0.002
NECTOPSYCHE	0.000	0.000	0.000	0.000	0.000	0.001	0.032	0.000	0.000
OECETIS	0.001	0.003	0.001	0.000	0.000	0.011	0.035	0.000	0.000
TURBELLARIA	0.003	0.005	0.002	0.000	0.000	0.013	0.150	0.000	0.005

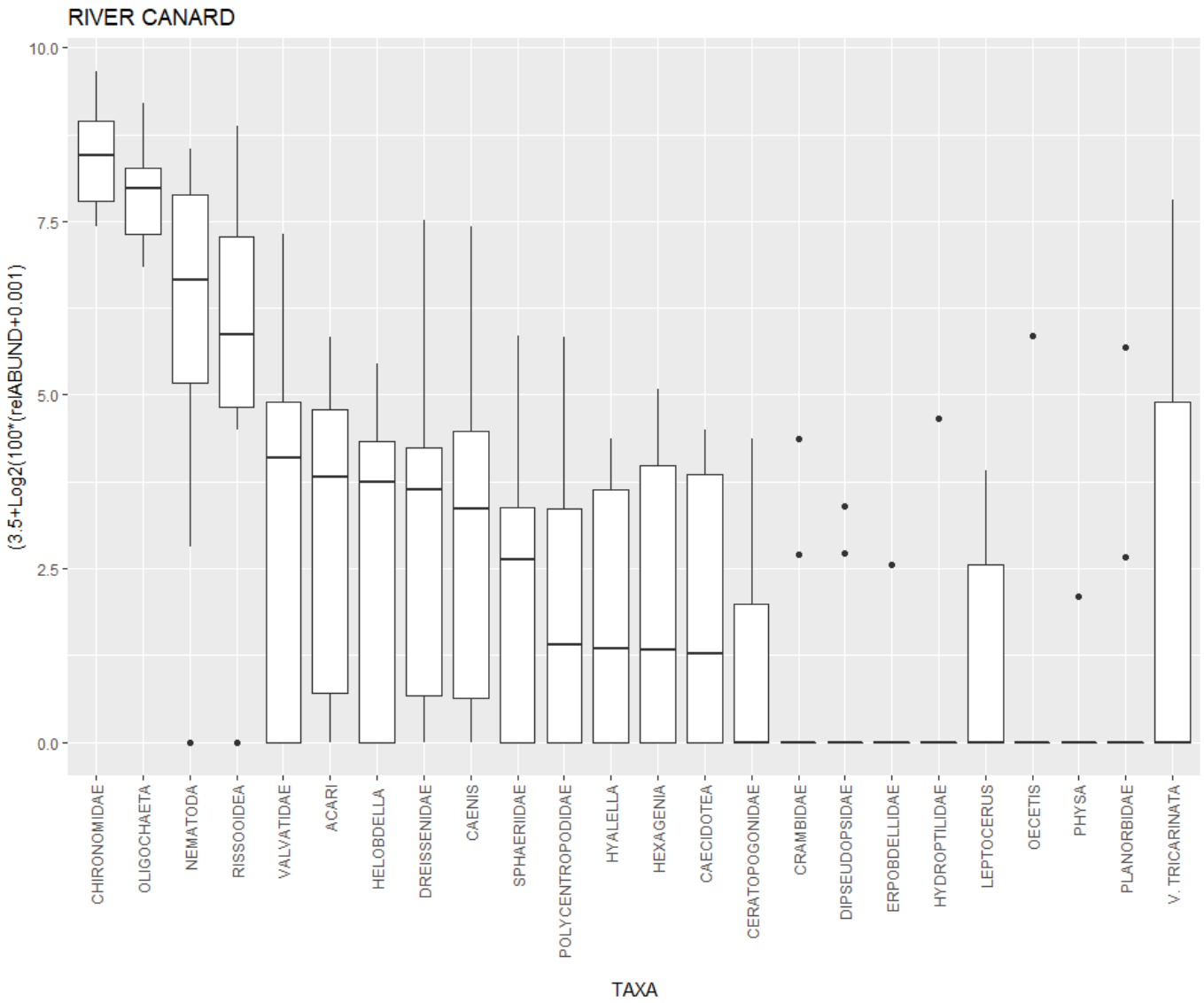
Peche Island sites boxplot



Statistics of taxon relative abundance of Peche Island sites

	Mean	Std. Dev.	Std. Error	Medians	Minimum	Maximum	Lower Quantile	Upper Quantile	Interquartile Range
CHIRONOMIDAE	0.286	0.120	0.038	0.267	0.092	0.454	0.027	0.020	0.181
OLIGOCHAETA	0.224	0.054	0.017	0.221	0.148	0.322	0.036	0.134	0.057
VALVATIDAE	0.048	0.055	0.017	0.024	0.000	0.170	0.052	0.000	0.038
NEMATODA	0.083	0.061	0.019	0.075	0.000	0.214	0.255	0.000	0.042
RISSOOIDEA	0.057	0.072	0.023	0.019	0.000	0.197	0.000	0.000	0.066
DREISSENIDAE	0.062	0.075	0.024	0.036	0.000	0.255	0.003	0.000	0.026
ACARI	0.023	0.023	0.007	0.015	0.000	0.062	0.015	0.004	0.032
SPHAERIIDAE	0.041	0.042	0.013	0.023	0.000	0.113	0.035	0.000	0.061
CAENIS	0.005	0.010	0.003	0.000	0.000	0.032	0.062	0.000	0.006
CERATOPOGONIDAE	0.027	0.037	0.012	0.017	0.000	0.117	0.000	0.000	0.021
HYALELLA	0.006	0.018	0.006	0.000	0.000	0.057	0.009	0.000	0.000
LEPTOCERUS	0.001	0.004	0.001	0.000	0.000	0.011	0.023	0.016	0.000
HELOBDELLA	0.039	0.035	0.011	0.039	0.000	0.113	0.071	0.000	0.045
PLANORBIDAE	0.012	0.018	0.006	0.000	0.000	0.047	0.113	0.002	0.027
V.TRICARINATA	0.021	0.041	0.013	0.000	0.000	0.124	0.000	0.011	0.015
HEXAGENIA	0.022	0.040	0.013	0.009	0.000	0.134	0.000	0.030	0.019
CRAMBIDAE	0.000	0.001	0.000	0.000	0.000	0.004	0.000	0.085	0.000
POLYCENTROPODIDAE	0.002	0.005	0.002	0.000	0.000	0.016	0.006	0.000	0.000
ERPOBDELLIDAE	0.021	0.027	0.009	0.011	0.000	0.085	0.032	0.000	0.028
HYDROPTILIDAE	0.005	0.016	0.005	0.000	0.000	0.052	0.000	0.000	0.000
A. HETEROCLITA	0.001	0.002	0.001	0.000	0.000	0.005	0.003	0.000	0.003
PHYSA	0.002	0.006	0.002	0.000	0.000	0.019	0.017	0.052	0.000
COENAGRIONIDAE	0.000	0.001	0.000	0.000	0.000	0.004	0.024	0.000	0.000
NECTOPSYCHE	0.003	0.005	0.002	0.000	0.000	0.012	0.117	0.000	0.004
DIPSEUDOPSIDAE	0.006	0.009	0.003	0.000	0.000	0.024	0.000	0.000	0.009
TURBELLARIA	0.001	0.002	0.001	0.000	0.000	0.006	0.000	0.003	0.000

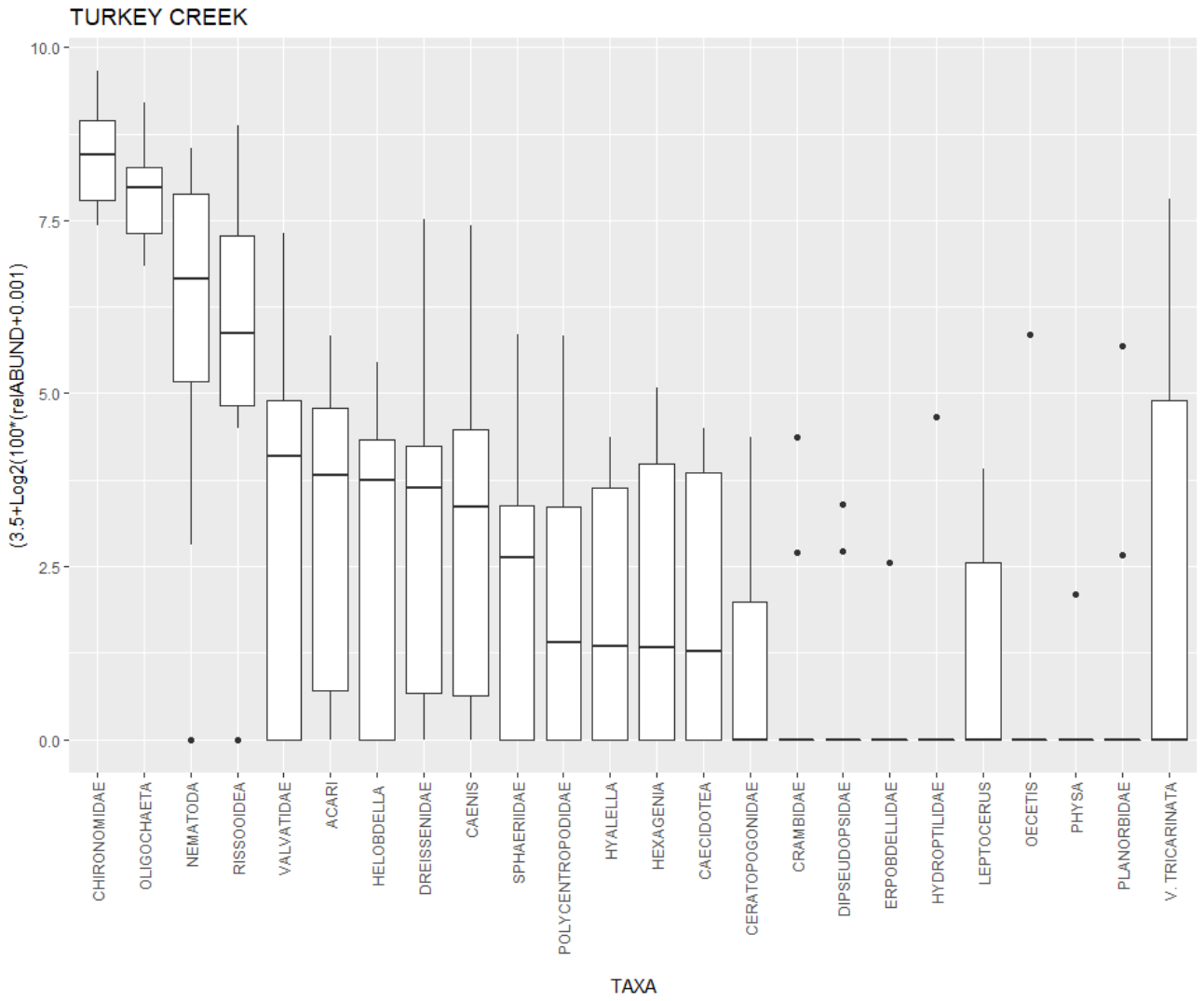
River Canard wetland sites boxplot



Statistics of taxon relative abundance of River Canard wetland sites

	Mean	Std. Dev.	Std. Error	Medians	Minimum	Maximum	Lower Quantile	Upper Quantile	Interquartile Range
CHIRONOMIDAE	0.343	0.182	0.057	0.310	0.152	0.708	0.410	0.000	0.241
OLIGOCHAETA	0.232	0.124	0.039	0.224	0.100	0.522	0.000	0.026	0.132
VALVATIDAE	0.026	0.043	0.013	0.014	0.000	0.140	0.001	0.198	0.026
NEMATODA	0.126	0.125	0.040	0.088	0.000	0.329	0.010	0.000	0.192
RISSOOIDEA	0.103	0.124	0.039	0.059	0.000	0.410	0.016	0.000	0.113
DREISSENIDAE	0.024	0.049	0.015	0.010	0.000	0.160	0.160	0.002	0.015
ACARI	0.016	0.017	0.005	0.011	0.000	0.049	0.000	0.014	0.022
SPHAERIIDAE	0.011	0.018	0.006	0.004	0.000	0.050	0.001	0.029	0.008
CAENIS	0.030	0.050	0.016	0.009	0.000	0.151	0.011	0.000	0.018
CERATOPOGONIDAE	0.003	0.006	0.002	0.000	0.000	0.017	0.023	0.000	0.003
HYALELLA	0.005	0.007	0.002	0.002	0.000	0.017	0.049	0.000	0.011
LEPTOCERUS	0.003	0.004	0.001	0.000	0.000	0.012	0.000	0.000	0.004
HELOBDELLA	0.013	0.014	0.004	0.011	0.000	0.038	0.000	0.017	0.017
PLANORBIDAE	0.005	0.014	0.004	0.000	0.000	0.045	0.004	0.000	0.000
V. TRICARINATA	0.027	0.061	0.019	0.000	0.000	0.198	0.008	0.000	0.026
HEXAGENIA	0.008	0.011	0.003	0.002	0.000	0.029	0.050	0.003	0.014
CRAMBIDAE	0.002	0.005	0.002	0.000	0.000	0.017	0.000	0.008	0.000
POLYCENTROPODIDAE	0.008	0.015	0.005	0.003	0.000	0.049	0.001	0.049	0.008
ERPOBDELLIDAE	0.000	0.001	0.000	0.000	0.000	0.004	0.009	0.000	0.000
HYDROPTILIDAE	0.002	0.007	0.002	0.000	0.000	0.021	0.019	0.000	0.000
CAECIDOTEA	0.006	0.008	0.003	0.002	0.000	0.019	0.151	0.000	0.013
PHYSA	0.000	0.001	0.000	0.000	0.000	0.003	0.000	0.000	0.000
OECETIS	0.005	0.016	0.005	0.000	0.000	0.050	0.000	0.004	0.000
DIPSEUDOPSIDAE	0.001	0.003	0.001	0.000	0.000	0.008	0.000	0.000	0.000

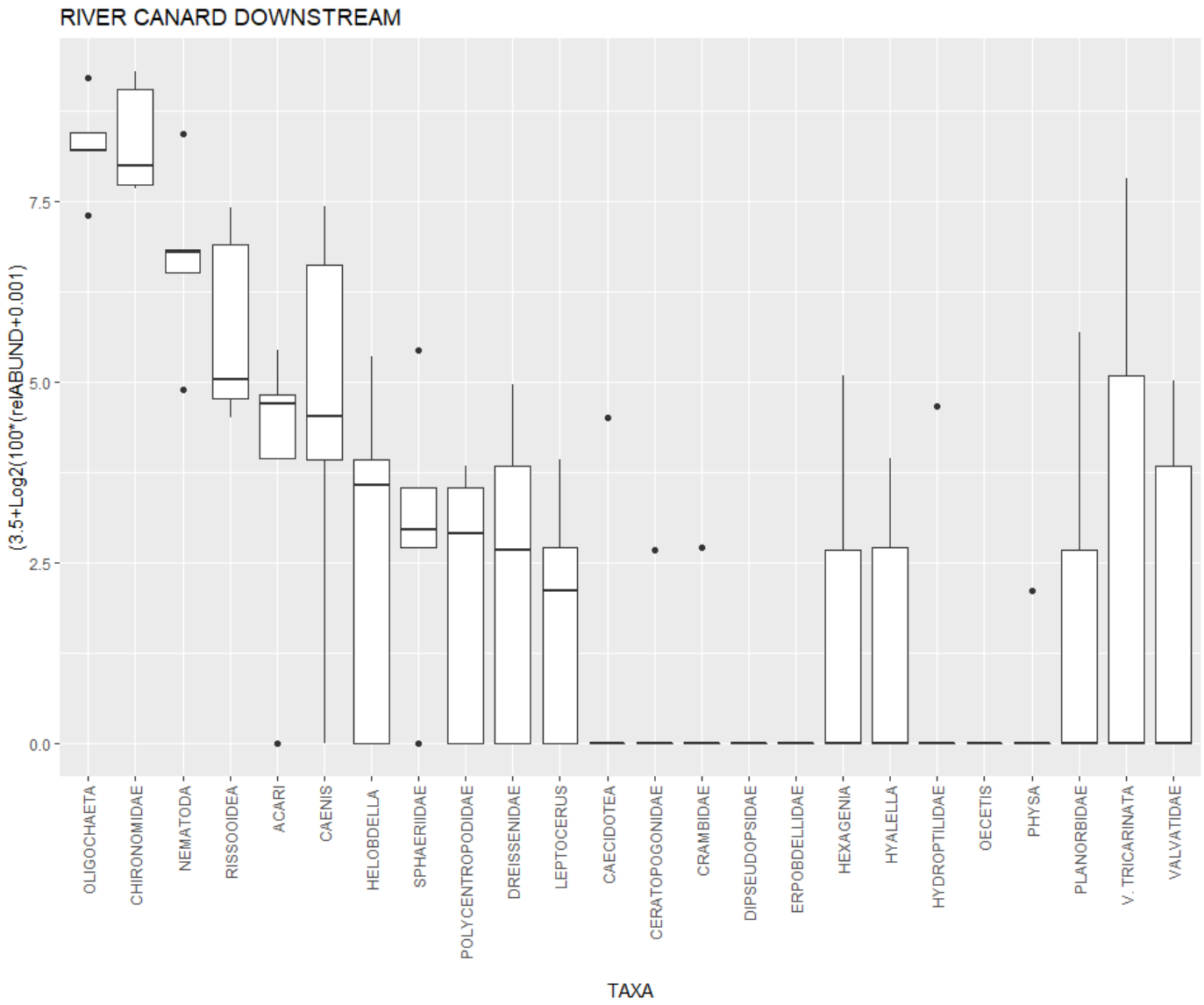
Turkey Creek wetland sites boxplot



Statistics of taxon relative abundance of Turkey Creek wetland sites

	Mean	Std. Dev.	Std. Error	Medians	Minimum	Maximum	Lower Quantile	Upper Quantile	Interquartile Range
CHIRONOMIDAE	0.463	0.137	0.043	0.488	0.279	0.644	0.065	0.000	0.223
OLIGOCHAETA	0.205	0.053	0.017	0.209	0.130	0.296	0.113	0.000	0.064
VALVATIDAE	0.032	0.030	0.010	0.021	0.002	0.107	0.127	0.010	0.019
NEMATODA	0.126	0.097	0.031	0.094	0.041	0.327	0.000	0.038	0.098
RISSOOIDEA	0.070	0.046	0.014	0.065	0.000	0.127	0.004	0.000	0.068
DREISSENIDAE	0.007	0.006	0.002	0.007	0.000	0.019	0.007	0.000	0.007
ACARI	0.012	0.012	0.004	0.011	0.000	0.029	0.011	0.000	0.023
SPHAERIIDAE	0.015	0.018	0.006	0.008	0.000	0.056	0.019	0.003	0.016
CAENIS	0.002	0.006	0.002	0.000	0.000	0.019	0.000	0.006	0.000
CERATOPOGONIDAE	0.001	0.002	0.001	0.000	0.000	0.006	0.000	0.000	0.000
LEPTOCERUS	0.004	0.013	0.004	0.000	0.000	0.042	0.011	0.000	0.000
HELOBDELLA	0.023	0.025	0.008	0.020	0.000	0.075	0.023	0.000	0.033
PLANORBIDAE	0.004	0.005	0.002	0.002	0.000	0.012	0.029	0.000	0.007
HEXAGENIA	0.007	0.012	0.004	0.000	0.000	0.038	0.000	0.045	0.010
CRAMBIDAE	0.001	0.002	0.001	0.000	0.000	0.006	0.002	0.000	0.003
POLYCENTROPODIDAE	0.006	0.014	0.004	0.000	0.000	0.045	0.008	0.001	0.000
ERPOBDELLIDAE	0.014	0.016	0.005	0.006	0.000	0.044	0.019	0.006	0.025
HYDROPTILIDAE	0.000	0.001	0.000	0.000	0.000	0.002	0.056	0.026	0.000
A. HETEROCLITA	0.004	0.008	0.003	0.000	0.000	0.026	0.000	0.044	0.004
CAECIDOTEA	0.002	0.005	0.002	0.000	0.000	0.015	0.000	0.000	0.000
PHYSA	0.001	0.002	0.001	0.000	0.000	0.006	0.000	0.000	0.002
OECETIS	0.000	0.001	0.000	0.000	0.000	0.002	0.000	0.000	0.000

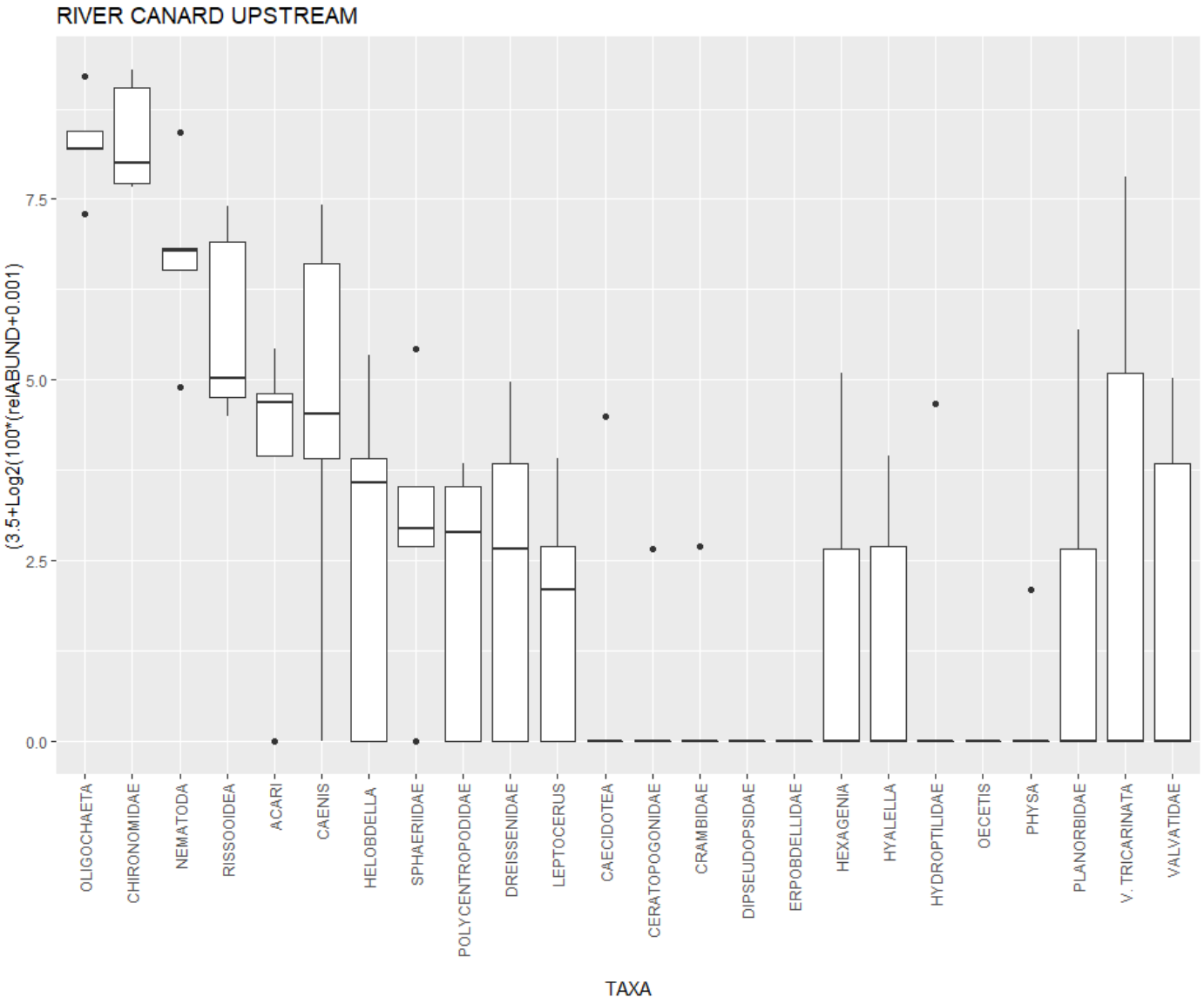
River Canard Downstream sites boxplot



Statistics of taxon relative abundance of River Canard Downstream sites

	Mean	Std. Dev.	Std. Error	Medians	Minimum	Maximum	Lower Quantile	Upper Quantile	Interquartile Range
CHIRONOMIDAE	0.365	0.207	0.092	0.335	0.152	0.708	0.029	0.017	0.062
OLIGOCHAETA	0.168	0.068	0.030	0.141	0.100	0.275	0.088	0.038	0.056
VALVATIDAE	0.044	0.056	0.025	0.019	0.000	0.140	0.181	0.000	0.025
NEMATODA	0.132	0.155	0.069	0.058	0.000	0.329	0.410	0.000	0.263
RISSOOIDEA	0.142	0.165	0.074	0.088	0.000	0.410	0.000	0.000	0.152
DREISSENIDAE	0.040	0.068	0.030	0.000	0.000	0.160	0.010	0.017	0.008
ACARI	0.013	0.021	0.009	0.005	0.000	0.049	0.010	0.031	0.010
SPHAERIIDAE	0.011	0.022	0.010	0.000	0.000	0.050	0.017	0.000	0.004
CAENIS	0.005	0.007	0.003	0.004	0.000	0.017	0.160	0.000	0.005
CERATOPOGONIDAE	0.004	0.007	0.003	0.000	0.000	0.017	0.000	0.005	0.005
HYALELLA	0.007	0.008	0.004	0.005	0.000	0.017	0.000	0.017	0.015
LEPTOCERUS	0.001	0.002	0.001	0.000	0.000	0.005	0.005	0.019	0.000
HELOBDELLA	0.014	0.016	0.007	0.016	0.000	0.038	0.010	0.000	0.017
V.TRICARINATA	0.010	0.014	0.006	0.000	0.000	0.031	0.049	0.000	0.017
HEXAGENIA	0.008	0.009	0.004	0.005	0.000	0.019	0.000	0.000	0.017
CRAMBIDAE	0.003	0.008	0.003	0.000	0.000	0.017	0.000	0.000	0.000
POLYCENTROPODIDAE	0.011	0.021	0.010	0.000	0.000	0.049	0.000	0.017	0.005
ERPOBDELLIDAE	0.001	0.002	0.001	0.000	0.000	0.004	0.004	0.000	0.000
CAECIDOTEA	0.008	0.008	0.003	0.005	0.000	0.017	0.050	0.000	0.011
OECETIS	0.010	0.022	0.010	0.000	0.000	0.050	0.000	0.000	0.000
DIPSEUDOPSIDAE	0.003	0.004	0.002	0.000	0.000	0.008	0.000	0.005	0.005

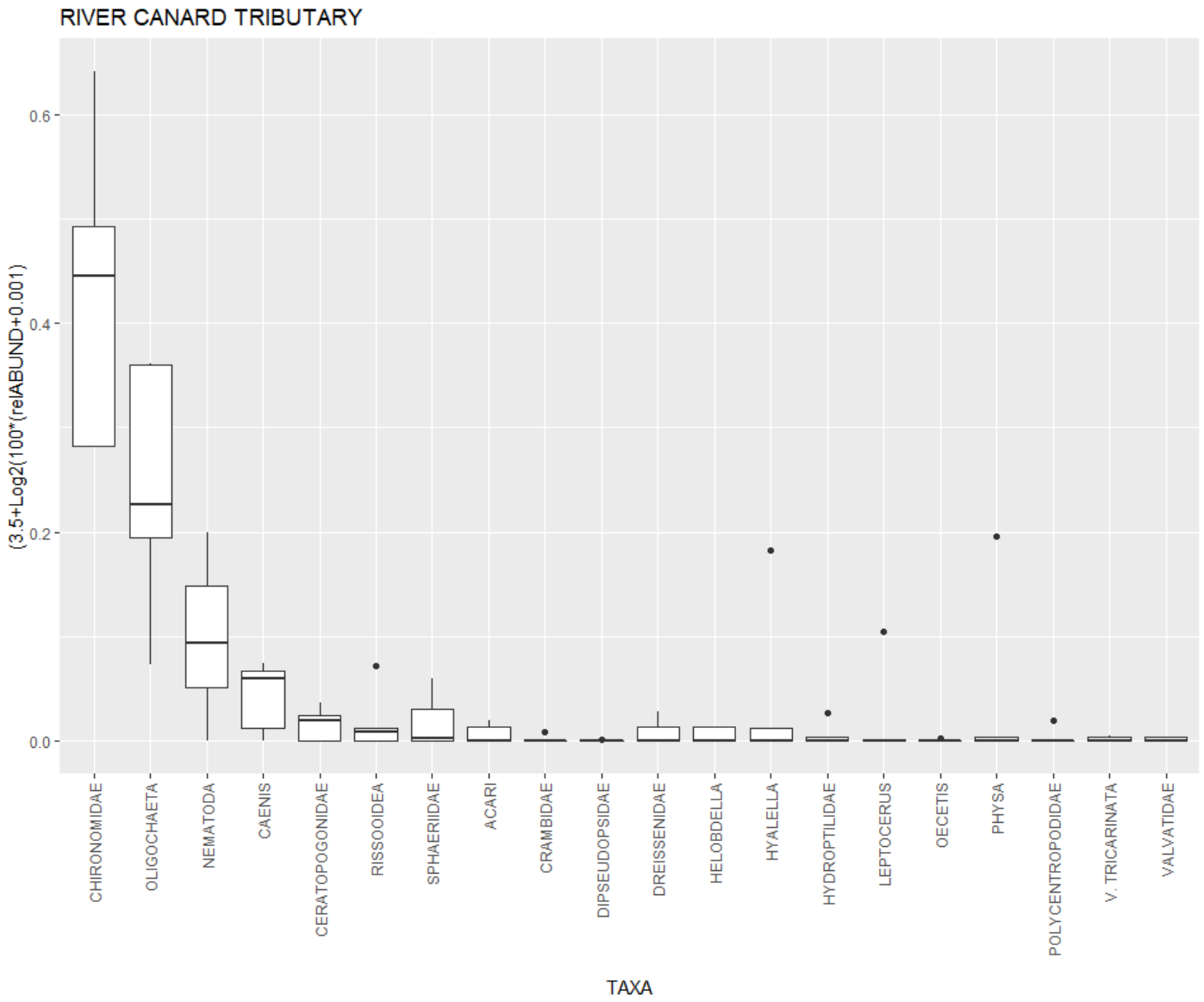
River Canard Upstream sites boxplot



Statistics of taxon relative abundance of River Canard Upstream sites

	Mean	Std. Dev.	Std. Error	Medians	Minimum	Maximum	Lower Quantile	Upper Quantile	Interquartile Range
CHIRONOMIDAE	0.321	0.174	0.078	0.225	0.179	0.549	0.023	0.012	0.281
OLIGOCHAETA	0.297	0.140	0.063	0.259	0.139	0.522	0.028	0.035	0.048
VALVATIDAE	0.008	0.012	0.005	0.000	0.000	0.028	0.105	0.000	0.012
NEMATODA	0.121	0.106	0.047	0.097	0.025	0.302	0.148	0.000	0.019
RISSOOIDEA	0.065	0.059	0.026	0.028	0.019	0.148	0.000	0.000	0.082
DREISSENIDAE	0.009	0.011	0.005	0.005	0.000	0.027	0.000	0.005	0.012
ACARI	0.019	0.014	0.006	0.022	0.000	0.037	0.005	0.045	0.011
SPHAERIIDAE	0.011	0.015	0.007	0.006	0.000	0.037	0.012	0.000	0.004
CAENIS	0.054	0.064	0.029	0.019	0.000	0.151	0.027	0.000	0.073
CERATOPOGONIDAE	0.001	0.002	0.001	0.000	0.000	0.005	0.000	0.000	0.000
HYALELLA	0.003	0.006	0.002	0.000	0.000	0.013	0.013	0.029	0.005
LEPTOCERUS	0.004	0.005	0.002	0.003	0.000	0.012	0.022	0.198	0.005
HELOBDELLA	0.011	0.014	0.006	0.009	0.000	0.035	0.024	0.000	0.012
PLANORBIDAE	0.010	0.020	0.009	0.000	0.000	0.045	0.037	0.000	0.005
V.TRICARINATA	0.045	0.086	0.038	0.000	0.000	0.198	0.000	0.000	0.029
HEXAGENIA	0.007	0.013	0.006	0.000	0.000	0.029	0.005	0.005	0.005
CRAMBIDAE	0.001	0.002	0.001	0.000	0.000	0.005	0.006	0.029	0.000
POLYCENTROPODIDAE	0.005	0.005	0.002	0.006	0.000	0.012	0.009	0.000	0.009
HYDROPTILIDAE	0.004	0.010	0.004	0.000	0.000	0.021	0.037	0.000	0.000
CAECIDOTEA	0.004	0.008	0.004	0.000	0.000	0.019	0.000	0.000	0.000
PHYSA	0.001	0.001	0.001	0.000	0.000	0.003	0.012	0.000	0.000

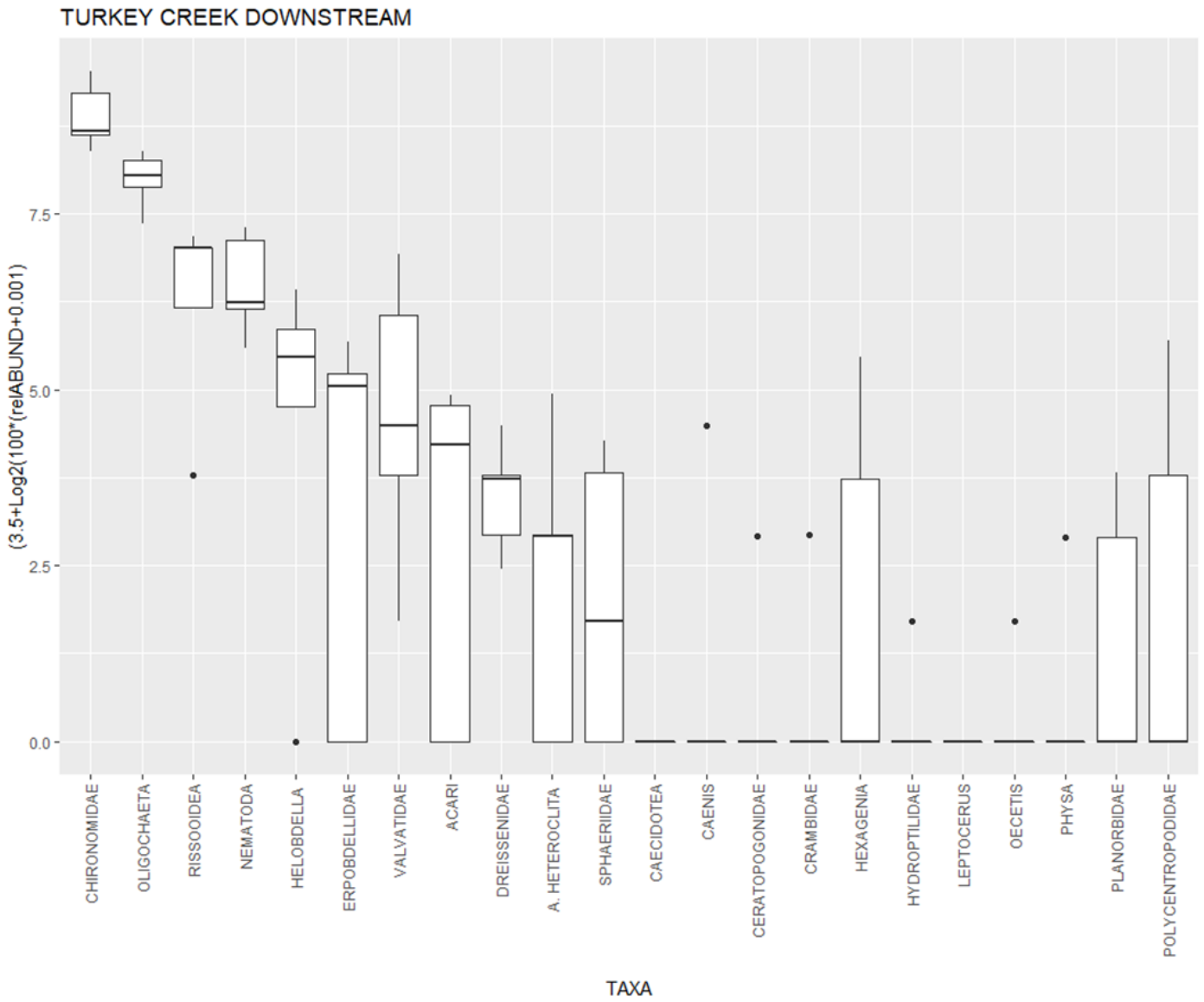
River Canard Tributary sites



Statistics of taxon relative abundance of River Canard Tributary sites

	Mean	Std. Dev.	Std. Error	Medians	Minimum	Maximum	Lower Quantile	Upper Quantile	Interquartile Range
CHIRONOMIDAE	0.429	0.152	0.068	0.445	0.282	0.641	0.000	0.000	0.210
OLIGOCHAETA	0.243	0.122	0.054	0.227	0.072	0.361	0.000	0.000	0.165
VALVATIDAE	0.002	0.002	0.001	0.000	0.000	0.004	0.009	0.000	0.004
NEMATODA	0.098	0.079	0.035	0.093	0.000	0.200	0.012	0.013	0.097
RISSOOIDEA	0.018	0.030	0.013	0.009	0.000	0.071	0.071	0.013	0.012
DREISSENIDAE	0.008	0.012	0.005	0.000	0.000	0.028	0.000	0.000	0.013
ACARI	0.006	0.009	0.004	0.000	0.000	0.019	0.000	0.000	0.013
SPHAERIIDAE	0.018	0.026	0.012	0.003	0.000	0.059	0.000	0.000	0.030
CAENIS	0.042	0.034	0.015	0.059	0.000	0.074	0.013	0.004	0.054
CERATOPOGONIDAE	0.016	0.016	0.007	0.020	0.000	0.037	0.028	0.004	0.024
HYALELLA	0.039	0.081	0.036	0.000	0.000	0.183	0.000	0.000	0.011
LEPTOCERUS	0.021	0.046	0.021	0.000	0.000	0.104	0.000	0.000	0.001
V.TRICARINATA	0.005	0.007	0.003	0.000	0.000	0.013	0.000	0.000	0.013
HEXAGENIA	0.002	0.002	0.001	0.000	0.000	0.004	0.013	0.000	0.004
CRAMBIDAE	0.002	0.004	0.002	0.000	0.000	0.009	0.019	0.009	0.000
POLYCENTROPODIDAE	0.004	0.009	0.004	0.000	0.000	0.020	0.000	0.000	0.000
HYDROPTILIDAE	0.006	0.011	0.005	0.000	0.000	0.026	0.000	0.000	0.004
PHYSA	0.040	0.087	0.039	0.000	0.000	0.195	0.003	0.000	0.004
OECETIS	0.001	0.001	0.001	0.000	0.000	0.003	0.030	0.000	0.000
DIPSEUDOPSIDAE	0.000	0.001	0.000	0.000	0.000	0.001	0.059	0.020	0.000

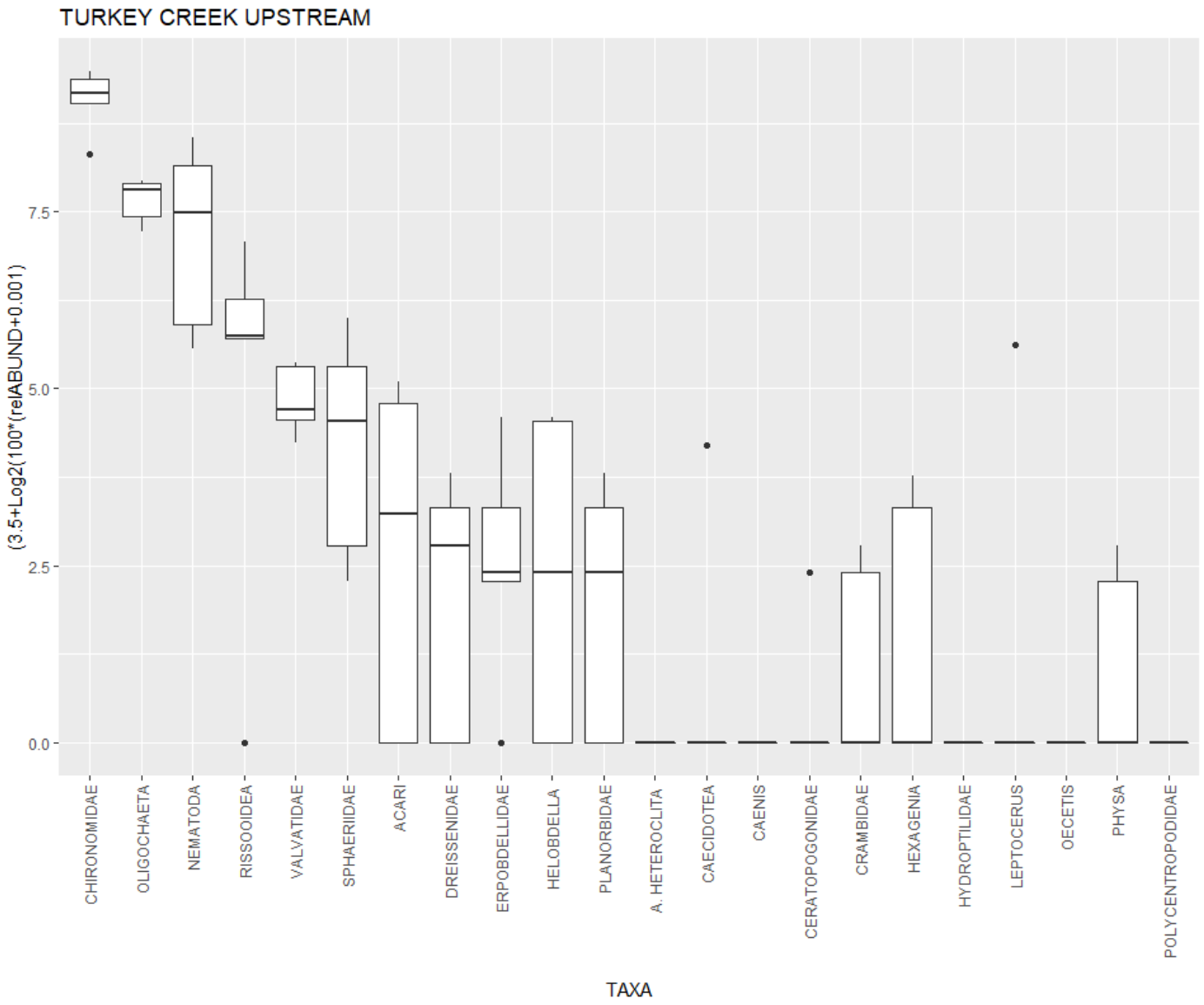
Turkey Creek Downstream sites



Statistics of taxon relative abundance of Turkey Creek Downstream sites

	Mean	Std. Dev.	Std. Error	Medians	Minimum	Maximum	Lower Quantile	Upper Quantile	Interquartile Range
CHIRONOMIDAE	0.433	0.147	0.066	0.359	0.293	0.644	0.011	0.000	0.180
OLIGOCHAETA	0.230	0.059	0.026	0.231	0.144	0.296	0.063	0.000	0.061
VALVATIDAE	0.039	0.043	0.019	0.019	0.002	0.107	0.113	0.000	0.047
NEMATODA	0.086	0.042	0.019	0.066	0.042	0.139	0.113	0.011	0.061
RISSOOIDEA	0.085	0.048	0.022	0.113	0.011	0.127	0.127	0.038	0.051
DREISSENIDAE	0.010	0.006	0.003	0.011	0.004	0.019	0.004	0.000	0.005
ACARI	0.013	0.012	0.006	0.016	0.000	0.026	0.006	0.000	0.023
SPHAERIIDAE	0.006	0.007	0.003	0.002	0.000	0.016	0.011	0.000	0.012
CAENIS	0.004	0.008	0.004	0.000	0.000	0.019	0.011	0.000	0.000
CERATOPOGONIDAE	0.001	0.003	0.001	0.000	0.000	0.006	0.019	0.006	0.000
HELOBDELLA	0.037	0.028	0.013	0.038	0.000	0.075	0.000	0.000	0.027
PLANORBIDAE	0.003	0.005	0.002	0.000	0.000	0.012	0.000	0.000	0.006
HEXAGENIA	0.010	0.016	0.007	0.000	0.000	0.038	0.016	0.000	0.011
CRAMBIDAE	0.001	0.003	0.001	0.000	0.000	0.006	0.023	0.011	0.000
POLYCENTROPODIDAE	0.011	0.019	0.009	0.000	0.000	0.045	0.026	0.045	0.011
ERPOBDELLIDAE	0.021	0.020	0.009	0.028	0.000	0.044	0.000	0.000	0.032
HYDROPTILIDAE	0.000	0.001	0.000	0.000	0.000	0.002	0.000	0.000	0.000
A. HETEROCLITA	0.008	0.011	0.005	0.006	0.000	0.026	0.002	0.028	0.006
PHYSA	0.001	0.003	0.001	0.000	0.000	0.006	0.012	0.032	0.000
OECETIS	0.000	0.001	0.000	0.000	0.000	0.002	0.016	0.044	0.000

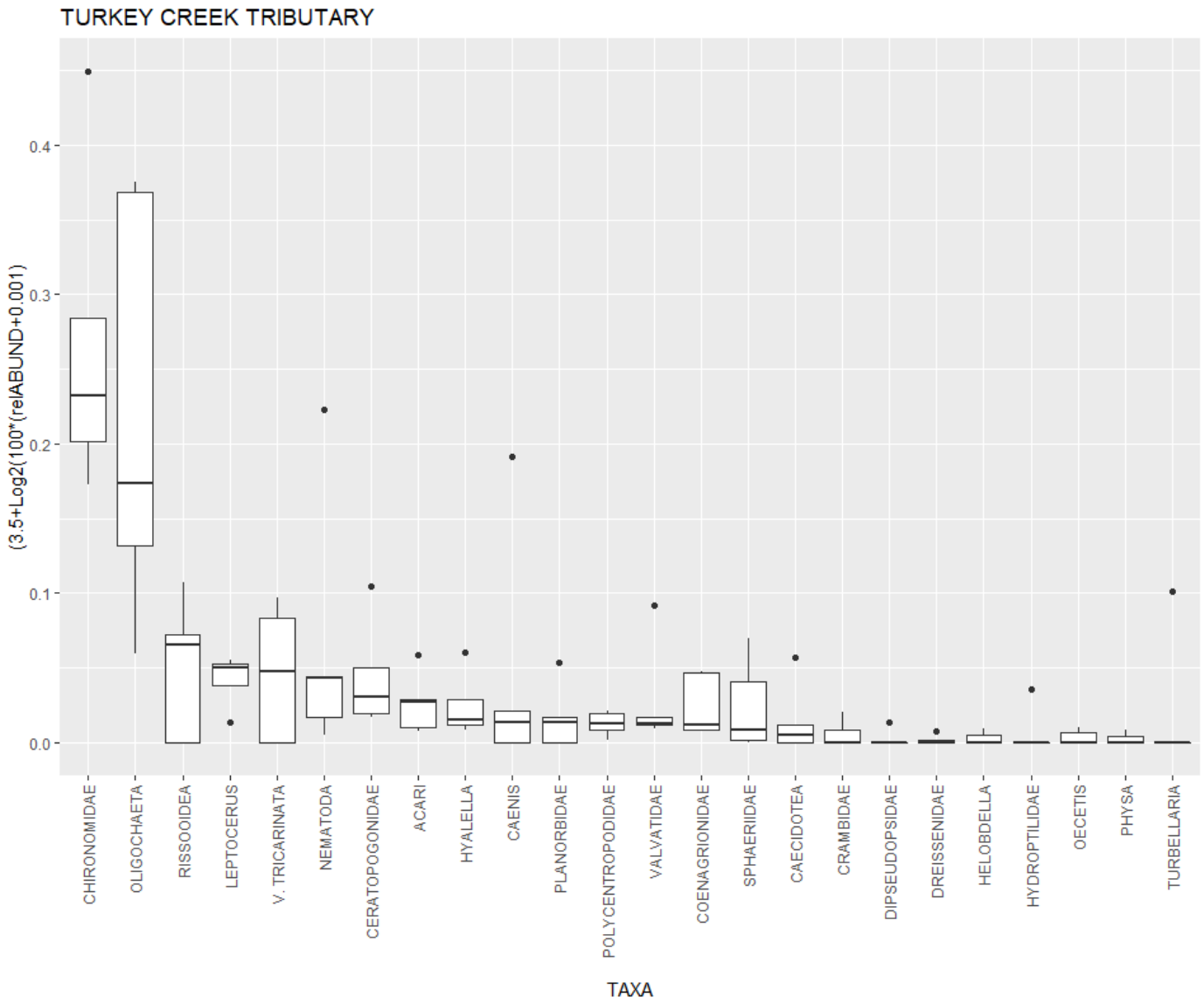
Relative abundance by taxon of Turkey Creek Upstream sites



Statistics of taxon relative abundance of Turkey Creek Upstream sites

	Mean	Std. Dev.	Std. Error	Medians	Minimum	Maximum	Lower Quantile	Upper Quantile	Interquartile Range
CHIRONOMIDAE	0.493	0.135	0.060	0.512	0.279	0.625	0.158	0.000	0.123
OLIGOCHAETA	0.181	0.038	0.017	0.196	0.130	0.216	0.250	0.004	0.058
VALVATIDAE	0.025	0.009	0.004	0.022	0.016	0.035	0.327	0.020	0.014
NEMATODA	0.166	0.124	0.056	0.158	0.041	0.327	0.000	0.020	0.198
RISSOOIDEA	0.055	0.043	0.019	0.046	0.000	0.117	0.045	0.000	0.021
DREISSENIDAE	0.005	0.005	0.002	0.005	0.000	0.011	0.046	0.000	0.008
ACARI	0.012	0.014	0.006	0.007	0.000	0.029	0.067	0.004	0.023
SPHAERIIDAE	0.023	0.022	0.010	0.020	0.003	0.056	0.117	0.008	0.029
CERATOPOGONIDAE	0.001	0.002	0.001	0.000	0.000	0.004	0.000	0.011	0.000
LEPTOCERUS	0.008	0.019	0.008	0.000	0.000	0.042	0.000	0.000	0.000
HELOBDELLA	0.009	0.010	0.005	0.004	0.000	0.020	0.005	0.000	0.020
PLANORBIDAE	0.005	0.005	0.002	0.004	0.000	0.011	0.008	0.000	0.008
HEXAGENIA	0.004	0.005	0.002	0.000	0.000	0.011	0.011	0.008	0.008
CRAMBIDAE	0.002	0.002	0.001	0.000	0.000	0.005	0.000	0.011	0.004
ERPOBDELLIDAE	0.007	0.008	0.004	0.004	0.000	0.020	0.000	0.000	0.005
CAECIDOTEA	0.003	0.007	0.003	0.000	0.000	0.015	0.007	0.000	0.000
PHYSA	0.002	0.002	0.001	0.000	0.000	0.005	0.023	0.000	0.003

Turkey Creek Tributary sites



Statistics of taxon relative abundance of Turkey Creek Tributary sites

	Mean	Std. Dev.	Std. Error	Medians	Minimum	Maximum	Lower Quantile	Upper Quantile	Interquartile Range
CHIRONOMIDAE	0.268	0.109	0.049	0.233	0.173	0.449	0.107	0.047	0.083
OLIGOCHAETA	0.222	0.143	0.064	0.173	0.060	0.375	0.000	0.083	0.236
VALVATIDAE	0.028	0.035	0.016	0.013	0.009	0.091	0.000	0.097	0.004
NEMATODA	0.066	0.089	0.040	0.044	0.005	0.223	0.000	0.000	0.028
RISSOOIDEA	0.049	0.047	0.021	0.066	0.000	0.107	0.002	0.000	0.072
DREISSENIDAE	0.002	0.003	0.001	0.000	0.000	0.008	0.008	0.000	0.002
ACARI	0.026	0.020	0.009	0.027	0.008	0.059	0.008	0.008	0.018
SPHAERIIDAE	0.024	0.030	0.014	0.008	0.000	0.069	0.010	0.020	0.039
CAENIS	0.045	0.082	0.037	0.014	0.000	0.191	0.027	0.002	0.021
CERATOPOGONIDAE	0.044	0.036	0.016	0.030	0.017	0.105	0.028	0.008	0.031
HYALELLA	0.025	0.021	0.010	0.015	0.008	0.060	0.059	0.013	0.017
LEPTOCERUS	0.042	0.017	0.008	0.050	0.013	0.055	0.000	0.019	0.014
HELOBDELLA	0.003	0.004	0.002	0.000	0.000	0.009	0.002	0.021	0.005
PLANORBIDAE	0.017	0.022	0.010	0.014	0.000	0.053	0.008	0.000	0.017
V. TRICARINATA	0.045	0.045	0.020	0.047	0.000	0.097	0.040	0.000	0.083
CRAMBIDAE	0.006	0.009	0.004	0.000	0.000	0.020	0.069	0.000	0.008
POLYCENTROPODIDAE	0.013	0.008	0.004	0.013	0.002	0.021	0.000	0.000	0.011
HYDROPTILIDAE	0.007	0.016	0.007	0.000	0.000	0.035	0.000	0.035	0.000
CAECIDOTEA	0.015	0.024	0.011	0.005	0.000	0.057	0.014	0.000	0.012
PHYSA	0.003	0.004	0.002	0.000	0.000	0.009	0.021	0.000	0.004
COENAGRIONIDAE	0.024	0.021	0.009	0.012	0.008	0.047	0.191	0.005	0.039
OECETIS	0.003	0.005	0.002	0.000	0.000	0.010	0.017	0.012	0.007
DIPSEUDOPSIDAE	0.003	0.006	0.003	0.000	0.000	0.013	0.019	0.057	0.000
TURBELLARIA	0.020	0.045	0.020	0.000	0.000	0.101	0.030	0.000	0.000

Appendix B-2: Alpha diversity by site (macroinvertebrates)

Sample Site	Shannon H'	Richness (n)	Pielou J
DRM-02	2.060	21	0.677
DRM-03	2.204	17	0.778
DRM-04	1.941	14	0.736
DRM-06	1.888	14	0.715
DRM-09	1.955	15	0.722
DRM-11	2.205	20	0.736
DRM-13	1.416	11	0.590
DRM-19	1.741	9	0.792
DRM-21	1.695	17	0.598
DRM-24	1.321	11	0.551
GI-01	1.719	14	0.651
GI-03	1.404	14	0.532
GI-04	2.140	15	0.790
GI-06	1.838	13	0.717
GI-09	1.808	14	0.685
GI-11	1.657	11	0.691
GI-13	2.195	19	0.745
GI-19	1.794	18	0.621
GI-21	2.040	19	0.693
GI-24	1.897	15	0.700
PI-02	1.542	8	0.741
PI-06	2.110	13	0.823
PI-07	1.659	11	0.692
PI-08	1.899	13	0.740
PI-09	1.973	9	0.898
PI-12	2.303	16	0.831
PI-15	2.212	15	0.817
PI-16	1.758	15	0.649
PI-17	2.148	14	0.814
PI-18	2.018	16	0.728
RC-U1	1.499	12	0.603
RC-U2	1.472	12	0.592
RC-U4	1.477	13	0.576
RC-U8	1.901	10	0.826

Sample Site	Shannon H'	Richness (n)	Pielou J
RC-U10	1.851	12	0.745
RC-D13	1.919	14	0.727
RC-D18	1.149	11	0.479
RC-D19	1.704	12	0.686
RC-D21	1.789	14	0.678
RC-D26	1.492	8	0.718
TRC-1	2.075	15	0.766
TRC-2	1.623	14	0.615
TRC-4	1.352	12	0.544
TRC-5	1.060	3	0.965
TRC-7	1.340	5	0.833
TC-U3	1.660	9	0.755
TC-U4	1.308	8	0.629
TC-U6	1.493	12	0.601
TC-U9	1.416	13	0.552
TC-U13	1.357	12	0.546
TC-U23	1.908	11	0.796
TC-U24	1.284	11	0.535
TC-U26	1.283	12	0.516
TC-U29	1.944	12	0.782
TC-U30	1.774	12	0.714
TTC-3	1.883	13	0.734
TTC-4	1.928	14	0.731
TTC-5	2.415	17	0.852
TTC-6	1.831	18	0.634

Appendix B-3: T-tests (macroinvertebrates)

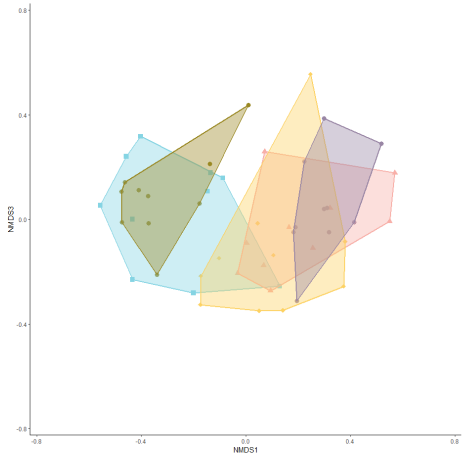
	RICHNESS			SHANNON			PIELOU J			EPT%		
	p	t	df	p	t	df	p	t	df	p	t	df
DRM-Grass	0.844	-0.200	15.813	0.957	-0.055	16.996	0.842	0.202	17.540	0.710	0.379	13.260
DRM-Peche	0.232	1.241	16.354	0.346	-0.968	17.343	**0.030**	-2.364	17.833	**0.030**	2.490	10.884
DRM-River Canard	**0.042**	2.266	12.669	0.096	1.759	17.342	0.527	0.645	17.372	0.054	2.141	11.722
DRM-Turkey Creek	**0.018**	2.613	18.000	0.009	2.911	17.811	0.868	0.170	14.450	0.123	1.630	15.786
Grass-Peche	0.090	1.792	17.931	0.310	-1.044	17.954	**0.012**	-2.781	17.921	**0.002**	3.670	15.433
Grass-River Canard	**0.004**	3.341	15.886	0.053	2.071	17.954	0.620	0.506	16.119	**0.009**	2.942	17.034
Grass-Turkey Creek	**0.005**	3.257	15.775	0.004	3.345	17.636	0.970	0.038	13.133	0.085	1.834	16.623
Peche-River Canard	0.276	1.129	15.330	0.007	3.040	18.000	**0.013**	2.778	16.676	0.541	-0.623	17.385
Peche-Turkey Creek	0.097	1.759	16.318	0.001	4.241	17.844	0.092	1.813	13.640	0.399	-0.872	12.972
River Canard-Turkey Creek	0.295	1.092	12.640	0.195	1.347	17.843	0.752	-0.321	16.161	0.675	-0.428	14.490
	CHIRON			**OLIGO**			**CERATO**			**NEMATO**		
DRM-Grass	0.324	1.014	18.000	0.464	-0.751	15.290	0.641	0.475	16.720	0.641	0.475	16.720
DRM-Peche	0.207	1.309	17.997	0.628	-0.496	12.571	0.832	-0.216	17.864	0.832	-0.216	17.864
DRM-River Canard	0.849	0.193	15.707	0.603	-0.529	17.951	0.286	-1.115	12.423	0.286	-1.115	12.423
DRM-Turkey Creek	0.924	0.097	17.271	0.663	-0.443	17.681	0.954	0.059	17.468	0.954	0.059	17.468
Grass-Peche	0.775	0.290	17.998	0.613	0.522	10.506	0.501	-0.689	15.982	0.501	-0.689	15.982
Grass-River Canard	0.555	-0.604	15.671	0.744	0.333	15.758	0.116	1.712	10.430	0.187	-1.408	10.990
Grass-Turkey Creek	0.431	-0.806	17.244	0.659	0.450	14.104	0.472	-0.738	14.525	0.727	-0.356	15.187
Peche-River Canard	0.418	-0.832	15.585	0.849	-0.194	12.242	0.065	2.089	9.396	0.350	-0.970	13.015
Peche-Turkey Creek	0.301	-1.067	17.180	0.966	-0.044	13.545	0.762	0.308	17.783	0.802	0.254	17.860
River Canard-Turkey Creek	0.922	-0.099	17.362	0.897	0.131	17.404	0.092	-1.872	9.493	0.284	1.114	13.689

	RICHNESS			SHANNON			PIELOU J			EPT%		
	p	t	df	p	t	df	p	t	df	p	t	df
Impacted-Unimpacted	**0.005**	-3.013	31.054	**0.000**	-4.095	41.952	**0.031**	-2.248	35.378	**0.005**	-3.013	31.054
RCDN-RCUP	0.521	-0.676	7.030	0.227	-1.309	7.955	0.372	-0.945	7.907	0.269	1.198	7.144
RCDN-TRC	0.568	0.609	5.260	0.863	0.179	6.843	0.242	-1.291	6.325	0.450	-0.803	6.447
RCUP-TRC	0.389	0.950	4.589	0.282	1.172	6.542	0.583	-0.577	6.750	0.156	-1.652	5.292
TCDN-TCUP	0.464	0.800	4.509	0.281	1.199	5.347	0.488	0.733	6.760	0.548	-0.641	5.300
TCDN-TTC	**0.019**	-3.660	4.289	0.051	-2.301	7.854	0.290	-1.151	6.626	0.165	-1.666	4.351
TCUP-TTC	**0.009**	-3.473	7.430	**0.005**	-4.503	5.735	**0.036**	-2.518	7.991	0.276	-1.199	5.968
	CHIRO			**OLIGO**			**CERATO**			**NEMATO**		
Impacted-Unimpacted	**0.051**	2.032	31.804	0.775	-0.288	47.440	**0.005**	-3.013	31.054	0.069	1.903	24.816
RCDN-RCUP	0.722	-0.369	7.773	0.114	1.859	5.794	0.355	-1.027	4.603	0.896	-0.135	7.058
RCDN-TRC	0.585	0.574	6.729	0.424	0.843	7.997	0.056	-2.653	4.026	0.408	0.876	7.773
RCUP-TRC	0.388	0.930	6.072	0.475	-0.764	5.730	0.070	-2.388	4.338	0.443	0.819	6.375
TCDN-TCUP	0.517	0.678	7.948	0.163	-1.563	6.842	0.770	-0.304	6.803	0.234	1.357	4.907
TCDN-TTC	0.497	0.712	7.892	0.328	-1.089	4.766	0.098	-2.141	4.083	0.342	1.023	6.763
TCUP-TTC	0.965	0.046	7.989	0.833	-0.220	5.760	0.104	-2.069	4.204	0.767	-0.309	6.118

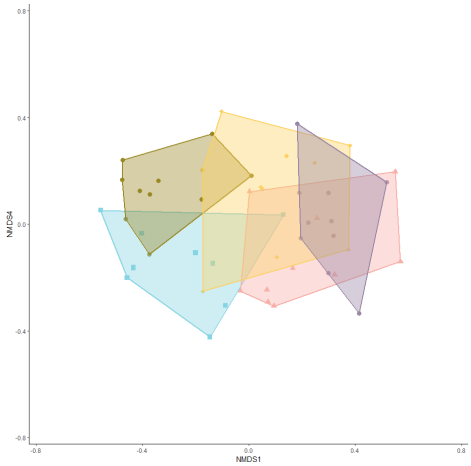
Appendix B-4: NMDS Ordinations on alternative dimensions (macroinvertebrates)

WETLANDS k=4; stress = 0.1342

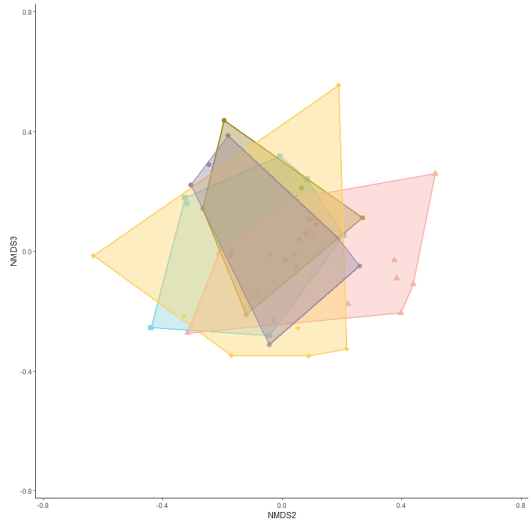
NMDS 1 – NMDS 3



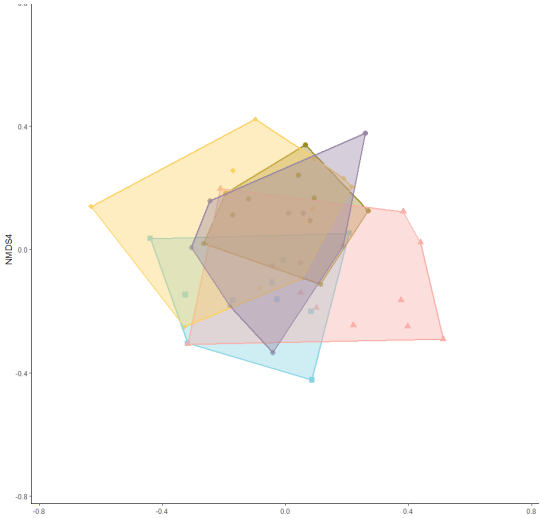
NMDS 1 – NMDS 4



NMDS2 – NMDS 3

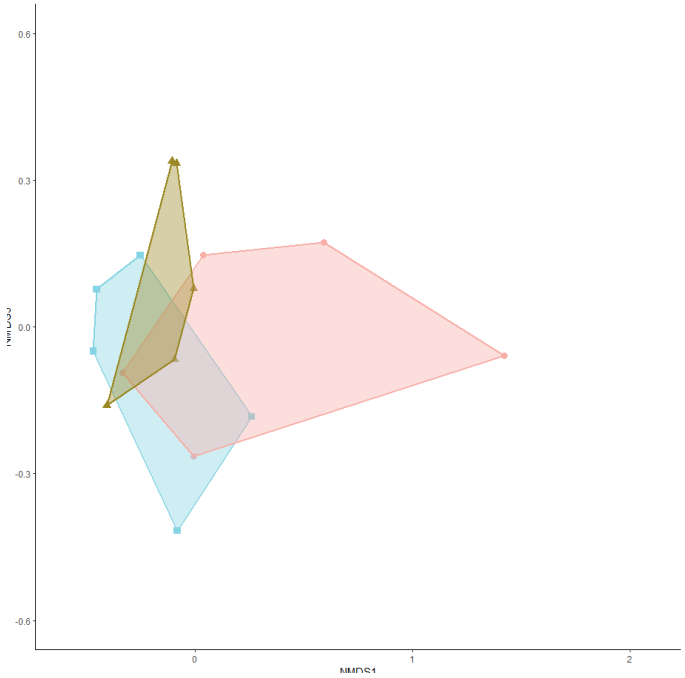


NMDS 2 – NMDS 4

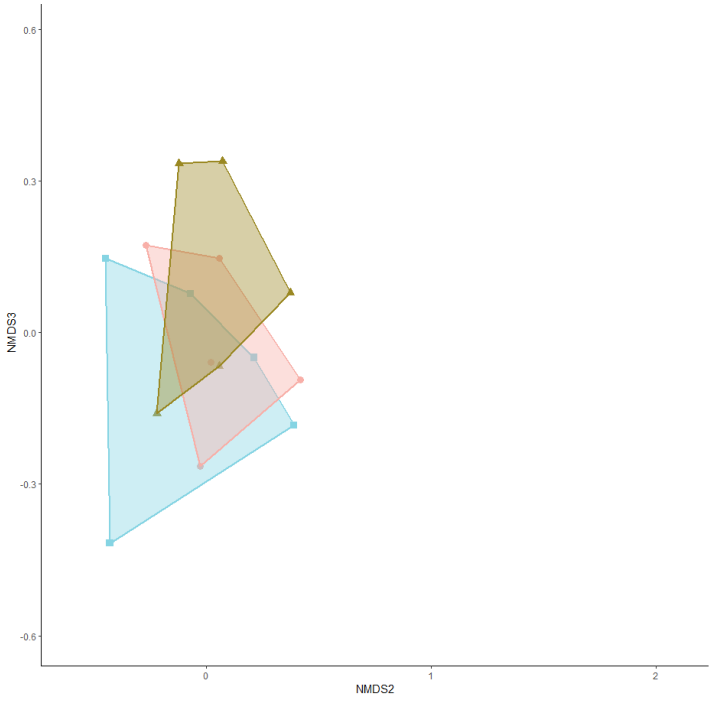


RIVER CANARD k=3; stress = 0.0612

NMDS 1 – NMDS 3

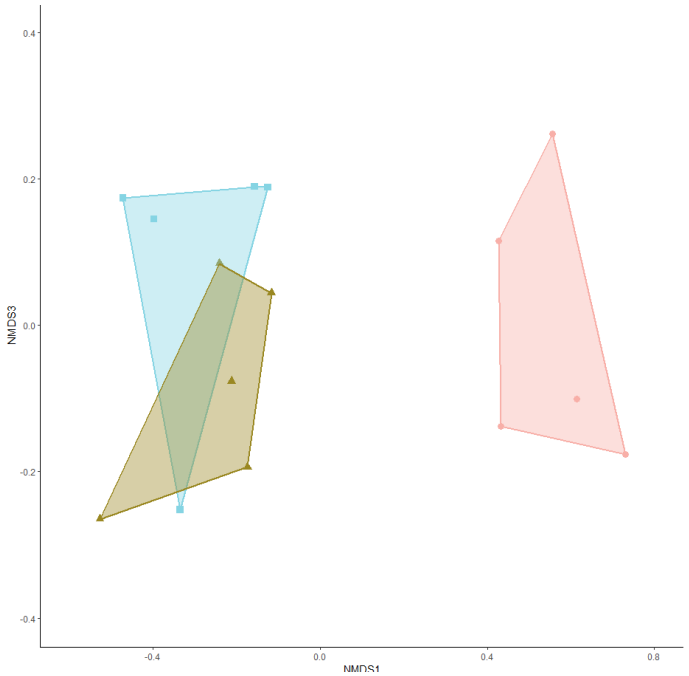


NMDS2 – NMDS 3

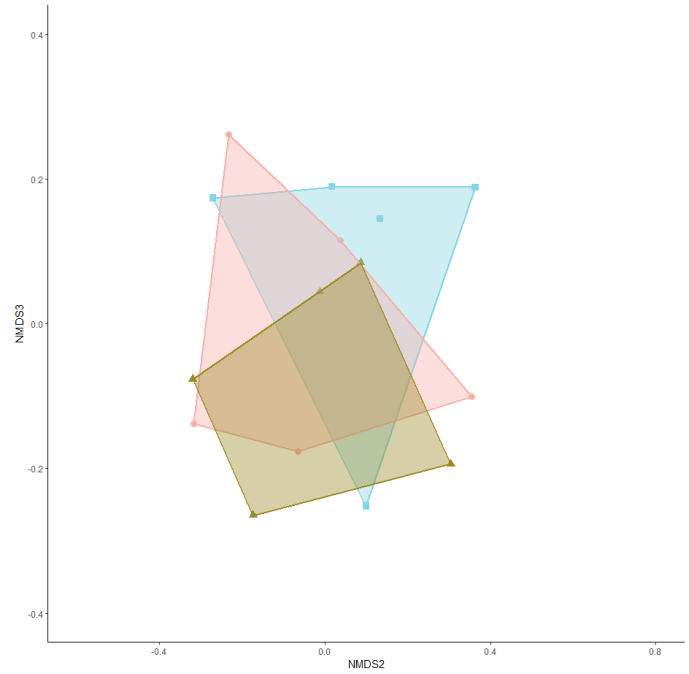


TURKEY CREEK k=3; stress 0.077

NMDS 1 – NMDS 3



NMDS2 – NMDS 3



Appendix B-5: Permutation test for homogeneity of multivariate dispersions (macroinvertebrates)

Wetland Strata

	Df	Sum of Squares (SS)	Mean SS	F	N. Perm	Pr (>F)
Groups	4	0.036044	0.009011	2.693	999	0.037
Residuals	45	0.150570	0.003346			

	DRM	GRASS	PECHE	RIVER CANARD	TURKEY CREEK
DRM		0.347000	0.449000	0.047000	0.392
GRASS	0.354152		0.169000	0.009000	0.987
PECHE	0.467824	0.184946		0.297000	0.179
RIVER CANARD	0.036841	0.010353	0.316977		0.015
TURKEY CREEK	0.381857	0.987035	0.198599	0.014013	

Tributary-Impacted Wetlands by Position

		Df	Sum of Squares (SS)	Mean SS	F	N. Perm	Pr (>F)
River Canard							
	Groups	2	0.008451	0.0042253	0.6195	999	0.587
	Residuals	12	0.081849	0.0068208			
Turkey Creek							
	Groups	2	0.001834	0.00091686	0.3002	999	0.74
	Residuals	12	0.036650	0.00305414			

Appendix C

Appendix C-1: PCR cycle reagents and amplification profiles

PCR cycle 1 and PCR cycle 2 reagents

PCR cycle 1			PCR cycle 2		
Reagent	Concentration	Volume (µl)	Reagent	Concentration	Volume (µl)
10x Buffer	10X	2.5	10x Buffer	10X	2.5
$MgSO_4$		1.0	MgSO4		3.5
dNTPs	10mM	0.5	dNTPs	10mM	0.5
F-Primer	10mM	0.5	F-UniA Primer	10mM	0.5
R-Primer	10mM	0.5	R-UniB Primer	10mM	0.5
TAQ Polymerase		0.1	TAQ Polymerase		0.1
diH2O		17.9	diH2O		7.4
DNA template (eDNA)		2.0	DNA template (Bead-cleaned PCR1 product)		10

PCR amplification profiles for cycle one and cycle two.

Stage	Time	Temperature (°C)	Cycles
First PCR			
Initial Denaturation	5 min	94	
Denaturation	15s	94	35
Annealing	15s	48	35
Elongation	30s	72	35
Final Elongation	1 min	72	
Second PCR			
Initial Denaturation	5 min	94	
Denaturation	15s	94	7
Annealing	15s	48	7
Elongation	30s	72	7
Final Elongation	1 min	72	

Appendix C-2: Statistics of most abundant taxa in relative abundance by taxonomic ranking

PHYLUM

	Acido-bacteria	Actino-bacteria	Bacter-oidetes	Chloro-flexi	Latesci-bacteria	Nitro-spinae	Nitro-spirae	Plancto-mycetes	Proteo-bacteria	Verruco-microbia
Detroit River	.	.	.	.	.	.	.	.	.	.
Mean	0.108	0.042	0.048	0.038	0.013	0.02	0.017	0.029	0.577	0.05
Std. Dev	0.022	0.018	0.012	0.016	0.004	0.008	0.005	0.013	0.071	0.013
Std Error.	0.003	0.003	0.002	0.002	0.001	0.001	0.001	0.002	0.01	0.002
Median	0.105	0.038	0.048	0.037	0.012	0.02	0.016	0.026	0.587	0.052
Minimum	0.061	0.007	0.029	0.006	0.005	0.001	0.007	0.006	0.454	0.02
Maximum	0.16	0.108	0.086	0.095	0.021	0.039	0.034	0.068	0.706	0.069
DRM										
Mean	0.112	0.052	0.056	0.038	0.014	0.02	0.015	0.029	0.536	0.054
Std. Dev	0.012	0.021	0.014	0.013	0.002	0.007	0.005	0.009	0.056	0.006
Std Error.	0.005	0.008	0.005	0.005	0.001	0.003	0.002	0.003	0.021	0.002
Median	0.113	0.046	0.056	0.032	0.014	0.021	0.014	0.026	0.518	0.053
Minimum	0.095	0.028	0.036	0.027	0.011	0.01	0.007	0.02	0.465	0.048
Maximum	0.128	0.089	0.082	0.064	0.018	0.03	0.021	0.041	0.623	0.066
Grass										
Mean	0.107	0.041	0.052	0.029	0.014	0.022	0.015	0.024	0.585	0.052
Std. Dev	0.018	0.012	0.014	0.01	0.005	0.006	0.004	0.009	0.069	0.013

	Acido-bacteria	Actino-bacteria	Bacter-oidetes	Chloro-flexi	Latesci-bacteria	Nitro-spinae	Nitro-spirae	Plancto-mycetes	Proteo-bacteria	Verruco-microbia
Std Error.	0.006	0.004	0.004	0.003	0.002	0.002	0.001	0.003	0.022	0.004
Median	0.105	0.039	0.051	0.028	0.013	0.021	0.015	0.025	0.577	0.053
Minimum	0.081	0.027	0.034	0.011	0.007	0.014	0.01	0.008	0.498	0.032
Maximum	0.133	0.064	0.086	0.046	0.021	0.032	0.021	0.034	0.679	0.069
Peche Island										
Mean	0.127	0.042	0.042	0.043	0.015	0.024	0.015	0.04	0.543	0.056
Std. Dev	0.031	0.009	0.007	0.013	0.005	0.009	0.002	0.017	0.088	0.013
Std Error.	0.01	0.003	0.002	0.004	0.001	0.003	0.001	0.005	0.028	0.004
Median	0.132	0.04	0.04	0.046	0.016	0.024	0.015	0.041	0.499	0.062
Minimum	0.081	0.032	0.033	0.021	0.008	0.012	0.013	0.016	0.454	0.031
Maximum	0.16	0.06	0.053	0.058	0.021	0.039	0.019	0.068	0.7	0.067
River Canard										
Mean	0.104	0.045	0.047	0.046	0.011	0.019	0.023	0.027	0.585	0.042
Std. Dev	0.014	0.026	0.014	0.018	0.003	0.011	0.006	0.012	0.058	0.012
Std Error.	0.004	0.008	0.004	0.006	0.001	0.003	0.002	0.004	0.018	0.004
Median	0.101	0.034	0.045	0.042	0.01	0.018	0.022	0.023	0.603	0.036
Minimum	0.09	0.026	0.029	0.028	0.008	0.001	0.012	0.017	0.482	0.029
Maximum	0.132	0.108	0.075	0.095	0.017	0.036	0.034	0.057	0.644	0.061
Turkey Creek										

	Acido-bacteria	Actino-bacteria	Bacter-oidetes	Chloro-flexi	Latesci-bacteria	Nitro-spinae	Nitro-spirae	Plancto-mycetes	Proteo-bacteria	Verruco-microbia
Mean	0.091	0.031	0.046	0.031	0.01	0.017	0.016	0.024	0.63	0.047
Std. Dev	0.014	0.018	0.009	0.017	0.002	0.005	0.005	0.01	0.041	0.014
Std Error.	0.005	0.006	0.003	0.006	0.001	0.002	0.002	0.003	0.014	0.005
Median	0.093	0.027	0.045	0.031	0.011	0.018	0.015	0.026	0.622	0.049
Minimum	0.061	0.007	0.029	0.006	0.005	0.01	0.01	0.006	0.586	0.02
Maximum	0.11	0.075	0.065	0.068	0.012	0.026	0.025	0.04	0.706	0.061
RC Downstream										
Mean	0.103	0.055	0.044	0.052	0.01	0.015	0.023	0.028	0.59	0.036
Std. Dev	0.013	0.034	0.012	0.024	0.001	0.008	0.006	0.017	0.066	0.005
Std Error.	0.006	0.015	0.005	0.011	0.001	0.004	0.003	0.007	0.029	0.002
Median	0.101	0.035	0.041	0.042	0.009	0.017	0.021	0.021	0.607	0.034
Minimum	0.093	0.029	0.029	0.038	0.008	0.001	0.018	0.017	0.483	0.031
Maximum	0.124	0.108	0.059	0.095	0.011	0.024	0.034	0.057	0.644	0.043
RC Tributary										
Mean	0.12	0.042	0.042	0.035	0.012	0.022	0.031	0.025	0.585	0.04
Std. Dev	0.044	0.018	0.01	0.007	0.004	0.011	0.016	0.009	0.081	0.01
Std Error.	0.02	0.008	0.005	0.003	0.002	0.005	0.007	0.004	0.036	0.005
Median	0.11	0.043	0.046	0.032	0.01	0.02	0.03	0.029	0.595	0.04
Minimum	0.073	0.014	0.027	0.029	0.007	0.011	0.01	0.012	0.465	0.027

	Acido-bacteria	Actino-bacteria	Bacter-oidetes	Chloro-flexi	Latesci-bacteria	Nitro-spinae	Nitro-spirae	Plancto-mycetes	Proteo-bacteria	Verruco-microbia
Maximum	0.189	0.06	0.053	0.046	0.019	0.035	0.051	0.032	0.67	0.052
RC Upstream										
Mean	0.105	0.035	0.05	0.039	0.012	0.023	0.023	0.026	0.581	0.048
Std. Dev	0.017	0.011	0.016	0.009	0.003	0.012	0.007	0.007	0.057	0.014
Std Error.	0.008	0.005	0.007	0.004	0.001	0.005	0.003	0.003	0.025	0.006
Median	0.101	0.032	0.049	0.04	0.011	0.026	0.026	0.023	0.598	0.054
Minimum	0.09	0.026	0.036	0.028	0.009	0.008	0.012	0.018	0.482	0.029
Maximum	0.132	0.052	0.075	0.051	0.017	0.036	0.029	0.037	0.624	0.061
TC Downstream										
Mean	0.096	0.026	0.046	0.027	0.011	0.015	0.019	0.023	0.627	0.048
Std. Dev	0.005	0.004	0.004	0.007	0.001	0.003	0.005	0.007	0.034	0.016
Std Error.	0.002	0.002	0.002	0.003	0.001	0.001	0.002	0.003	0.015	0.007
Median	0.094	0.025	0.044	0.024	0.011	0.014	0.02	0.023	0.622	0.055
Minimum	0.092	0.022	0.042	0.019	0.009	0.013	0.014	0.013	0.586	0.02
Maximum	0.105	0.032	0.051	0.037	0.012	0.018	0.025	0.031	0.68	0.06
TC Tributary										
Mean	0.093	0.026	0.041	0.035	0.014	0.015	0.009	0.024	0.617	0.046
Std. Dev	0.014	0.005	0.01	0.003	0.004	0.003	0.006	0.003	0.054	0.01
Std Error.	0.006	0.002	0.005	0.002	0.002	0.001	0.003	0.001	0.024	0.005

	Acido-bacteria	Actino-bacteria	Bacter-oidetes	Chloro-flexi	Latesci-bacteria	Nitro-spinae	Nitro-spirae	Plancto-mycetes	Proteo-bacteria	Verruco-microbia
Median	0.09	0.026	0.047	0.035	0.015	0.016	0.009	0.024	0.601	0.045
Minimum	0.076	0.019	0.028	0.03	0.01	0.012	0	0.02	0.555	0.035
Maximum	0.114	0.033	0.051	0.039	0.02	0.017	0.017	0.028	0.691	0.062
TC Upstream										
Mean	0.105	0.035	0.05	0.039	0.012	0.023	0.023	0.026	0.581	0.048
Std. Dev	0.017	0.011	0.016	0.009	0.003	0.012	0.007	0.007	0.057	0.014
Std Error.	0.008	0.005	0.007	0.004	0.001	0.005	0.003	0.003	0.025	0.006
Median	0.101	0.032	0.049	0.04	0.011	0.026	0.026	0.023	0.598	0.054
Minimum	0.09	0.026	0.036	0.028	0.009	0.008	0.012	0.018	0.482	0.029
Maximum	0.132	0.052	0.075	0.051	0.017	0.036	0.029	0.037	0.624	0.061

CLASS

	Acidi-microbiia	α Proteo-bacteria	Anaero-lineae	Bact-eroidia	δ-Proteo-bacteria	γ Proteo-bacteria	KD4.96	P9X2b3D02	Subgroup 6	Verruco-microbiae
Detroit River										
Mean	0.02	0.033	0.02	0.035	0.102	0.445	0.018	0.021	0.049	0.051
Std. Dev	0.008	0.008	0.007	0.012	0.02	0.063	0.01	0.008	0.015	0.013
Std Error.	0.001	0.001	0.001	0.002	0.003	0.009	0.001	0.001	0.002	0.002
Medians	0.018	0.033	0.02	0.033	0.103	0.447	0.016	0.02	0.047	0.053
Minimum	0.007	0.018	0.005	0.02	0.058	0.341	0.001	0.001	0.025	0.02
Maximum	0.051	0.059	0.036	0.074	0.143	0.605	0.06	0.039	0.088	0.07
DRM										
Mean	0.021	0.033	0.019	0.044	0.102	0.404	0.018	0.02	0.052	0.055
Std. Dev	0.004	0.005	0.005	0.014	0.017	0.043	0.008	0.007	0.008	0.006
Std Error.	0.002	0.002	0.002	0.005	0.006	0.016	0.003	0.003	0.003	0.002
Medians	0.021	0.033	0.017	0.041	0.106	0.38	0.015	0.021	0.054	0.053
Minimum	0.017	0.025	0.013	0.028	0.066	0.37	0.011	0.01	0.039	0.048
Maximum	0.028	0.039	0.028	0.074	0.114	0.484	0.034	0.031	0.064	0.066
Grass Island										
Mean	0.018	0.03	0.016	0.039	0.104	0.454	0.013	0.023	0.048	0.053
Std. Dev	0.003	0.006	0.005	0.013	0.022	0.059	0.006	0.007	0.014	0.013
Std Error.	0.001	0.002	0.002	0.004	0.007	0.019	0.002	0.002	0.004	0.004
Medians	0.018	0.029	0.016	0.039	0.105	0.452	0.012	0.021	0.049	0.054
Minimum	0.014	0.023	0.007	0.022	0.058	0.363	0.003	0.014	0.025	0.032
Maximum	0.024	0.039	0.023	0.071	0.143	0.54	0.023	0.032	0.07	0.07
Peche Island										
Mean	0.019	0.034	0.022	0.03	0.091	0.422	0.021	0.024	0.06	0.057
Std. Dev	0.003	0.005	0.006	0.006	0.013	0.075	0.007	0.009	0.021	0.013

	Acidi-microbiia	α Proteo-bacteria	Anaero-lineae	Bact-eroidia	δ-Proteo-bacteria	γ Proteo-bacteria	KD4.96	P9X2b3D02	Subgroup 6	Verruco-microbiae
Std Error.	0.001	0.002	0.002	0.002	0.004	0.024	0.002	0.003	0.007	0.004
Medians	0.019	0.034	0.024	0.029	0.087	0.386	0.022	0.024	0.066	0.063
Minimum	0.016	0.023	0.012	0.023	0.08	0.344	0.009	0.012	0.026	0.031
Maximum	0.025	0.041	0.03	0.041	0.12	0.562	0.032	0.039	0.088	0.069
River Canard										
Mean	0.024	0.035	0.024	0.032	0.107	0.447	0.022	0.019	0.046	0.042
Std. Dev	0.013	0.013	0.008	0.014	0.021	0.056	0.014	0.011	0.01	0.012
Std Error.	0.004	0.004	0.002	0.004	0.007	0.018	0.004	0.003	0.003	0.004
Medians	0.018	0.03	0.024	0.028	0.111	0.454	0.018	0.018	0.043	0.036
Minimum	0.013	0.02	0.007	0.02	0.065	0.341	0.011	0.001	0.033	0.03
Maximum	0.051	0.059	0.036	0.065	0.134	0.51	0.06	0.037	0.066	0.062
Turkey Creek										
Mean	0.018	0.034	0.017	0.032	0.108	0.489	0.013	0.017	0.039	0.048
Std. Dev	0.011	0.009	0.009	0.009	0.025	0.05	0.009	0.005	0.007	0.014
Std Error.	0.004	0.003	0.003	0.003	0.008	0.017	0.003	0.002	0.002	0.005
Medians	0.014	0.032	0.018	0.031	0.108	0.477	0.013	0.018	0.038	0.05
Minimum	0.007	0.018	0.005	0.021	0.067	0.431	0.001	0.011	0.032	0.02
Maximum	0.044	0.05	0.035	0.053	0.134	0.605	0.034	0.027	0.054	0.065
RC Downstream										
Mean	0.026	0.036	0.026	0.029	0.102	0.456	0.026	0.015	0.048	0.036
Std. Dev	0.015	0.014	0.005	0.01	0.023	0.059	0.019	0.009	0.011	0.005
Std Error.	0.007	0.006	0.002	0.004	0.01	0.026	0.009	0.004	0.005	0.002
Medians	0.019	0.034	0.025	0.024	0.104	0.458	0.019	0.017	0.045	0.034
Minimum	0.015	0.02	0.022	0.021	0.065	0.364	0.013	0.001	0.039	0.032

	Acidi-microbiia	α Proteo-bacteria	Anaero-lineae	Bact-eroidia	δ-Proteo-bacteria	γ Proteo-bacteria	KD4.96	P9X2b3D02	Subgroup 6	Verruco-microbiae
Maximum	0.051	0.058	0.036	0.044	0.124	0.51	0.06	0.024	0.066	0.044
RC Tributary										
Mean	0.023	0.043	0.018	0.029	0.105	0.441	0.016	0.022	0.064	0.04
Std. Dev	0.011	0.028	0.003	0.009	0.026	0.068	0.007	0.011	0.027	0.01
Std Error.	0.005	0.013	0.001	0.004	0.012	0.03	0.003	0.005	0.012	0.005
Medians	0.02	0.05	0.019	0.031	0.096	0.446	0.014	0.02	0.062	0.04
Minimum	0.012	0.011	0.013	0.015	0.076	0.344	0.009	0.011	0.034	0.028
Maximum	0.037	0.078	0.02	0.04	0.147	0.516	0.027	0.035	0.107	0.053
RC Upstream										
Mean	0.022	0.033	0.021	0.035	0.113	0.439	0.017	0.024	0.044	0.048
Std. Dev	0.011	0.014	0.009	0.017	0.02	0.058	0.004	0.012	0.009	0.014
Std Error.	0.005	0.006	0.004	0.008	0.009	0.026	0.002	0.005	0.004	0.006
Medians	0.017	0.028	0.022	0.032	0.118	0.45	0.018	0.027	0.042	0.054
Minimum	0.013	0.024	0.007	0.02	0.091	0.341	0.011	0.008	0.033	0.03
Maximum	0.039	0.059	0.032	0.065	0.134	0.495	0.021	0.037	0.058	0.062
TC Downstream										
Mean	0.014	0.033	0.015	0.03	0.126	0.472	0.011	0.015	0.036	0.049
Std. Dev	0.002	0.006	0.005	0.004	0.011	0.03	0.003	0.003	0.003	0.017
Std Error.	0.001	0.003	0.002	0.002	0.005	0.014	0.001	0.001	0.001	0.007
Medians	0.014	0.03	0.012	0.031	0.131	0.473	0.012	0.015	0.038	0.055
Minimum	0.012	0.029	0.012	0.025	0.108	0.431	0.007	0.013	0.033	0.02
Maximum	0.017	0.043	0.023	0.036	0.134	0.515	0.013	0.018	0.039	0.061
TC Tributary										
Mean	0.013	0.028	0.023	0.027	0.149	0.444	0.01	0.015	0.031	0.047

	Acidi-microbiia	α Proteo-bacteria	Anaero-lineae	Bact-eroidia	δ-Proteo-bacteria	γ Proteo-bacteria	KD4.96	P9X2b3D02	Subgroup 6	Verruco-microbiae
Std. Dev	0.003	0.004	0.005	0.009	0.026	0.053	0.002	0.003	0.009	0.01
Std Error.	0.001	0.002	0.002	0.004	0.012	0.024	0.001	0.001	0.004	0.005
Medians	0.013	0.027	0.022	0.028	0.147	0.434	0.01	0.017	0.031	0.047
Minimum	0.009	0.024	0.017	0.018	0.119	0.381	0.006	0.012	0.019	0.035
Maximum	0.016	0.035	0.029	0.041	0.191	0.513	0.012	0.017	0.044	0.062
TC Downstream										
Mean	0.022	0.033	0.021	0.035	0.113	0.439	0.017	0.024	0.044	0.048
Std. Dev	0.011	0.014	0.009	0.017	0.02	0.058	0.004	0.012	0.009	0.014
Std Error.	0.005	0.006	0.004	0.008	0.009	0.026	0.002	0.005	0.004	0.006
Medians	0.017	0.028	0.022	0.032	0.118	0.45	0.018	0.027	0.042	0.054
Minimum	0.013	0.024	0.007	0.02	0.091	0.341	0.011	0.008	0.033	0.03
Maximum	0.039	0.059	0.032	0.065	0.134	0.495	0.021	0.037	0.058	0.062

ORDER

	β-proteo-bacteriales	Chitino-phagales	Desulfo-bacterales	Desulfuro-monadales	Myxo-coccales	Pedo-sphaerales	Steroido-bacterales	Syntro-phobacterales	uncult. pro. Subgroup 6	Uncult. Pro. (P9X2b3 D02)
Detroit River										
Mean	0.286	0.025	0.022	0.016	0.016	0.04	0.118	0.021	0.022	0.019
Std. Dev	0.032	0.012	0.007	0.006	0.007	0.009	0.028	0.008	0.007	0.007
Std Error.	0.005	0.002	0.001	0.001	0.001	0.001	0.004	0.001	0.001	0.001
Median	0.289	0.021	0.022	0.016	0.017	0.04	0.113	0.02	0.022	0.019
Minimum	0.23	0.011	0.006	0.001	0.002	0.017	0.065	0.004	0.009	0
Maximum	0.348	0.065	0.044	0.03	0.029	0.065	0.211	0.043	0.039	0.036
DRM										
Mean	0.271	0.034	0.023	0.017	0.012	0.04	0.095	0.022	0.025	0.02
Std. Dev	0.03	0.014	0.005	0.007	0.005	0.003	0.017	0.008	0.005	0.008
Std Error.	0.011	0.005	0.002	0.003	0.002	0.001	0.007	0.003	0.002	0.003
Median	0.267	0.03	0.021	0.019	0.013	0.041	0.094	0.021	0.024	0.022
Minimum	0.242	0.024	0.016	0.005	0.002	0.034	0.072	0.01	0.017	0.008
Maximum	0.332	0.065	0.029	0.027	0.017	0.043	0.121	0.032	0.035	0.033
Grass Island										
Mean	0.296	0.027	0.02	0.02	0.013	0.041	0.121	0.023	0.023	0.02
Std. Dev	0.033	0.014	0.006	0.005	0.005	0.007	0.022	0.009	0.007	0.006
Std Error.	0.01	0.004	0.002	0.002	0.002	0.002	0.007	0.003	0.002	0.002
Median	0.294	0.024	0.021	0.02	0.014	0.04	0.115	0.021	0.024	0.02
Minimum	0.237	0.012	0.012	0.011	0.003	0.03	0.088	0.007	0.012	0.012
Maximum	0.348	0.063	0.032	0.026	0.019	0.054	0.154	0.043	0.034	0.031
Peche Island										
Mean	0.269	0.023	0.019	0.013	0.02	0.04	0.123	0.014	0.026	0.019
Std. Dev	0.036	0.007	0.005	0.004	0.002	0.007	0.032	0.003	0.01	0.009

	β-proteo-bacteriales	Chitino-phagales	Desulfo-bacterales	Desulfuro-monadales	Myxo-coccales	Pedo-sphaerales	Steroido-bacterales	Syntro-phobacterales	uncult. pro. Subgroup 6	Uncult. Pro. (P9X2b3D02)
Std Error.	0.011	0.002	0.002	0.001	0.001	0.002	0.01	0.001	0.003	0.003
Median	0.255	0.02	0.018	0.012	0.021	0.04	0.112	0.014	0.026	0.019
Minimum	0.23	0.015	0.014	0.008	0.017	0.028	0.086	0.009	0.009	0.008
Maximum	0.331	0.039	0.03	0.021	0.023	0.052	0.186	0.017	0.039	0.036
River Canard										
Mean	0.294	0.025	0.024	0.012	0.015	0.035	0.109	0.026	0.02	0.018
Std. Dev	0.027	0.015	0.004	0.005	0.007	0.011	0.021	0.008	0.004	0.009
Std Error.	0.008	0.005	0.001	0.002	0.002	0.003	0.007	0.002	0.001	0.003
Median	0.294	0.021	0.023	0.013	0.013	0.033	0.111	0.028	0.019	0.017
Minimum	0.252	0.011	0.019	0.001	0.004	0.017	0.065	0.011	0.014	0
Maximum	0.324	0.065	0.032	0.018	0.022	0.052	0.141	0.04	0.029	0.03
Turkey Creek										
Mean	0.298	0.019	0.027	0.017	0.019	0.043	0.137	0.018	0.017	0.017
Std. Dev	0.028	0.004	0.01	0.007	0.01	0.014	0.032	0.007	0.005	0.006
Std Error.	0.009	0.001	0.003	0.002	0.003	0.005	0.011	0.002	0.002	0.002
Median	0.299	0.019	0.029	0.015	0.02	0.043	0.129	0.018	0.016	0.019
Minimum	0.251	0.014	0.006	0.006	0.005	0.02	0.103	0.004	0.011	0.01
Maximum	0.341	0.026	0.044	0.03	0.029	0.065	0.211	0.026	0.029	0.026
RC Downstream										
Mean	0.295	0.021	0.023	0.009	0.013	0.029	0.112	0.027	0.02	0.014
Std. Dev	0.027	0.006	0.005	0.006	0.006	0.006	0.019	0.005	0.003	0.009
Std Error.	0.012	0.003	0.002	0.003	0.003	0.003	0.009	0.002	0.001	0.004
Median	0.296	0.021	0.023	0.009	0.012	0.03	0.113	0.028	0.02	0.015
Minimum	0.252	0.014	0.019	0.001	0.006	0.017	0.093	0.022	0.018	0

	β-proteo-bacteriales	Chitino-phagales	Desulfo-bacterales	Desulfuro-monadales	Myxo-coccales	Pedo-sphaerales	Steroido-bacterales	Syntro-phobacterales	uncult. pro. Subgroup 6	Uncult. Pro. (P9X2b3 D02)
Maximum	0.324	0.03	0.032	0.018	0.022	0.033	0.141	0.032	0.026	0.024
RC Tributary										
Mean	0.297	0.021	0.02	0.012	0.016	0.033	0.103	0.022	0.03	0.021
Std. Dev	0.046	0.01	0.011	0.005	0.003	0.006	0.028	0.012	0.016	0.009
Std Error.	0.021	0.004	0.005	0.002	0.001	0.003	0.012	0.005	0.007	0.004
Median	0.276	0.024	0.017	0.012	0.018	0.034	0.104	0.017	0.027	0.021
Minimum	0.255	0.004	0.01	0.006	0.012	0.026	0.059	0.011	0.014	0.012
Maximum	0.365	0.029	0.035	0.018	0.02	0.042	0.131	0.042	0.057	0.031
RC Upstream										
Mean	0.292	0.029	0.024	0.015	0.016	0.041	0.105	0.026	0.02	0.021
Std. Dev	0.029	0.021	0.003	0.003	0.008	0.011	0.025	0.01	0.006	0.009
Std Error.	0.013	0.01	0.001	0.001	0.004	0.005	0.011	0.005	0.003	0.004
Median	0.289	0.024	0.026	0.014	0.02	0.043	0.108	0.027	0.019	0.022
Minimum	0.252	0.011	0.02	0.011	0.004	0.027	0.065	0.011	0.014	0.008
Maximum	0.323	0.065	0.027	0.018	0.022	0.052	0.132	0.04	0.029	0.03
TC Downstream										
Mean	0.288	0.018	0.032	0.019	0.023	0.043	0.128	0.02	0.014	0.015
Std. Dev	0.03	0.003	0.008	0.006	0.009	0.013	0.02	0.006	0.003	0.004
Std Error.	0.014	0.001	0.003	0.003	0.004	0.006	0.009	0.003	0.001	0.002
Median	0.291	0.018	0.032	0.018	0.027	0.05	0.123	0.019	0.014	0.012
Minimum	0.251	0.014	0.023	0.014	0.008	0.02	0.103	0.012	0.011	0.01
Maximum	0.333	0.021	0.044	0.03	0.029	0.051	0.153	0.026	0.018	0.02
TC Tributary										
Mean	0.325	0.012	0.027	0.031	0.024	0.043	0.079	0.034	0.015	0.014

	β-proteo-bacteriales	Chitino-phagales	Desulfo-bacterales	Desulfuro-monadales	Myxo-coccales	Pedo-sphaerales	Steroido-bacterales	Syntro-phobacterales	uncult. pro. Subgroup 6	Uncult. Pro. (P9X2b3D02)
Std. Dev	0.046	0.006	0.003	0.008	0.005	0.007	0.007	0.009	0.004	0.003
Std Error.	0.021	0.003	0.001	0.004	0.002	0.003	0.003	0.004	0.002	0.001
Median	0.327	0.009	0.026	0.03	0.026	0.044	0.082	0.036	0.015	0.015
Minimum	0.268	0.006	0.022	0.024	0.018	0.034	0.072	0.018	0.01	0.011
Maximum	0.373	0.022	0.031	0.043	0.029	0.052	0.085	0.043	0.021	0.019
TC Upstream										
Mean	0.292	0.029	0.024	0.015	0.016	0.041	0.105	0.026	0.02	0.021
Std. Dev	0.029	0.021	0.003	0.003	0.008	0.011	0.025	0.01	0.006	0.009
Std Error.	0.013	0.01	0.001	0.001	0.004	0.005	0.011	0.005	0.003	0.004
Median	0.289	0.024	0.026	0.014	0.02	0.043	0.108	0.027	0.019	0.022
Minimum	0.252	0.011	0.02	0.011	0.004	0.027	0.065	0.011	0.014	0.008
Maximum	0.323	0.065	0.027	0.018	0.022	0.052	0.132	0.04	0.029	0.03

FAMILY:

	Burkholderiaceae	Chitinophagaceae	Desulfobacteraceae	Hydrogenophilaceae	Pedosphaeraceae	Rhodocyclaceae	SC.I.84	Steroidobacteraceae	Uncult. Subgroup 6	Uncult. (P9X2b3D02)
Detroit River										
Mean	0.1	0.026	0.019	0.022	0.043	0.034	0.088	0.129	0.024	0.021
Std. Dev	0.013	0.013	0.005	0.011	0.01	0.009	0.019	0.031	0.008	0.008
Std Error.	0.002	0.002	0.001	0.002	0.001	0.001	0.003	0.005	0.001	0.001
Median	0.104	0.023	0.018	0.021	0.044	0.033	0.087	0.125	0.024	0.021
Minimum	0.074	0.011	0.001	0.006	0.019	0.017	0.047	0.072	0.011	0
Maximum	0.127	0.068	0.031	0.05	0.072	0.056	0.151	0.233	0.042	0.039
DRM										
Mean	0.107	0.035	0.019	0.02	0.043	0.036	0.071	0.103	0.027	0.021
Std. Dev	0.006	0.014	0.004	0.013	0.003	0.009	0.013	0.019	0.006	0.009
Std Error.	0.002	0.005	0.001	0.005	0.001	0.003	0.005	0.007	0.002	0.003
Median	0.105	0.031	0.018	0.017	0.044	0.035	0.065	0.103	0.027	0.024
Minimum	0.1	0.026	0.015	0.006	0.037	0.023	0.057	0.079	0.019	0.009
Maximum	0.116	0.067	0.026	0.043	0.047	0.049	0.092	0.133	0.037	0.036
Grass Island										
Mean	0.109	0.029	0.018	0.022	0.045	0.03	0.09	0.133	0.025	0.022
Std. Dev	0.01	0.015	0.005	0.011	0.007	0.01	0.019	0.023	0.007	0.006
Std Error.	0.003	0.005	0.002	0.003	0.002	0.003	0.006	0.007	0.002	0.002
Median	0.109	0.025	0.018	0.022	0.045	0.027	0.09	0.13	0.026	0.022
Minimum	0.091	0.013	0.009	0.006	0.032	0.017	0.059	0.096	0.014	0.014
Maximum	0.127	0.068	0.029	0.036	0.059	0.046	0.129	0.167	0.037	0.034
Peche Island										

	Burkholderiaceae	Chitinophagaceae	Desulfobacteraceae	Hydrogenophilaceae	Pedosphaeraceae	Rhodocyclaceae	SC.I.84	Steroidobacteraceae	Uncult. Subgroup 6	Uncult. (P9X2b3D02)
Mean	0.092	0.024	0.017	0.014	0.044	0.032	0.085	0.136	0.028	0.021
Std. Dev	0.012	0.007	0.004	0.004	0.008	0.005	0.014	0.037	0.011	0.009
Std Error.	0.004	0.002	0.001	0.001	0.003	0.002	0.004	0.012	0.003	0.003
Median	0.091	0.021	0.016	0.012	0.044	0.031	0.083	0.124	0.028	0.021
Minimum	0.074	0.016	0.014	0.009	0.031	0.026	0.068	0.095	0.011	0.009
Maximum	0.112	0.04	0.027	0.022	0.058	0.041	0.116	0.208	0.042	0.039
River Canard										
Mean	0.099	0.025	0.022	0.031	0.038	0.038	0.086	0.118	0.022	0.019
Std. Dev	0.015	0.016	0.005	0.012	0.012	0.007	0.017	0.023	0.005	0.01
Std Error.	0.005	0.005	0.001	0.004	0.004	0.002	0.006	0.007	0.001	0.003
Median	0.105	0.022	0.021	0.03	0.035	0.04	0.089	0.122	0.02	0.018
Minimum	0.075	0.011	0.017	0.016	0.019	0.027	0.047	0.072	0.015	0
Maximum	0.121	0.067	0.031	0.05	0.057	0.046	0.11	0.153	0.032	0.033
Turkey Creek										
Mean	0.096	0.019	0.02	0.022	0.047	0.036	0.101	0.15	0.018	0.019
Std. Dev	0.011	0.004	0.009	0.008	0.015	0.01	0.023	0.036	0.006	0.007
Std Error.	0.004	0.001	0.003	0.003	0.005	0.003	0.008	0.012	0.002	0.002
Median	0.094	0.019	0.022	0.021	0.048	0.034	0.099	0.139	0.018	0.021
Minimum	0.079	0.014	0.001	0.012	0.022	0.024	0.079	0.111	0.012	0.011
Maximum	0.111	0.027	0.03	0.032	0.072	0.056	0.151	0.233	0.031	0.029
RC Downstream										
Mean	0.102	0.021	0.022	0.026	0.031	0.035	0.093	0.122	0.022	0.016
Std. Dev	0.007	0.007	0.006	0.01	0.007	0.007	0.01	0.021	0.003	0.01

	Burkholderiaceae	Chitinophagaceae	Desulfobacteraceae	Hydrogenophilaceae	Pedosphaeraceae	Rhodocyclaceae	SC.I.84	Steroidobacteraceae	Uncult. Subgroup 6	Uncult. (P9X2b3D02)
Std Error.	0.003	0.003	0.003	0.004	0.003	0.003	0.004	0.009	0.001	0.004
Median	0.105	0.022	0.021	0.023	0.033	0.033	0.091	0.124	0.021	0.017
Minimum	0.089	0.014	0.017	0.016	0.019	0.027	0.087	0.099	0.019	0
Maximum	0.106	0.031	0.031	0.042	0.036	0.045	0.11	0.153	0.027	0.026
RC Tributary										
Mean	0.108	0.022	0.017	0.032	0.037	0.041	0.083	0.113	0.033	0.023
Std. Dev	0.011	0.01	0.008	0.022	0.007	0.013	0.01	0.029	0.018	0.01
Std Error.	0.005	0.005	0.004	0.01	0.003	0.006	0.004	0.013	0.008	0.004
Median	0.103	0.026	0.015	0.03	0.037	0.035	0.082	0.119	0.03	0.023
Minimum	0.095	0.004	0.007	0.012	0.029	0.027	0.071	0.065	0.015	0.013
Maximum	0.121	0.03	0.028	0.068	0.046	0.06	0.096	0.141	0.063	0.035
RC Upstream										
Mean	0.096	0.029	0.022	0.036	0.045	0.041	0.079	0.114	0.022	0.023
Std. Dev	0.02	0.022	0.004	0.013	0.012	0.004	0.022	0.026	0.006	0.01
Std Error.	0.009	0.01	0.002	0.006	0.005	0.002	0.01	0.012	0.003	0.004
Median	0.092	0.024	0.021	0.038	0.046	0.042	0.077	0.12	0.02	0.025
Minimum	0.075	0.011	0.017	0.017	0.03	0.035	0.047	0.072	0.015	0.009
Maximum	0.121	0.067	0.026	0.05	0.057	0.046	0.104	0.14	0.032	0.033
TC Downstream										
Mean	0.091	0.018	0.025	0.024	0.047	0.042	0.086	0.14	0.016	0.016
Std. Dev	0.009	0.004	0.005	0.008	0.015	0.009	0.008	0.022	0.003	0.005
Std Error.	0.004	0.002	0.002	0.003	0.006	0.004	0.003	0.01	0.001	0.002
Median	0.09	0.018	0.026	0.028	0.054	0.041	0.084	0.135	0.015	0.014

	Burkholderiaceae	Chitinophagaceae	Desulfobacteraceae	Hydrogenophilaceae	Pedosphaeraceae	Rhodocyclaceae	SC.I.84	Steroidobacteraceae	Uncult. Subgroup 6	Uncult. (P9X2b3D02)
Minimum	0.079	0.014	0.017	0.013	0.022	0.034	0.079	0.111	0.012	0.011
Maximum	0.104	0.023	0.03	0.032	0.056	0.056	0.099	0.166	0.019	0.022
TC Tributary										
Mean	0.088	0.013	0.022	0.067	0.047	0.06	0.053	0.087	0.016	0.016
Std. Dev	0.014	0.007	0.004	0.017	0.008	0.012	0.009	0.007	0.004	0.003
Std Error.	0.006	0.003	0.002	0.008	0.003	0.005	0.004	0.003	0.002	0.001
Median	0.084	0.01	0.021	0.073	0.049	0.066	0.051	0.092	0.016	0.016
Minimum	0.072	0.007	0.015	0.049	0.037	0.045	0.044	0.079	0.01	0.012
Maximum	0.107	0.024	0.027	0.084	0.057	0.072	0.068	0.093	0.023	0.021
TC Upstream										
Mean	0.096	0.029	0.022	0.036	0.045	0.041	0.079	0.114	0.022	0.023
Std. Dev	0.02	0.022	0.004	0.013	0.012	0.004	0.022	0.026	0.006	0.01
Std Error.	0.009	0.01	0.002	0.006	0.005	0.002	0.01	0.012	0.003	0.004
Median	0.092	0.024	0.021	0.038	0.046	0.042	0.077	0.12	0.02	0.025
Minimum	0.075	0.011	0.017	0.017	0.03	0.035	0.047	0.072	0.015	0.009
Maximum	0.121	0.067	0.026	0.05	0.057	0.046	0.104	0.14	0.032	0.033

Appendix C-3: Alpha diversity by site (bacteria)

	shannon	richness	pielou
DRM13	4.13825	118	0.867433
DRM19	4.654181	193	0.884373
DRM2	4.4955	209	0.841486
DRM21	4.337875	159	0.855782
DRM3	5.025944	355	0.8559
DRM4	5.032015	323	0.870945
DRM6	5.373858	467	0.87432
GI1	4.274046	134	0.872639
GI11	5.019948	315	0.872644
GI13	4.339141	161	0.853926
GI19	5.090866	332	0.876959
GI21	4.787675	232	0.878999
GI24	4.517908	210	0.844926
GI3	4.13431	124	0.857691
GI4	4.631184	212	0.864578
GI6	4.712294	210	0.881279
GI9	3.935199	94	0.866155
PI12	4.775026	328	0.824273
PI15	5.351318	448	0.876576
PI16	4.642281	259	0.835419
PI17	5.466434	715	0.831741
PI18	5.425854	628	0.842192
PI2	5.114943	317	0.88818
PI6	5.279253	522	0.843646
PI7	4.95371	394	0.828885
PI8	5.320402	557	0.841494
PI9	5.374858	549	0.852057

	shannon	richness	pielou
RCD13	4.745614	251	0.858864
RCD18	4.451817	215	0.828918
RCD19	5.019306	303	0.878464
RCD21	4.852351	283	0.859516
RCD26	4.084646	117	0.857727
RCU1	4.158021	115	0.876308
RCU10	4.110008	110	0.87438
RCU2	5.096383	467	0.829175
RCU4	5.030664	380	0.846889
RCU8	4.794523	276	0.853057
TCD23	5.190482	331	0.894584
TCD24	4.016786	106	0.861336
TCD26	5.360331	399	0.895035
TCD29	5.376583	406	0.895149
TCD30	4.904309	283	0.868719
TCU3	4.370076	150	0.87216
TCU4	3.32	64	0.798291
TCU6	4.234933	131	0.868669
TCU9	5.063458	295	0.89036
TRC1	4.518072	224	0.834879
TRC2	4.292366	136	0.873736
TRC4	5.095984	441	0.83691
TRC5	3.655226	83	0.827191
TRC7	4.366236	206	0.819508
TTC3	4.402046	140	0.890806
TTC4	4.753954	269	0.849723
TTC5	5.413834	501	0.870866
TTC6	5.288907	484	0.855522
TTC7	4.869276	298	0.854695

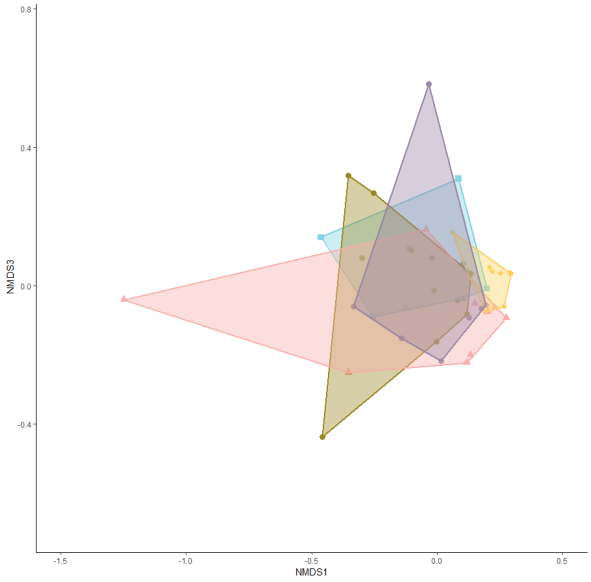
Appendix C-4: T-tests (bacteria)

	pielou J			richness			shannon		
	p	t	df	p	t	df	p	t	df
Impacted-Unimpacted	0.702	0.385	43.791	0.047	-2.021	69.326	0.167	-1.398	58.053
DRM-Grass	0.654	-0.405	11.415	0.302	1.093	9.278	0.400	0.873	11.693
DRM-Peche	0.026	2.105	14.999	0.006	-3.184	14.339	0.041	-2.365	9.579
DRM-River Canard	0.160	1.028	14.600	0.885	0.148	12.582	0.680	0.422	12.243
DRM-Turkey Creek	0.730	-0.630	11.864	0.759	0.313	13.296	0.801	0.257	13.493
Grass-Peche	0.008	2.719	14.307	0.000	-5.103	13.675	0.001	-4.178	16.851
Grass-River Canard	0.067	1.581	15.709	0.287	-1.102	15.613	0.610	-0.518	17.932
Grass-Turkey Creek	0.660	-0.425	10.141	0.458	-0.766	12.843	0.699	-0.396	11.929
Peche-River Canard	0.866	-1.146	17.570	0.002	3.683	17.171	0.003	3.438	16.353
Peche-Turkey Creek	0.971	-2.075	13.878	0.002	3.632	16.996	0.064	2.069	10.375
River Canard-Turkey Creek	0.894	-1.312	12.542	0.424	0.195	16.303	0.900	-1.395	7.906
RCUP-RCDN	0.623	-0.498	26.488	0.611	-0.515	26.075	0.761	-0.308	26.223
RCDN-TRC	0.226	1.248	21.436	0.278	-1.112	21.914	0.422	0.819	21.915
TRC-RCUP	0.572	0.575	19.490	0.515	0.662	20.676	0.515	0.662	20.676
TCUP-TCDN	0.761	-0.308	26.223	0.611	-0.515	26.075	0.761	-0.308	26.223
TCDN-TTC	0.395	-0.866	22.824	0.668	0.435	20.109	0.395	-0.866	22.824
TTC-TCUP	0.192	-1.347	21.850	0.361	-0.939	16.797	0.192	-1.347	21.850

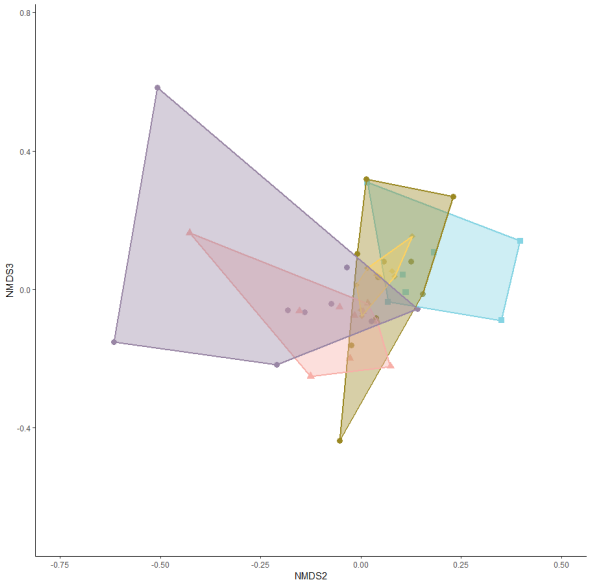
Appendix C-5: NMDS Wetland Ordinations on alternative dimensions (bacteria)

WETLANDS k=3; stress = 0.1034

NMDS 1 – NMDS 3



NMDS2 – NMDS 3



Appendix C-6: Permutation test for homogeneity of multivariate dispersions (bacteria)

	Df	Sum of Squares	R^2	F	Pr(>F)
Wetlands					
GROUPS	4	0.078	0.020	2.790	0.042*
Residual	41	0.286	0.007	NA	NA
River Canard					
GROUPS	1	0.007	0.007	0.975	0.322
Residual	13	0.088	0.007	NA	NA
Turkey Creek					
GROUPS	2	0.017	0.008	0.679	0.606
Residual	11	0.135	0.012	NA	NA

	DRM	GRASS	PECHE	RIVER CANARD	TURKEY CREEK
DRM		0.829	0.81	0.807	0.006
GRASS	0.821		0.927	0.894	0.002
PECHE	0.793	0.913		0.973	0.007
RIVER CANARD	0.811	0.897	0.968		0.035
TURKEY CREEK	0.010	0.002	0.009	0.0317	

Appendix C-7 PERMANOVA pairwise results by strata (bacteria)

	Df	Sums of Squares	F.Model	R^2	p	p adjusted
WETLAND PAIRS						
DRM vs GRASS ISLAND	1	0.122	0.940	0.059	0.536	1.000
DRM vs PECHE ISLAND	1	0.221	1.659	0.100	0.012*	0.120
DRM vs RIVER CANARD	1	0.215	1.562	0.094	0.048*	0.479
DRM vs TURKEY CREEK	1	0.261	2.799	0.167	0.002*	**0.022***
GRASS ISLAND vs PECHE ISLAND	1	0.214	1.620	0.083	0.013*	0.132
GRASS ISLAND vs RIVER CANARD	1	0.204	1.503	0.077	0.057*	0.565
GRASS ISLAND vs TURKEY CREEK	1	0.377	3.802	0.183	0.000*	**0.002***
PECHE ISLAND vs RIVER CANARD	1	0.139	1.000	0.053	0.373	1.000
PECHE ISLAND vs TURKEY CREEK	1	0.296	2.885	0.145	0.000*	0.004*
RIVER CANARD vs TURKEY CREEK	1	0.280	2.633	0.134	0.002*	0.020*
RIVER CANARD PAIRS						
UPSTREAM vs DOWNSTREAM	1	0.155	1.044	0.115	0.319	0.956
UPSTREAM vs TRIBUTARY	1	0.207	1.134	0.124	0.310	0.930
DOWNSTREAM vs TRIBUTARY	1	0.236	1.366	0.146	0.039	0.116
TURKEY CREEK PAIRS						
DOWNSTREAM vs UPSTREAM	1	0.223	1.485	0.175	0.092	0.275
DOWNSTREAM vs TRIBUTARY	1	0.314	2.519	0.240	0.008	0.023*
UPSTREAM vs TRIBUTARY	1	0.404	2.491	0.262	0.007	0.021*

Appendix C-8: Differential abundance - DESeq2 results – significantly different taxa by strata

	Phylum	Class	Order	Family	Genus	Species	Base Mean	log2 Fold Change	lfcSE	stat	padj
Impaired to non-impaired											
	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingorhabdus	Sphingomonas sp. 201	1.205	2.826	0.650	4.345	0.004
	Actinobacteria	Acidimicrobiia	Microtrichales	Ilumatobacteraceae	CL500-29 marine group	marine metagenome	1.767	2.561	0.550	4.658	0.002
	Actinobacteria	Acidimicrobiia	Microtrichales	Ilumatobacteraceae	CL500-29 marine group	uncultured Acidimicrobiales bacterium	0.938	2.622	0.689	3.807	0.030
	Verrucomicrobia	Verrucomicrobiae	Chthoniobacterales	Terrimicrobiaceae	Terrimicrobium	uncultured bacterium	1.936	2.236	0.526	4.253	0.005
	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	uncultured Bacteroidetes bacterium	2.446	2.624	0.485	5.413	0.000
WETLANDS											
	Actinobacteria	Acidimicrobiia	Microtrichales	Ilumatobacteraceae	CL500-29 marine group	marine metagenome	1.767	4.017	0.835	4.808	0.000
	Verrucomicrobia	Verrucomicrobiae	Chthoniobacterales	Terrimicrobiaceae	Terrimicrobium	uncultured bacterium	1.936	4.391	0.882	4.981	0.000
	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	uncultured Bacteroidetes bacterium	2.446	4.814	0.809	5.948	0.000
RIVER CANARD											
	Proteobacteria	Gammaproteobacteria	Methylococcales	Methylococcaceae	Methylocaldum	uncultured bacterium	3.194	4.670	1.075	4.346	0.009
	Chloroflexi	Anaerolineae	SJA-15	uncultured Chloroflexi bacterium	uncultured Chloroflexi bacterium	uncultured Chloroflexi bacterium	3.042	4.602	1.194	3.855	0.036
	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	TRA3-20	bacterium TG161	bacterium TG161	2.940	4.550	1.217	3.739	0.038
TURKEY CREEK											
	Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobulbaceae	Candidatus Electronema	uncultured soil bacterium	2.641	4.514	1.264	3.572	0.044
	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Sulfuricellaceae	**Sulfuricella**	Ambiguous taxa	1.877	4.828	1.300	3.715	0.033

	Phylum	Class	Order	Family	Genus	Species	Base Mean	log2 Fold Change	lfcSE	stat	padj
	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Sulfuricellaceae	**Ferritrophicum**	Ambiguous taxa	2.673	3.875	1.097	3.533	0.044
	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Rhodocyclaceae	uncultured	uncultured gamma proteobacterium	1.947	4.881	1.268	3.848	0.026
	Spirochaetes	Spirochaetia	Spirochaetales	Spirochaetaceae	uncultured	uncultured soil bacterium	2.155	5.027	1.190	4.224	0.008
	Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobacteraceae	Desulfatirhabdium	Ambiguous_taxa	2.365	5.161	1.125	4.588	0.003

Appendix C-9: Differential abundance - DeSEQ2 results – significantly different taxa by sample site (MRA)

RCU 10	Verrucomicrobia	Verrucomicrobiae	Chthoniobacterales	Terrimicrobiaceae	Terrimicrobium	98.885	-20.177	5.592	-3.608	0.035
	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	158.440	-20.808	5.705	-3.647	0.035
RCD 18	Kiritimatiellaeota	Kiritimatiellae	WCHB1-41	uncult. eubacterium WCHB1-41	uncult. eubacterium WCHB1-41	61.137	-20.426	5.838	-3.499	0.047
	Verrucomicrobia	Verrucomicrobiae	Chthoniobacterales	Terrimicrobiaceae	Terrimicrobium	98.885	-21.077	5.574	-3.781	0.023
	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	158.440	-21.737	5.687	-3.822	0.023
RCD 26	Nitrospinae	P9X2b3D02	uncult. prokaryote	uncult. prokaryote	uncult. prokaryote	927.774	-10.360	2.402	-4.314	0.004
TCU 3	Proteobacteria	Gammaproteobacteria	SZB30	uncult. prokaryote	uncult. prokaryote	213.770	-9.349	2.880	-3.247	0.050
	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Hydrogenophilaceae	Thiobacillus	43.759	-19.711	5.664	-3.480	0.042
	Latescibacteria	uncult. soil bacterium PRR-10	uncult. soil bacterium PRR-10	uncult. soil bacterium PRR-10	uncult. soil bacterium PRR-10	36.912	-19.429	5.973	-3.253	0.050
	Kiritimatiellaeota	Kiritimatiellae	WCHB1-41	uncult. eubacterium WCHB1-41	uncult. eubacterium WCHB1-41	61.137	-20.165	5.847	-3.449	0.042
	Proteobacteria	Deltaproteobacteria	Desulfuromonadales	Geobacteraceae	Geothermobacter	40.974	-19.628	5.968	-3.289	0.050
	Verrucomicrobia	Verrucomicrobiae	Chthoniobacterales	Terrimicrobiaceae	Terrimicrobium	98.885	-20.774	5.582	-3.721	0.030
	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	158.440	-21.429	5.695	-3.763	0.030
TCU 4	Chloroflexi	KD4-96	metagenome	metagenome	metagenome	541.065	-9.669	2.583	-3.744	0.028
	Acidobacteria	Subgroup 22	uncult. bacterium	uncult. bacterium	uncult. bacterium	172.059	-8.018	2.485	-3.227	0.047
	Kiritimatiellaeota	Kiritimatiellae	WCHB1-41	uncult. eubacterium WCHB1-41	uncult. eubacterium WCHB1-41	61.137	-19.210	5.862	-3.277	0.045
	Verrucomicrobia	Verrucomicrobiae	Chthoniobacterales	Terrimicrobiaceae	Terrimicrobium	98.885	-19.863	5.598	-3.548	0.028
	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Comamonas	292.776	-8.782	2.459	-3.572	0.028

	Spirochaetes	Spirochaetia	Spirochaetales	Spirochaetaceae	Spirochaeta 2	243.488	-8.517	2.437	-3.496	0.028
	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	158.440	-20.488	5.710	-3.588	0.028
	Proteobacteria	Deltaproteobacteria	Syntrophobacterales	Syntrophobacteraceae	uncultured	240.697	-8.499	2.577	-3.298	0.045
TCD 24	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Hydrogenophilaceae	Thiobacillus	43.759	-18.866	5.679	-3.322	0.032
	Acidobacteria	Subgroup 6	uncult. Candidatus Desulforudis sp.	uncult. Candidatus Desulforudis sp.	uncult. Candidatus Desulforudis sp.	192.863	-8.327	2.455	-3.391	0.031
	Latescibacteria	uncult. soil bacterium PRR-10	uncult. soil bacterium PRR-10	uncult. soil bacterium PRR-10	uncult. soil bacterium PRR-10	36.912	-18.651	5.988	-3.115	0.047
	Proteobacteria	Deltaproteobacteria	Desulfuromonadales	Geobacteraceae	Geothermobacter	40.974	-18.795	5.983	-3.141	0.047
	Verrucomicrobia	Verrucomicrobiae	Chthoniobacterales	Terrimicrobiaceae	Terrimicrobium	98.885	-19.852	5.596	-3.547	0.031
	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	SC-I-84	uncult. Derxia sp.	316.609	-9.040	2.602	-3.475	0.031
	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	158.440	-20.624	5.709	-3.613	0.031
TCD 30	Verrucomicrobia	Verrucomicrobiae	Chthoniobacterales	Terrimicrobiaceae	Terrimicrobium	98.885	-21.427	5.569	-3.848	0.015
	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Sediminibacterium	158.440	-22.073	5.681	-3.885	0.015
TRC 1	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingorhabdus	62.92517	-20.5297	6.577879	-3.12102	0.039691
	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiales Incertae Sedis	uncultured	62.58036	-20.5224	5.919925	-3.46666	0.027086
	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	Roseomonas	65.3994	-20.5771	5.856748	-3.5134	0.026066
	Acidobacteria	Thermoanaerobaculia	Thermoanaerobaculales	Thermoanaerobaculaceae	Thermoanaerobaculum	58.27933	-20.4172	6.576264	-3.10467	0.039691
	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Hydrogenophilaceae	Thiobacillus	87.51862	-20.318	5.987358	-3.39348	0.032247

	Actinobacteria	Acidimicrobiia	Microtrichales	Ilumatobacteraceae	CL500-29 marine group	122.5632	-21.4276	5.751683	-3.72544	0.020043
	Actinobacteria	Acidimicrobiia	Microtrichales	Ilumatobacteraceae	CL500-29 marine group	39.43059	-19.9002	6.445746	-3.08734	0.039691
	Chloroflexi	Anaerolineae	MSB-5E12	uncultured Chloroflexi bacterium	uncultured Chloroflexi bacterium	29.6584	-19.5068	6.423019	-3.03701	0.039691
	Proteobacteria	Deltaproteobacteria	Myxococcales	Phaselicystidaceae	Phaselicystis	37.28059	-19.887	6.49544	-3.06168	0.039691
	Acidobacteria	Holophagae	Subgroup 7	uncultured soil bacterium	uncultured soil bacterium	38.37777	-19.8286	6.428908	-3.08428	0.039691
	Acidobacteria	Subgroup 18	uncultured Acidobacteria bacterium	uncultured Acidobacteria bacterium	uncultured Acidobacteria bacterium	45.22662	-20.077	6.129883	-3.27526	0.038305
	Acidobacteria	Aminicenantia	Aminicenantales	uncultured Desulfovibrio sp.	uncultured Desulfovibrio sp.	139.3913	-21.4966	5.495459	-3.9117	0.020043
	Latescibacteria	uncultured soil bacterium PRR-10	uncultured soil bacterium PRR-10	uncultured soil bacterium PRR-10	uncultured soil bacterium PRR-10	73.82385	-20.3748	6.318391	-3.22469	0.039078
	Verrucomicrobia	Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae	ADurb.Bin063-1	120.9668	-21.4092	5.694562	-3.75958	0.020043
	Proteobacteria	Gammaproteobacteria	Steroidobacterales	Steroidobacteraceae	uncultured	25.9396	-19.2806	6.052429	-3.1856	0.039078
	Proteobacteria	Deltaproteobacteria	Desulfuromonadales	Geobacteraceae	Geothermobacter	81.94705	-20.8859	6.314214	-3.30776	0.038305
	Nitrospirae	Thermodesulfovibrionia	uncultured	uncultured Magnetobacterium sp.	uncultured Magnetobacterium sp.	43.13061	-20.0193	6.142664	-3.25905	0.038305
	Proteobacteria	Gammaproteobacteria	PLTA13	uncultured gamma proteobacterium	uncultured gamma proteobacterium	121.0121	-21.3611	5.699838	-3.74767	0.020043

	Verrucomicrobia	Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae	Oikopleura dioica	25.30097	-19.2818	6.391172	-3.01694	0.041014
	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Rhodocyclaceae	Candidatus Accumulibacter	58.85794	-20.4329	6.669785	-3.06351	0.039691
	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Comamonas	52.33086	-20.2743	6.504055	-3.11717	0.039691
	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Nitrosomonadaceae	oc32	211.07	-9.6793	3.056938	-3.16634	0.039674
	Actinobacteria	Actinobacteria	Frankiales	Sporichthyaceae	hgcI clade	148.3035	-21.6794	7.271023	-2.98162	0.04466
	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidetes vadinHA17	uncultured Sphingobacteriia bacterium	34.98185	-19.7361	6.499739	-3.03645	0.039691
	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidetes vadinHA17	uncultured eubacterium WCHB1-53	35.02811	-19.7231	6.132176	-3.21633	0.039078
	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methyloligellaceae	uncultured	25.39595	-19.2882	6.319065	-3.05238	0.039691
	Chloroflexi	Anaerolineae	RBG-13-54-9	uncultured Caldilineae bacterium	uncultured Caldilineae bacterium	107.9243	-8.71294	2.728014	-3.19388	0.039078
	Planctomycetes	Planctomycetacia	Pirellulales	Pirellulaceae	Pirellula	36.26589	-19.789	6.388905	-3.09739	0.039691
	Planctomycetes	Planctomycetacia	Pirellulales	Pirellulaceae	Candidatus Anammoximicrobium	102.7526	-20.5982	5.688964	-3.62072	0.021572
	Proteobacteria	Deltaproteobacteria	NB1-j	Ambiguous_taxa	Ambiguous_taxa	37.21718	-19.8183	6.075442	-3.26204	0.038305
	Proteobacteria	Deltaproteobacteria	Syntrophobacterales	Syntrophaceae	uncultured	425.2212	-10.6889	2.9414	-3.63394	0.021572

	Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobacteraceae	Sva0081 sediment group	208.8015	-22.101	5.204638	-4.2464	0.011166
TRC2	Proteobacteria	Deltaproteobacteria	Sva0485	uncultured prokaryote	uncultured prokaryote	123.8284	-7.61403	2.173775	-3.50267	0.048433
	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Arenimonas	149.0876	-7.87796	2.27818	-3.45801	0.048433
TRC5	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Hydrogenophilaceae	Thiobacillus	43.84167	-18.9744	5.916887	-3.20682	0.017335
	Actinobacteria	Actinobacteria	Frankiales	Cryptosporangiaceae	Fodinicola	99.35069	-7.45558	2.424705	-3.07484	0.017335
	Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	uncultured	72.11382	-7.00201	2.737097	-2.55819	0.048949
	Chloroflexi	Anaerolineae	20-Jan	Ambiguous_taxa	Ambiguous_taxa	94.60539	-7.39292	2.792638	-2.64729	0.04341
	Acidobacteria	Subgroup 6	uncultured Candidatus Desulforudis sp.	uncultured Candidatus Desulforudis sp.	uncultured Candidatus Desulforudis sp.	169.9782	-8.23612	2.238618	-3.67911	0.012522
	Acidobacteria	Holophagae	Subgroup 7	uncultured bacterium SJA-36	uncultured bacterium SJA-36	70.82221	-6.97502	2.709812	-2.57399	0.048896
	Acidobacteria	Subgroup 22	uncultured bacterium	uncultured bacterium	uncultured bacterium	147.8109	-8.03724	2.328727	-3.45135	0.013396
	Acidobacteria	Aminicenantia	Aminicenantales	uncultured prokaryote	uncultured prokaryote	130.7975	-7.8626	2.509111	-3.13362	0.017335
	Latescibacteria	metagenome	metagenome	metagenome	metagenome	158.4461	-8.13239	2.509703	-3.24038	0.017335
	Kiritimatiellaeota	Kiritimatiellae	WCHB1-41	metagenome	metagenome	40.62232	-18.0269	5.855302	-3.07873	0.017335

	Kiritimatiellaeota	Kiritimatiellae	WCHB1-41	uncultured eubacterium WCHB1-41	uncultured eubacterium WCHB1-41	45.77797	-19.0083	6.003353	-3.16628	0.017335
	Proteobacteria	Gammaproteobacteria	Methylococcales	Methylomonaceae	Crenothrix	46.62544	-18.6596	5.885677	-3.17034	0.017335
	Proteobacteria	Gammaproteobacteria	Cellvibrionales	Spongiibacteraceae	BD1-7 clade	161.6307	-8.16163	2.386366	-3.42011	0.013396
	Verrucomicrobia	Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae	uncultured prokaryote	53.56022	-19.2402	5.385234	-3.57277	0.012598
	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Comamonas	291.3496	-9.01229	2.39741	-3.75918	0.012522
	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Nitrosomonadaceae	oc32	86.50663	-7.26457	2.737853	-2.65338	0.04341
	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Hydrogenophilaceae	uncultured	104.9016	-7.54138	2.801355	-2.69205	0.042214
	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	B1-7BS	uncultured soil bacterium	77.00858	-7.0974	2.52195	-2.81425	0.034875
	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	SC-I-84	uncultured Derxia sp.	219.2041	-8.60387	2.870551	-2.99729	0.020819
	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Dinghuibacter	113.4577	-7.65068	2.784526	-2.74757	0.040151
	Planctomycetes	Planctomycetacia	Pirellulales	Pirellulaceae	Candidatus Anammoximicrobium	40.19604	-18.868	6.063328	-3.11182	0.017335
	Proteobacteria	Deltaproteobacteria	NB1-j	uncultured delta proteobacterium	uncultured delta proteobacterium	119.856	-7.73388	2.859527	-2.7046	0.042214
	Actinobacteria	Thermoleophilia	Gaiellales	uncultured	uncultured Solirubrobacterales bacterium	95.46215	-7.40571	2.874937	-2.57595	0.048896

TRC 7	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Hydrogenophilaceae	Thiobacillus (spp: Thiobacillus denitrificans ATCC 25259)	43.84167	-20.2237	5.897606	-3.42914	0.01433
	Actinobacteria	MB-A2-108	uncultured Nocardioidaceae bacterium	uncultured Nocardioidaceae bacterium	uncultured Nocardioidaceae bacterium	47.37675	-7.73973	2.657725	-2.91216	0.033421
	Acidobacteria	Aminicenantia	Aminicenantales	uncultured soil bacterium	uncultured soil bacterium	38.31045	-7.43826	2.788618	-2.66736	0.049344
	Acidobacteria	Aminicenantia	Aminicenantales	uncultured Anaerolineae bacterium	uncultured Anaerolineae bacterium	103.8661	-8.87604	3.178052	-2.79292	0.041207
	Acidobacteria	Aminicenantia	Aminicenantales	uncultured prokaryote	uncultured prokaryote	130.7975	-9.21149	2.488329	-3.70188	0.009117
	Latescibacteria	uncultured soil bacterium PRR-10	uncultured soil bacterium PRR-10	uncultured soil bacterium PRR-10	uncultured soil bacterium PRR-10	28.77227	-19.639	6.64662	-2.95473	0.033421
	Kiritimatiellaeota	Kiritimatiellae	WCHB1-41	metagenome	metagenome	40.62232	-18.0045	5.835945	-3.0851	0.027089
	Kiritimatiellaeota	Kiritimatiellae	WCHB1-41	uncultured eubacterium WCHB1-41	uncultured eubacterium WCHB1-41	45.77797	-20.2832	5.984487	-3.3893	0.014925
	Proteobacteria	Gammaproteobacteria	Methylococcales	Methylomonaceae	Crenothrix	46.62544	-20.3133	5.866236	-3.46274	0.014236
	Proteobacteria	Gammaproteobacteria	Cellvibrionales	Halieaceae	OM60(NOR5) clade	209.8115	-9.8884	2.378732	-4.157	0.002289
	Proteobacteria	Deltaproteobacteria	Sva0485	uncultured prokaryote	uncultured prokaryote	123.8284	2.057288	0.747264	2.753093	0.04336
	Planctomycetes	Phycisphaerae	Phycisphaerales	Phycisphaeraceae	SM1A02	28.76875	-7.01972	2.579212	-2.72165	0.044631
	Proteobacteria	Gammaproteobacteria	PLTA13	uncultured gamma proteobacterium	uncultured gamma proteobacterium	32.1873	-19.8148	7.241544	-2.73627	0.04412
	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Rhodanobacteraceae	uncultured	69.19977	-8.28889	2.829924	-2.929	0.033421

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	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Arenimonas	80.66717	-8.50772	3.160948	-2.69151	0.047346
	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Rhodanobacteraceae	uncultured	31.30772	-7.13639	2.466338	-2.89352	0.033805
	Latescibacteria	Ambiguous_taxa	Ambiguous_taxa	Ambiguous_taxa	Ambiguous_taxa	62.57211	-8.14589	2.798823	-2.91047	0.033421
	Verrucomicrobia	Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae	uncultured prokaryote	53.56022	-17.9536	5.366673	-3.34539	0.015378
	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Comamonas	17.24193	-18.967	6.887439	-2.75385	0.04336
	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Comamonas	291.3496	-10.3607	2.37668	-4.35932	0.001389
	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	SC-I-84	uncultured Burkholderia sp.	849.3473	-11.9057	2.187264	-5.44322	1.11E-05
	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	SC-I-84	uncultured Derxia sp.	219.2041	-9.95236	2.853842	-3.48735	0.014236
	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Nitrosomonadaceae	DSSD61	123.8669	-9.1288	3.172785	-2.87722	0.033805
	Proteobacteria	Deltaproteobacteria	Syntrophobacterales	Syntrophobacteraceae	Syntrophobacter	26.81416	-19.5672	6.644032	-2.94509	0.033421
	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidetes BD2-2	Bacteroidetes bacterium GWF2_49_14	35.39892	-7.32246	2.359599	-3.10326	0.027089
	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidetes BD2-2	uncultured prokaryote	47.99823	-7.75694	2.621673	-2.95877	0.033421
	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Dinghuibacter	113.4577	-8.99921	2.766564	-3.252	0.01872

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	Proteobacteria	Deltaproteobacteria	Desulfuromonadales	Geobacteraceae	Geobacter	216.3194	-9.93347	3.463159	-2.86833	0.033805
	Planctomycetes	Planctomycetacia	Pirellulales	Pirellulaceae	Candidatus Anammoximicrobium	40.19604	-20.129	6.043549	-3.33067	0.015378
	Proteobacteria	Deltaproteobacteria	NB1-j	uncultured delta proteobacterium	uncultured delta proteobacterium	119.856	-9.08248	2.840887	-3.19706	0.021123
	Proteobacteria	Deltaproteobacteria	Syntrophobacterales	Syntrophaceae	uncultured	209.3338	-9.88632	2.647501	-3.73421	0.009117
	Actinobacteria	Thermoleophilia	Gaiellales	uncultured	uncultured Solirubrobacterales bacterium	95.46215	-8.75437	2.856669	-3.06454	0.027315
	Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobacteraceae	Sva0081 sediment group	336.1403	-10.57	2.993343	-3.53117	0.014236
TTC 3	Actinobacteria	Actinobacteria	Frankiales	Cryptosporangiaceae	Fodinicola	99.35069	-7.56217	2.421629	-3.12276	0.017108
	Chloroflexi	Anaerolineae	Anaerolineales	Anaerolineaceae	uncultured	72.11382	-7.10866	2.733414	-2.60065	0.045255
	Acidobacteria	Holophagae	Subgroup 7	uncultured bacterium SJA-36	uncultured bacterium SJA-36	70.82221	-7.08166	2.706056	-2.61697	0.045255
	Kiritimatiellaeota	Kiritimatiellae	WCHB1-41	metagenome	metagenome	40.62232	-18.9881	5.85053	-3.24554	0.015679
	Kiritimatiellaeota	Kiritimatiellae	WCHB1-41	uncultured eubacterium WCHB1-41	uncultured eubacterium WCHB1-41	45.77797	-19.147	5.99857	-3.19192	0.016803
	Proteobacteria	Gammaproteobacteria	Methylococcales	Methylomonaceae	Crenothrix	46.62544	-19.1669	5.88071	-3.25928	0.015679
	Proteobacteria	Gammaproteobacteria	Cellvibrionales	Spongiibacteraceae	BD1-7 clade	161.6307	-8.26823	2.383442	-3.469	0.011178

									03	
	Bacteroidetes	Ignavibacteria	Kryptoniales	BSV26	uncultured Chlorobi bacterium	111.871	-7.74851	2.203615	-3.51627	0.011178
	Nitrospirae	Thermodesulfovibrionia	uncultured	uncultured prokaryote	uncultured prokaryote	160.3104	-8.26158	2.799142	-2.95147	0.02256
	Verrucomicrobia	Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae	uncultured prokaryote	53.56022	-19.3297	5.380394	-3.59263	0.011178
	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Nitrosomonadaceae	oc32	86.50663	-7.37121	2.734155	-2.69597	0.039524
	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	SC-I-84	uncultured Derxia sp.	219.2041	-8.71047	2.867409	-3.03775	0.019007
	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	B1-7BS	uncultured beta proteobacterium	374.9004	-9.48376	2.251877	-4.21149	0.002714
	Spirochaetes	Spirochaetia	Spirochaetales	Spirochaetaceae	Spirochaeta 2	209.9548	-8.65107	2.255242	-3.83598	0.006691
	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Dinghuibacter	113.4577	-7.75728	2.781267	-2.78912	0.034953
	Proteobacteria	Deltaproteobacteria	Syntrophobacterales	Syntrophobacteraceae	uncultured	165.0549	-8.30219	2.744591	-3.02493	0.019007
	Bacteroidetes	Ignavibacteria	Kryptoniales	BSV26	uncultured bacterium	44.95783	-6.43744	2.489979	-2.58534	0.045258
	Planctomycetes	Planctomycetacia	Pirellulales	Pirellulaceae	Candidatus Anammoximicrobium	40.19604	-18.9512	6.058336	-3.12813	0.017108
	Proteobacteria	Deltaproteobacteria	NB1-j	uncultured delta proteobacterium	uncultured delta proteobacterium	119.856	-7.84049	2.856057	-2.74521	0.035947
	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	uncultured	82.09294	-7.28821	2.628221	-2.773	0.034953

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	Proteobacteria	Deltaproteobacteria	Syntrophobacterales	Syntrophaceae	uncultured	209.3338	-8.64438	2.663432	-3.24558	0.015679
	Actinobacteria	Thermoleophilia	Gaiellales	uncultured	uncultured Solirubrobacterales bacterium	95.46215	-7.51233	2.871493	-2.61617	0.045255
	Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobacteraceae	Sva0081 sediment group	336.1403	-9.32814	3.006611	-3.10254	0.017108
TTC4	Chloroflexi	Anaerolineae	20-Jan	Ambiguous_taxa	Ambiguous_taxa	94.60539	-8.71638	2.772612	-3.14374	0.039476
	Proteobacteria	Deltaproteobacteria	Syntrophobacterales	Syntrophobacteraceae	uncultured	34.37005	-19.8858	6.439194	-3.08824	0.040027
	Kiritimatiellaeota	Kiritimatiellae	WCHB1-41	metagenome	metagenome	40.62232	-17.9774	5.83603	-3.08041	0.040027
	Kiritimatiellaeota	Kiritimatiellae	WCHB1-41	uncultured eubacterium WCHB1-41	uncultured eubacterium WCHB1-41	45.77797	-20.0401	5.984666	-3.34858	0.027505
	Proteobacteria	Gammaproteobacteria	Cellvibrionales	Spongiibacteraceae	BD1-7 clade	161.6307	-9.48495	2.365339	-4.00998	0.012934
	Nitrospirae	Thermodesulfovibrionia	uncultured	uncultured prokaryote	uncultured prokaryote	160.3104	-9.47833	2.782729	-3.40613	0.027505
	Verrucomicrobia	Verrucomicrobiae	Pedosphaerales	Pedosphaeraceae	uncultured prokaryote	53.56022	-19.3096	5.366536	-3.59816	0.026813
	Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	SC-I-84	uncultured Derxia sp.	219.2041	-9.92711	2.85354	-3.47888	0.026813
	Bacteroidetes	Bacteroidia	Chitinophagales	Chitinophagaceae	Dinghuibacter	113.4577	-8.97397	2.766268	-3.24407	0.031374
	Proteobacteria	Deltaproteobacteria	Syntrophobacterales	Syntrophobacteraceae	uncultured	165.0549	-9.51892	2.729003	-3.488	0.026813

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	Planctomycetes	Planctomycetacia	Pirellulales	Pirellulaceae	Candidatus Anammoximicrobium	40.19604	-20.0584	6.043778	-3.31885	0.027505

Appendix C-10: Unique species at strata – between wetland and tributary strata

TAXA: PHYLUM_CLASS_ORDER_FAMILY_GENUS_SPECIES

DRM

- Acidobacteria_Acidobacteriia_Subgroup.13_uncultured.Solibacterales.bacterium_uncultured.Solibacterales.bacterium_uncultured.Solibacterales.bacterium
- Acidobacteria_Blastocatellia..Subgroup.4._11.24_uncultured.soil.bacterium_uncultured.soil.bacterium_uncultured.soil.bacterium
- Acidobacteria_Blastocatellia..Subgroup.4._Blastocatellales_Blastocatellaceae_Stenotrophobacter_uncultured.Acidobacteriales.bacterium
- Acidobacteria_Blastocatellia..Subgroup.4._Blastocatellales_Blastocatellaceae_uncultured_uncultivated.soil.bacterium.clone.C105
- Actinobacteria_Acidimicrobiia_IMCC26256_uncultured.Iamia.sp._uncultured.Iamia.sp._uncultured.Iamia.sp.
- Actinobacteria_Actinobacteria_Streptosporangiales_Streptosporangiaceae_Sphaerisporangium_Sphaerisporangium.aureirubrum
- Altiarchaeota_Altiarchaeia_uncultured.euryarchaeote_uncultured.euryarchaeote_uncultured.euryarchaeote_uncultured.euryarchaeote
- Armatimonadetes_uncultured_uncultured.bacterium.SJA.171_uncultured.bacterium.SJA.171_uncultured.bacterium.SJA.171_uncultured.bacterium.SJA.171
- Bacteroidetes_Ignavibacteria_OPB56_uncultured.Bacteroidetes.Chlorobi.group.bacterium_uncultured.Bacteroidetes.Chlorobi.group.bacterium_uncultured.Bacteroidetes.Chlorobi.group.bacterium
- BRC1_metagenome_metagenome_metagenome_metagenome_metagenome
- Chlamydiae_Chlamydiae_Chlamydiales_cvE6_Gammaproteobacteria.bacterium.RIFCSPLOWO2_02_FULL_47_50_Gammaproteobacteria.bacterium.RIFCSPLOWO2_02_FULL_47_50
- Chloroflexi_Chloroflexia_Chloroflexales_Roseiflexaceae_uncultured_uncultured.Roseiflexus.sp.
- Chloroflexi_Chloroflexia_Thermomicrobiales_JG30.KF.CM45_uncultured.anaerobic.ammonium.oxidizing.bacterium_uncultured.anaerobic.ammonium.oxidizing.bacterium
- Chloroflexi_Dehalococcoidia_FW22_uncultured.organism_uncultured.organism_uncultured.organism
- Chloroflexi_Dehalococcoidia_GIF9_uncultured.Chloroflexi.bacterium_uncultured.Chloroflexi.bacterium_uncultured.Chloroflexi.bacterium
- Chloroflexi_SHA.26_uncultured.soil.bacterium_uncultured.soil.bacterium_uncultured.soil.bacterium_uncultured.soil.bacterium
- Cyanobacteria_Oxyphotobacteria_Nostocales_Nostocaceae_Aphanizomenon.NIES81_Ambiguous_taxa
- Firmicutes_Clostridia_Clostridiales_Lachnospiraceae_Anaerosporobacter_uncultured.rumen.bacterium
- Firmicutes_Clostridia_Clostridiales_Ruminococcaceae_Ruminococcaceae.UCG.012_bacterium.enrichment.culture.clone.E34
- Firmicutes_Erysipelotrichia_Erysipelotrichales_Erysipelotrichaceae_Erysipelothrix_metagenome
- Nanoarchaeaeota_Nanohaloarchaeia_Aenigmarchaeales_uncultured.archaeon_uncultured.archaeon_uncultured.archaeon
- Nanoarchaeaeota_Woesearchaeia_uncultured.crenarchaeote_uncultured.crenarchaeote_uncultured.crenarchaeote_uncultured.crenarchaeote
- Patescibacteria_CPR2_uncultured.Clostridium.sp._uncultured.Clostridium.sp._uncultured.Clostridium.sp._uncultured.Clostridium.sp.
- Patescibacteria_Parcubacteria_Candidatus.Nomurabacteria_uncultured.bacterium_uncultured.bacterium_uncultured.bacterium
- Patescibacteria_Parcubacteria_Candidatus.Vogelbacteria_uncultured.bacterium_uncultured.bacterium_uncultured.bacterium

- Patescibacteria_WWE3_uncultured.anaerobic.bacterium_uncultured.anaerobic.bacterium_uncultured.anaerobic.bacterium_uncultured.anaerobic.bacterium
- Planctomycetes_Phycisphaerae_mle1.8_uncultured.Schlesneria.sp._uncultured.Schlesneria.sp._uncultured.Schlesneria.sp.
- Proteobacteria_Alphaproteobacteria_Caulobacterales_Parvularculaceae_Amphiplicatus_uncultured.bacterium
- Proteobacteria_Alphaproteobacteria_Micavibrionales_uncultured_uncultured.Rhizobiales.bacterium_uncultured.Rhizobiales.bacterium
- Proteobacteria_Alphaproteobacteria_Micropepsales_Micropepsaceae_uncultured.Hyphomicrobiaceae.bacterium_uncultured.Hyphomicrobiaceae.bacterium
- Proteobacteria_Alphaproteobacteria_Rhizobiales_Beijerinckiaceae_Microvirga_Mesorhizobium.sp..CCNWQLS7
- Proteobacteria_Alphaproteobacteria_Rhodobacterales_Rhodobacteraceae_Amaricoccus_Amaricoccus.veronensis
- Proteobacteria_Alphaproteobacteria_Rhodospirillales_uncultured_Ambiguous_taxa_Ambiguous_taxa
- Proteobacteria_Alphaproteobacteria_Rhodospirillales_uncultured_uncultured.Rhizobiaceae.bacterium_uncultured.Rhizobiaceae.bacterium
- Proteobacteria_Alphaproteobacteria_Sphingomonadales_Sphingomonadaceae_Sphingomonas_Sphingomonas.sediminicola
- Proteobacteria_Deltaproteobacteria_Bdellovibrionales_Bacteriovoracaceae_Peredibacter_uncultured.bacterium
- Proteobacteria_Deltaproteobacteria_Desulfarculales_Desulfarculaceae_Desulfatiglans_uncultured.hydrocarbon.seep.bacterium.GCA017
- Proteobacteria_Deltaproteobacteria_Myxococcales_27F.1492R_uncultured.delta.proteobacterium_uncultured.delta.proteobacterium
- Proteobacteria_Deltaproteobacteria_Myxococcales_Haliangiaceae_Haliangium_uncultured.organism
- Proteobacteria_Deltaproteobacteria_Oligoflexales_0319.6G20_metagenome_metagenome
- Proteobacteria_Deltaproteobacteria_Sva0485_uncultured.Desulfobacteraceae.bacterium_uncultured.Desulfobacteraceae.bacterium_uncultured.Desulfobacteraceae.bacterium
- Proteobacteria_Deltaproteobacteria_Syntrophobacterales_Syntrophaceae_Desulfobacca_uncultured.archaeon
- Proteobacteria_Deltaproteobacteria_Syntrophobacterales_Syntrophaceae_Desulfomonile_uncultured.delta.proteobacterium
- Proteobacteria_Gammaproteobacteria_Beggiatoales_Beggiatoaceae_Beggiatoa_uncultured.gamma.proteobacterium
- Proteobacteria_Gammaproteobacteria_Betaproteobacteriales_Rhodocyclaceae_uncultured_uncultured.proteobacterium
- Proteobacteria_Gammaproteobacteria_Competibacterales_Competibacteraceae_Plasticicumulans_Plasticicumulans.lactativorans
- Proteobacteria_Gammaproteobacteria_Coxiellales_Coxiellaceae_Coxiella_uncultured.bacterium
- Proteobacteria_Gammaproteobacteria_Gammaproteobacteria.Incertae.Sedis_Unknown.Family_uncultured.gamma.proteobacterium_uncultured.gamma.proteobacterium
- Proteobacteria_Gammaproteobacteria_Xanthomonadales_Rhodanobacteraceae_uncultured_Ambiguous_taxa
- Spirochaetes_Spirochaetia_Spirochaetales_Spirochaetaceae_uncultured_uncultured.Leptospiraceae.bacterium
- WS4_uncultured.Armatimonadetes.bacterium_uncultured.Armatimonadetes.bacterium_uncultured.Armatimonadetes.bacterium_uncultured.Armatimonadetes.bacterium_uncultured.Armatimonadetes.bacterium

GRASS

- Acidobacteria_Acidobacteriia_Solibacterales_Solibacteraceae..Subgroup.3._Candidatus.Solibacter_bacterium.Ellin6505
- Actinobacteria_Acidimicrobiia_IMCC26256_uncultured.Ferrimicrobium.sp._uncultured.Ferrimicrobium.sp._uncultured.Ferrimicrobium.sp.
- Armatimonadetes_uncultured_uncultured.bacterium..0319.6A24_uncultured.bacterium..0319.6A24_uncultured.bacterium..0319.6A24_uncultured.bacterium..0319.6A24
- Bacteroidetes_Bacteroidia_Bacteroidales_SB.5_uncultured.bacterium_uncultured.bacterium
- Bacteroidetes_Bacteroidia_Chitinophagales_Chitinophagaceae_Ferruginibacter_Ferruginibacter.yonginensis
- Bacteroidetes_Bacteroidia_Chitinophagales_Chitinophagaceae_Lacibacter_uncultured.Bacteroidetes.bacterium
- Bacteroidetes_Bacteroidia_Chitinophagales_Chitinophagaceae_Sediminibacterium_uncultured.Sphingobacteriia.bacterium
- Bacteroidetes_Bacteroidia_Chitinophagales_Saprospiraceae_uncultured_uncultured.bacterium.BS2
- Bacteroidetes_Bacteroidia_Sphingobacteriales_Lentimicrobiaceae_Lentimicrobium_uncultured.soil.bacterium
- Bacteroidetes_Bacteroidia_Sphingobacteriales_ST.12K33_uncultured.Bacteroidetes.bacterium_uncultured.Bacteroidetes.bacterium
- BRC1_uncultured.soil.bacterium.PBS.III.4_uncultured.soil.bacterium.PBS.III.4_uncultured.soil.bacterium.PBS.III.4_uncultured.soil.bacterium.PBS.III.4_uncultured.soil.bacterium.PBS.III.4
- Chlamydiae_Chlamydiae_Chlamydiales_Parachlamydiaceae_Candidatus.Protochlamydia_uncultured.soil.bacterium
- Chlamydiae_Chlamydiae_Chlamydiales_Parachlamydiaceae_metagenome_metagenome
- Chlamydiae_Chlamydiae_Chlamydiales_Simkaniaceae_metagenome_metagenome
- Chloroflexi_Dehalococcoidia_Sh765B.AG.111_uncultured.Chlorobi.bacterium_uncultured.Chlorobi.bacterium_uncultured.Chlorobi.bacterium
- Dependentiae_Babeliae_Babeliales_Vermiphilaceae_uncultured.soil.bacterium_uncultured.soil.bacterium
- Euryarchaeota_Thermoplasmata_Methanomassiliicoccales_uncultured_uncultured.archaeon_uncultured.archaeon
- FCPU426_uncultured.Syntrophaceae.bacterium_uncultured.Syntrophaceae.bacterium_uncultured.Syntrophaceae.bacterium_uncultured.Syntrophaceae.bacterium_uncultured.Syntrophaceae.bacterium
- Firmicutes_Clostridia_Clostridiales_Christensenellaceae_Christensenellaceae.R.7.group_iron.reducing.bacterium.enrichment.culture.clone.HN117
- Firmicutes_Clostridia_Clostridiales_Christensenellaceae_uncultured_Ambiguous_taxa
- Firmicutes_Clostridia_Clostridiales_Peptostreptococcaceae_Sporacetigenium_Clostridium.sp..Gec1.52.ana4.2
- Gemmatimonadetes_Gemmatimonadetes_Gemmatimonadales_Gemmatimonadaceae_uncultured_uncultured.proteobacterium
- Latescibacteria_Latescibacteria_Latescibacterales_Latescibacteraceae_Candidatus.Latescibacter_Latescibacteria.bacterium.SCGC.AAA252.D10
- Patescibacteria_ABY1_Candidatus.Buchananbacteria_uncultured.bacterium_uncultured.bacterium_uncultured.bacterium
- Patescibacteria_Gracilibacteria_Absconditabacteriales..SR1._uncultured.organism_uncultured.organism_uncultured.organism
- Patescibacteria_Parcubacteria_Candidatus.Azambacteria_uncultured.bacterium_uncultured.bacterium_uncultured.bacterium
- Patescibacteria_Parcubacteria_Candidatus.Kaiserbacteria_bacterium.SH4.10_bacterium.SH4.10_bacterium.SH4.10
- Planctomycetes_Phycisphaerae_Phycisphaerales_Phycisphaeraceae_AKYG587_uncultured.planctomycete
- Planctomycetes_Phycisphaerae_Phycisphaerales_Phycisphaeraceae_uncultured_uncultured.bacterium

- Planctomycetes_Phycisphaerae_Phycisphaerales_Phycisphaeraceae_uncultured_uncultured.bacterium..0319.7F4
- Planctomycetes_Planctomycetacia_Pirellulales_Pirellulaceae_uncultured_uncultured.sludge.bacterium.A40
- Planctomycetes_Planctomycetacia_Planctomycetales_Rubinisphaeraceae_Rubinisphaera_uncultured.planctomycete
- Proteobacteria_Alphaproteobacteria_Holosporales_Holosporaceae_uncultured_uncultured.Holosporaceae.bacterium
- Proteobacteria_Alphaproteobacteria_Micavibrionales_uncultured_Alphaproteobacteria.bacterium.CG1_02_46_17_Alphaproteobacteria.bacterium.CG1_02_46_17
- Proteobacteria_Alphaproteobacteria_Rhizobiales_Hyphomicrobiaceae_Hyphomicrobium_Ambiguous_taxa
- Proteobacteria_Deltaproteobacteria_Myxococcales_bacteriap25_uncultured.sludge.bacterium_uncultured.sludge.bacterium
- Proteobacteria_Deltaproteobacteria_NB1.j_uncultured.prokaryote_uncultured.prokaryote_uncultured.prokaryote
- Proteobacteria_Deltaproteobacteria_Oligoflexales_0319.6G20_uncultured.marine.bacterium_uncultured.marine.bacterium
- Proteobacteria_Deltaproteobacteria_Oligoflexales_0319.6G20_uncultured.Syntrophobacteraceae.bacterium_uncultured.Syntrophobacteraceae.bacterium
- Proteobacteria_Deltaproteobacteria_Oligoflexales_Oligoflexaceae_Silvanigrella_bacterium.GLA1
- Proteobacteria_Deltaproteobacteria_Syntrophobacterales_Syntrophaceae_uncultured_uncultured.Smithella.sp.
- Proteobacteria_Gammaproteobacteria_Betaproteobacteriales_Chitinibacteraceae_Formivibrio_Formivibrio.citricus
- Proteobacteria_Gammaproteobacteria_Betaproteobacteriales_Methylophilaceae_Methylotenera_Methylotenera.sp..73s
- Proteobacteria_Gammaproteobacteria_Competibacterales_Competibacteraceae_Candidatus.Competibacter_uncultured.soil.bacterium
- Spirochaetes_Leptospirae_Leptospirales_Leptospiraceae_Leptospira_uncultured.spirochete
- Zixibacteria_uncultured.candidate.division.TA06.bacterium_uncultured.candidate.division.TA06.bacterium_uncultured.candidate.division.TA06.bacterium_uncultured.candidate.division.TA06.bacterium_uncultured.candidate.division.TA06.bacterium

PECHE

- Acidobacteria_Holophagae_Holophagales_Holophagaceae_uncultured_uncultured.Acidobacteriaceae.bacterium
- Acidobacteria_Thermoanaerobaculia_Thermoanaerobaculales_Thermoanaerobaculaceae_Subgroup.10_uncultured.alpha.proteobacterium
- Actinobacteria_Acidimicrobiia_Microtrichales_Ilumatobacteraceae_CL500.29.marine.group_groundwater.metagenome
- Actinobacteria_Actinobacteria_Micrococcales_Demequinaceae_Demequina_uncultured.bacterium
- Armatimonadetes_Chthonomonadetes_Chthonomonadales_Armatimonadetes.bacterium.JGI.0000077.K19_Armatimonadetes.bacterium.JGI.0000077.K19_Armatimonadetes.bacterium.JGI.0000077.K19
- Armatimonadetes_Chthonomonadetes_Chthonomonadales_Chthonomonadaceae_Chthonomonas_uncultured.bacterium
- Armatimonadetes_uncultured_uncultured.Firmicutes.bacterium_uncultured.Firmicutes.bacterium_uncultured.Firmicutes.bacterium_uncultured.Firmicutes.bacterium
- Bacteroidetes_Bacteroidia_Bacteroidales_Bacteroidetes.BD2.2_uncultured.gamma.proteobacterium_uncultured.gamma.proteobacterium
- Bacteroidetes_Bacteroidia_Bacteroidales_Dysgonomonadaceae_uncultured_uncultured.soil.bacterium

- Bacteroidetes_Bacteroidia_Bacteroidales_Marinilabiliaceae_Alkaliflexus_uncultured.Mangroviflexus.sp.
- Bacteroidetes_Bacteroidia_Bacteroidales_Rikenellaceae_Acetobacteroides_uncultured.bacterium
- Bacteroidetes_Bacteroidia_Chitinophagales_Chitinophagaceae_Ferruginibacter_uncultured.Sphingobacterium.sp.
- Bacteroidetes_Bacteroidia_Chitinophagales_Chitinophagaceae_uncultured_uncultured.Bacteroides.sp.
- Bacteroidetes_Bacteroidia_Cytophagales_Cyclobacteriaceae_uncultured_uncultured.soil.bacterium
- Bacteroidetes_Bacteroidia_Flavobacteriales_Crocinitomicaceae_Fluviicola_marine.metagenome
- Bacteroidetes_Bacteroidia_Flavobacteriales_Crocinitomicaceae_Fluviicola_uncultured.Cytophagales.bacterium
- Bacteroidetes_Bacteroidia_Flavobacteriales_Flavobacteriaceae_Flavobacterium_Flavobacterium.columnare.ATCC.49512
- Bacteroidetes_Bacteroidia_Flavobacteriales_NS9.marine.group_uncultured.bacterium.PHOS.HE28_uncultured.bacterium.PHOS.HE28
- Bacteroidetes_Bacteroidia_Sphingobacteriales_NS11.12.marine.group_uncultured.Cytophagales.bacterium_uncultured.Cytophagales.bacterium
- Bacteroidetes_Bacteroidia_Sphingobacteriales_ST.12K33_uncultured.Cytophagales.bacterium_uncultured.Cytophagales.bacterium
- Chlamydiae_Chlamydiae_Chlamydiales_Chlamydiaceae_uncultured.Chlamydiales.bacterium_uncultured.Chlamydiales.bacterium
- Chlamydiae_Chlamydiae_Chlamydiales_Parachlamydiaceae_uncultured.bacterium_uncultured.bacterium
- Chlamydiae_Chlamydiae_Chlamydiales_Parachlamydiaceae_uncultured_metagenome
- Chlamydiae_Chlamydiae_Chlamydiales_Simkaniaceae_uncultured_Chlamydiae.bacterium.SM23_39
- Chlamydiae_LD1.PA32_metagenome_metagenome_metagenome_metagenome
- Chloroflexi_Chloroflexia_Thermomicrobiales_JG30.KF.CM45_Ambiguous_taxa_Ambiguous_taxa
- Chloroflexi_Dehalococcoidia_vadinBA26_uncultured.Chloroflexi.bacterium_uncultured.Chloroflexi.bacterium_uncultured.Chloroflexi.bacterium
- Chloroflexi_Gitt.GS.136_uncultured.Chloroflexi.bacterium_uncultured.Chloroflexi.bacterium_uncultured.Chloroflexi.bacterium_uncultured.Chloroflexi.bacterium
- Chloroflexi_JG30.KF.CM66_uncultured.soil.bacterium_uncultured.soil.bacterium_uncultured.soil.bacterium_uncultured.soil.bacterium
- Chloroflexi_KD4.96_uncultured.Longilinea.sp._uncultured.Longilinea.sp._uncultured.Longilinea.sp._uncultured.Longilinea.sp.
- Crenarchaeota_Bathyarchaeia_uncultured.Thermoproteales.archaeon_uncultured.Thermoproteales.archaeon_uncultured.Thermoproteales.archaeon_uncultured.Thermoproteales.archaeon
- Cyanobacteria_Oxyphotobacteria_Nostocales_Cyanobacteriaceae_Geminocystis.PCC.6308_uncultured.cyanobacterium
- Cyanobacteria_Oxyphotobacteria_Nostocales_Cyanobacteriaceae_Merismopedia.AICB1015_Ambiguous_taxa
- Dadabacteria_Dadabacteriia_Dadabacteriales_uncultured.candidate.division.SBR1093.bacterium_uncultured.candidate.division.SBR1093.bacterium_uncultured.candidate.division.SBR1093.bacterium
- Dependentiae_Babeliae_Babeliales_UBA12409_candidate.division.TM6.bacterium.RIFCSPHIGHO2_12_FULL_36_22_candidate.division.TM6.bacterium.RIFCSPHIGHO2_12_FULL_36_22
- Dependentiae_Babeliae_Babeliales_UBA12409_uncultured.organism_uncultured.organism
- Dependentiae_Babeliae_Babeliales_uncultured.deep.sea.bacterium_uncultured.deep.sea.bacterium_uncultured.deep.sea.bacterium
- Dependentiae_Babeliae_Babeliales_Vermiphilaceae_candidate.division.TM6.bacterium.GW2011_GWF2_30_66_candidate.division.TM6.bacterium.GW2011_GWF2_30_66
- Dependentiae_Babeliae_Babeliales_Vermiphilaceae_uncultured.delta.proteobacterium_uncultured.delta.proteobacterium
- Dependentiae_Babeliae_Babeliales_Vermiphilaceae_uncultured.prokaryote_uncultured.prokaryote

- Diapherotrites_Iainarchaeia_Iainarchaeales_Diapherotrites.archaeon.SCGC.AAA011.K09_Diapherotrites.archaeon.SCGC.AAA011.K09_Diapherotrites.archaeon.SCGC.AAA011.K09
- Elusimicrobia_Lineage.IIc_Elusimicrobia.bacterium.RIFCSPHIGHO2_02_FULL_39_36_Elusimicrobia.bacterium.RIFCSPHIGHO2_02_FULL_39_36_Elusimicrobia.bacterium.RIFCSPHIGHO2_02_FULL_39_36_Elusimicrobia.bacterium.RIFCSPHIGHO2_02_FULL_39_36
- Fibrobacteres_Fibrobacteria_Fibrobacterales_Fibrobacteraceae_possible.genus.04_uncultured.bacterium
- Firmicutes_Clostridia_Clostridiales_Christensenellaceae_Christensenellaceae.R.7.group_uncultured.microorganism
- Firmicutes_Clostridia_Clostridiales_Christensenellaceae_uncultured.Firmicutes.bacterium_uncultured.Firmicutes.bacterium
- Firmicutes_Negativicutes_Selenomonadales_Veillonellaceae_uncultured_uncultured.bacterium.SJA.143
- Hadesarchaeaeota_uncultured.archaeon.SAGMA.I_uncultured.archaeon.SAGMA.I_uncultured.archaeon.SAGMA.I_uncultured.archaeon.SAGMA.I_uncultured.archaeon.SAGMA.I
- Kiritimatiellaeota_Kiritimatiellae_WCHB1.41_uncultured.marine.bacterium_uncultured.marine.bacterium_uncultured.marine.bacterium
- Latescibacteria_uncultured.soil.bacterium.PBS.III.32a_uncultured.soil.bacterium.PBS.III.32a_uncultured.soil.bacterium.PBS.III.32a_uncultured.soil.bacterium.PBS.III.32a_uncultured.soil.bacterium.PBS.III.32a
- Margulisbacteria_microbial.mat.metagenome_microbial.mat.metagenome_microbial.mat.metagenome_microbial.mat.metagenome_microbial.mat.metagenome
- Margulisbacteria_uncultured.Gram.positive.bacterium_uncultured.Gram.positive.bacterium_uncultured.Gram.positive.bacterium_uncultured.Gram.positive.bacterium_uncultured.Gram.positive.bacterium
- Omnitrophicaeota_Omnitrophia_Omnitrophales_Omnitrophaceae_Candidatus.Omnitrophus_Candidatus.Omnitrophus.fodinae.SCGC.AAA011.A17
- Patescibacteria_ABY1_Candidatus.Falkowbacteria_Candidatus.Falkowbacteria.bacterium.GW2011_GWF2_43_32_Candidatus.Falkowbacteria.bacterium.GW2011_GWF2_43_32_Candidatus.Falkowbacteria.bacterium.GW2011_GWF2_43_32
- Patescibacteria_ABY1_Candidatus.Kerfeldbacteria_Parcubacteria.group.bacterium.ADurb.Bin326_Parcubacteria.group.bacterium.ADurb.Bin326_Parcubacteria.group.bacterium.ADurb.Bin326
- Patescibacteria_ABY1_Candidatus.Magasanikbacteria_Candidatus.Uhrbacteria.bacterium.RIFCSPHIGHO2_12_FULL_60_25_Candidatus.Uhrbacteria.bacterium.RIFCSPHIGHO2_12_FULL_60_25_Candidatus.Uhrbacteria.bacterium.RIFCSPHIGHO2_12_FULL_60_25
- Patescibacteria_ABY1_Candidatus.Uhrbacteria_marine.metagenome_marine.metagenome_marine.metagenome
- Patescibacteria_Gracilibacteria_Absconditabacteriales..SR1._metagenome_metagenome_metagenome
- Patescibacteria_Gracilibacteria_Ambiguous_taxa_Ambiguous_taxa_Ambiguous_taxa_Ambiguous_taxa
- Patescibacteria_Gracilibacteria_Candidatus.Peregrinibacteria_Candidatus.Peregrinibacteria.bacterium.RIFOXYB2_FULL_41_88_Candidatus.Peregrinibacteria.bacterium.RIFOXYB2_FULL_41_88_Candidatus.Peregrinibacteria.bacterium.RIFOXYB2_FULL_41_88
- Patescibacteria_Parcubacteria_Candidatus.Kaiserbacteria_Parcubacteria.group.bacterium.CG1_02_50_68_Parcubacteria.group.bacterium.CG1_02_50_68_Parcubacteria.group.bacterium.CG1_02_50_68
- Patescibacteria_Parcubacteria_Candidatus.Yanofskybacteria_uncultured.bacterium_uncultured.bacterium_uncultured.bacterium
- Patescibacteria_Parcubacteria_uncultured.bacterium_uncultured.bacterium_uncultured.bacterium_uncultured.bacterium
- Patescibacteria_Parcubacteria_uncultured.soil.bacterium_uncultured.soil.bacterium_uncultured.soil.bacterium_uncultured.soil.bacterium
- Patescibacteria_Saccharimonadia_Saccharimonadales_bacterium.LWQ8_bacterium.LWQ8_bacterium.LWQ8
- Patescibacteria_Saccharimonadia_Saccharimonadales_uncultured.bacterium.SBR2013_uncultured.bacterium.SBR2013_uncultured.bacterium.SBR2013

- Patescibacteria_Saccharimonadia_Saccharimonadales_uncultured.candidate.division.WS5.bacterium_uncultured.candidate.division.WS5.bacterium_uncultured.candidate.division.WS5.bacterium
- Patescibacteria_Saccharimonadia_Saccharimonadales_uncultured.Epsilonproteobacteria.bacterium_uncultured.Epsilonproteobacteria.bacterium_uncultured.Epsilonproteobacteria.bacterium
- Patescibacteria_WS6..Dojkabacteria._uncultured.eubacterium.WCHB1.01_uncultured.eubacterium.WCHB1.01_uncultured.eubacterium.WCHB1.01_uncultured.eubacterium.WCHB1.01
- Planctomycetes_028H05.P.BN.P5_uncultured.bacterium_uncultured.bacterium_uncultured.bacterium_uncultured.bacterium
- Planctomycetes_ODP123_uncultured.planctomycete_uncultured.planctomycete_uncultured.planctomycete_uncultured.planctomycete
- Planctomycetes_Phycisphaerae_Phycisphaerales_AKAU3564.sediment.group_marine.sediment.metagenome_marine.sediment.metagenome
- Planctomycetes_Phycisphaerae_Phycisphaerales_Phycisphaeraceae_AKYG587_uncultured.Planctomycetales.bacterium
- Planctomycetes_Phycisphaerae_Phycisphaerales_Phycisphaeraceae_AKYG587_uncultured.soil.bacterium
- Planctomycetes_Phycisphaerae_Phycisphaerales_Phycisphaeraceae_Phycisphaera_uncultured.Planctomycetales.bacterium
- Planctomycetes_Phycisphaerae_Tepidisphaerales_CPla.3.termite.group_uncultured.planctomycete_uncultured.planctomycete
- Planctomycetes_Planctomycetacia_Gemmatales_Gemmataceae_uncultured_uncultured.Ktedonobacterales.bacterium
- Planctomycetes_Planctomycetacia_Gemmatales_Gemmataceae_uncultured_uncultured.sludge.bacterium.A12
- Planctomycetes_Planctomycetacia_Isosphaerales_Isosphaeraceae_uncultured_uncultured.soil.bacterium
- Planctomycetes_Planctomycetacia_Pirellulales_Pirellulaceae_Pir2.lineage_uncultured.soil.bacterium.PRR.1B
- Planctomycetes_Planctomycetacia_Planctomycetales_Schlesneriaceae_Planctopirus_uncultured.Planctomyces.sp.
- Proteobacteria_Alphaproteobacteria_Acetobacterales_Acetobacteraceae_uncultured_uncultured.proteobacterium
- Proteobacteria_Alphaproteobacteria_Caedibacterales_Caedibacteraceae_Caedibacter_uncultured.alpha.proteobacterium
- Proteobacteria_Alphaproteobacteria_Paracaedibacterales_Paracaedibacteraceae_Candidatus.Captivus_Candidatus.Captivus.acidiprotistae
- Proteobacteria_Alphaproteobacteria_Paracaedibacterales_Paracaedibacteraceae_Candidatus.Captivus_uncultured.bacterium
- Proteobacteria_Alphaproteobacteria_Reyranellales_Reyranellaceae_Reyranella_alpha.proteobacterium.Gsoil.1161
- Proteobacteria_Alphaproteobacteria_Rhizobiales_Xanthobacteraceae_uncultured_alpha.proteobacterium.Shinshu.th1
- Proteobacteria_Alphaproteobacteria_Rhodospirillales_Magnetospirillaceae_Magnetospirillum.sp..enrichment.culture.clone.Van25_Magnetospirillum.sp..enrichment.culture.clone.Van25
- Proteobacteria_Alphaproteobacteria_Rhodospirillales_Rhodospirillaceae_uncultured_uncultured.bacterium
- Proteobacteria_Alphaproteobacteria_Rhodospirillales_uncultured_metagenome_metagenome
- Proteobacteria_Alphaproteobacteria_Rickettsiales_Mitochondria_uncultured.bacterium_uncultured.bacterium
- Proteobacteria_Alphaproteobacteria_Rickettsiales_Rickettsiaceae_uncultured_metagenome
- Proteobacteria_Alphaproteobacteria_Sphingomonadales_Sphingomonadaceae_Sandarakinorhabdus_metagenome
- Proteobacteria_Deltaproteobacteria_Bradymonadales_Ambiguous_taxa_Ambiguous_taxa_Ambiguous_taxa

- Proteobacteria_Deltaproteobacteria_Deltaproteobacteria.Incertae.Sedis_Unknown.Family_Deferrisoma_uncultured.bacterium
- Proteobacteria_Deltaproteobacteria_Deltaproteobacteria.Incertae.Sedis_Unknown.Family_Deferrisoma_uncultured.delta.proteobacterium
- Proteobacteria_Deltaproteobacteria_Desulfovibrionales_Desulfovibrionaceae_Desulfovibrio_Ephemera.danica
- Proteobacteria_Deltaproteobacteria_Desulfuromonadales_Desulfuromonadaceae_Desulfuromonas_uncultured.prokaryote
- Proteobacteria_Deltaproteobacteria_Myxococcales_Haliangiaceae_Haliangium_Betaproteobacteria.bacterium.RIFCSPLOWO2_12_FULL_64_23
- Proteobacteria_Deltaproteobacteria_Myxococcales_P3OB.42_uncultured.Myxococcales.bacterium_uncultured.Myxococcales.bacterium
- Proteobacteria_Deltaproteobacteria_Myxococcales_Sandaracinaceae_Sandaracinus_uncultured.Sandaracinus.sp.
- Proteobacteria_Deltaproteobacteria_Myxococcales_Sandaracinaceae_uncultured_uncultured.Sorangiineae.bacterium
- Proteobacteria_Deltaproteobacteria_Oligoflexales_0319.6G20_uncultured.delta.proteobacterium_uncultured.delta.proteobacterium
- Proteobacteria_Deltaproteobacteria_Oligoflexales_053A03.B.DI.P58_metagenome_metagenome
- Proteobacteria_Deltaproteobacteria_Oligoflexales_053A03.B.DI.P58_uncultured.bacterium_uncultured.bacterium
- Proteobacteria_Deltaproteobacteria_Oligoflexales_053A03.B.DI.P58_uncultured.delta.proteobacterium_uncultured.delta.proteobacterium
- Proteobacteria_Deltaproteobacteria_Oligoflexales_Oligoflexaceae_uncultured_uncultured.organism
- Proteobacteria_Deltaproteobacteria_SAR324.clade.Marine.group.B._uncultured.delta.proteobacterium.Sva0853_uncultured.delta.proteobacterium.Sva0853_uncultured.delta.proteobacterium.Sva0853
- Proteobacteria_Deltaproteobacteria_SAR324.clade.Marine.group.B._uncultured.prokaryote_uncultured.prokaryote_uncultured.prokaryote
- Proteobacteria_Deltaproteobacteria_Syntrophobacterales_Syntrophaceae_Desulfomonile_Ambiguous_taxa
- Proteobacteria_Deltaproteobacteria_Syntrophobacterales_Syntrophaceae_uncultured_uncultured.Myxococcales.bacterium
- Proteobacteria_Gammaproteobacteria_Cellvibrionales_Cellvibrionaceae_uncultured_Teredinibacter.sp..1162T.S.0a.05
- Proteobacteria_Gammaproteobacteria_EC3_uncultured.proteobacterium_uncultured.proteobacterium_uncultured.proteobacterium
- Proteobacteria_Gammaproteobacteria_Gammaproteobacteria.Incertae.Sedis_Unknown.Family_Candidatus.Ovatusbacter_Candidatus.Ovatusbacter.abovo
- Rokubacteria_NC10_Rokubacteriales_uncultured.Thermanaeromonas.sp._uncultured.Thermanaeromonas.sp._uncultured.Thermanaeromonas.sp.
- Spirochaetes_Leptospirae_Leptospirales_Leptospiraceae_Leptospira_metagenome
- Spirochaetes_Leptospirae_Leptospirales_Leptospiraceae_RBG.16.49.21_Spirochaetes.bacterium.RBG_16_49_21
- Verrucomicrobia_Verrucomicrobiae_Chthoniobacterales_Chthoniobacteraceae_uncultured.Verrucomicrobia.bacterium_uncultured.Verrucomicrobia.bacterium
- Verrucomicrobia_Verrucomicrobiae_Pedosphaerales_Pedosphaeraceae_uncultured.verrucomicrobium.DEV008_uncultured.verrucomicrobium.DEV008
- Verrucomicrobia_Verrucomicrobiae_Pedosphaerales_Pedosphaeraceae_uncultured.verrucomicrobium.DEV064_uncultured.verrucomicrobium.DEV064
- Verrucomicrobia_Verrucomicrobiae_uncultured_marine.metagenome_marine.metagenome_marine.metagenome

- Verrucomicrobia_Verrucomicrobiae_Verrucomicrobiales_DEV007_uncultured.bacterium_uncultured.bacterium
- WPS.2_bacterium.enrichment.culture.clone.Anammox_3_bacterium.enrichment.culture.clone.Anammox_3_bacterium.enrichment.culture.clone.Anammox_3_bacterium.enrichment.culture.clone.Anammox_3_bacterium.enrichment.culture.clone.Anammox_3

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- Acidobacteria_Subgroup.17_uncultured.Holophaga.sp._uncultured.Holophaga.sp._uncultured.Holophaga.sp._uncultured.Holophaga.sp.
- Actinobacteria_Acidimicrobiia_Microtrichales_Ilumatobacteraceae_uncultured_Ambiguous_taxa
- Bacteroidetes_Bacteroidia_Bacteroidales_Bacteroidetes.BD2.2_Bacteroidetes.bacterium.GWF2_40_13_Bacteroidetes.bacterium.GWF2_40_13
- Bacteroidetes_Bacteroidia_Bacteroidales_Prolixibacteraceae_uncultured_uncultured.Cytophagales.bacterium
- Bacteroidetes_Bacteroidia_Chitinophagales_Chitinophagaceae_uncultured_uncultured.Sphingobacterium.sp.
- Bacteroidetes_Bacteroidia_Flavobacteriales_NS9.marine.group_metagenome_metagenome
- Bacteroidetes_Bacteroidia_Sphingobacteriales_Lentimicrobiaceae_metagenome_metagenome
- Bacteroidetes_Ignavibacteria_OPB56_Ambiguous_taxa_Ambiguous_taxa_Ambiguous_taxa
- BRC1_uncultured.soil.bacterium.PBS.III.29_uncultured.soil.bacterium.PBS.III.29_uncultured.soil.bacterium.PBS.III.29_uncultured.soil.bacterium.PBS.III.29_uncultured.soil.bacterium.PBS.III.29
- Crenarchaeota_Bathyarchaeia_uncultured.crenarchaeote_uncultured.crenarchaeote_uncultured.crenarchaeote_uncultured.crenarchaeote
- Dependentiae_Babeliae_Babeliales_UBA12411_metagenome_metagenome
- Elusimicrobia_Elusimicrobia_Lineage.IV_Elusimicrobia.bacterium.RIFCSPHIGHO2_02_FULL_61_10_Elusimicrobia.bacterium.RIFCSPHIGHO2_02_FULL_61_10_Elusimicrobia.bacterium.RIFCSPHIGHO2_02_FULL_61_10
- Euryarchaeota_Methanomicrobia_Methanomicrobiales_uncultured_Ambiguous_taxa_Ambiguous_taxa
- Euryarchaeota_Thermoplasmata_uncultured_uncultured.eukaryote_uncultured.eukaryote_uncultured.eukaryote
- Firmicutes_Bacilli_Bacillales_Paenibacillaceae_Paenibacillus_Paenibacillus.pectinilyticus
- Firmicutes_Clostridia_Clostridiales_Clostridiaceae.1_Clostridium.sensu.stricto.5_Clostridium.algidicarnis
- Firmicutes_Clostridia_Clostridiales_Family.XI_Anaerococcus_Ambiguous_taxa
- Lentisphaerae_Lentisphaeria_Victivallales_PRD18C08_uncultured.bacterium_uncultured.bacterium
- Margulisbacteria_uncultured.candidate.division.ZB3.bacterium_uncultured.candidate.division.ZB3.bacterium_uncultured.candidate.division.ZB3.bacterium_uncultured.candidate.division.ZB3.bacterium_uncultured.candidate.division.ZB3.bacterium
- Margulisbacteria_uncultured.delta.proteobacterium_uncultured.delta.proteobacterium_uncultured.delta.proteobacterium_uncultured.delta.proteobacterium_uncultured.delta.proteobacterium
- Patescibacteria_Saccharimonadia_Saccharimonadales_uncultured.bacterium.SBR1071_uncultured.bacterium.SBR1071_uncultured.bacterium.SBR1071
- Planctomycetes_Phycisphaerae_Phycisphaerales_Phycisphaeraceae_SM1A02_uncultured.deep.sea.bacterium
- Planctomycetes_Planctomycetacia_Planctomycetales_Schlesneriaceae_Schlesneria_Planctomyces.sp.
- Proteobacteria_Alphaproteobacteria_Caulobacterales_Hyphomonadaceae_UKL13.1_uncultured.alpha.proteobacterium
- Proteobacteria_Alphaproteobacteria_Rhizobiales_Beijerinckiaceae_FFCH5858_uncultured.Rhizobiales.bacterium
- Proteobacteria_Alphaproteobacteria_Sphingomonadales_Sphingomonadaceae_Sandaracinobacter_metagenome

- Proteobacteria_Alphaproteobacteria_Tistrellales_Geminicoccaceae_Candidatus.Alysiosphaera_uncultured.bacterium
- Proteobacteria_Deltaproteobacteria_Myxococcales_BIrii41_uncultured.proteobacterium_uncultured.proteobacterium
- Proteobacteria_Gammaproteobacteria_Betaproteobacteriales_Nitrosomonadaceae_966.1_metagenome
- Proteobacteria_Gammaproteobacteria_Immundisolibacterales_Immundisolibacteraceae_Immundisolibacter_uncultured.bacterium
- Spirochaetes_Spirochaetia_Spirochaetales_Spirochaetaceae_uncultured_uncultured.Spirochaetes.bacterium

TURKEY CREEK TRIBUTARY

- Acidobacteria_Acidobacteriia_Subgroup.2_uncultured.forest.soil.bacterium_uncultured.forest.soil.bacterium_uncultured.forest.soil.bacterium
- Asgardaeota_Lokiarchaeia_unidentified.archaeon_unidentified.archaeon_unidentified.archaeon_unidentified.archaeon
- Bacteroidetes_Bacteroidia_Bacteroidales_Rikenellaceae_Blvii28.wastewater.sludge.group_uncultured.soil.bacterium
- Bacteroidetes_Bacteroidia_Flavobacteriales_NS9.marine.group_uncultured.Bacteroidetes.bacterium_uncultured.Bacteroidetes.bacterium
- Bacteroidetes_Bacteroidia_Sphingobacteriales_env.OPS.17_uncultured.Sphingobacteriales.bacterium_uncultured.Sphingobacteriales.bacterium
- BRC1_uncultured.soil.bacterium.PBS.II.34_uncultured.soil.bacterium.PBS.II.34_uncultured.soil.bacterium.PBS.II.34_uncultured.soil.bacterium.PBS.II.34_uncultured.soil.bacterium.PBS.II.34
- Chlamydiae_Chlamydiae_Chlamydiales_Chlamydiaceae_Candidatus.Amphibiichlamydia_Candidatus.Amphibiichlamydia.ranarum
- Chlamydiae_Chlamydiae_Chlamydiales_uncultured.organism_uncultured.organism_uncultured.organism
- Chloroflexi_Anaerolineae_Anaerolineales_Anaerolineaceae_Leptolinea_uncultured.bacterium.SJA.116
- Chloroflexi_Anaerolineae_Anaerolineales_Anaerolineaceae_Leptolinea_uncultured.eubacterium.WCHB1.43
- Chloroflexi_Anaerolineae_Anaerolineales_Anaerolineaceae_Longilinea_uncultured.anaerobic.bacterium
- Chloroflexi_Anaerolineae_SBR1031_uncultured.sludge.bacterium.H5_uncultured.sludge.bacterium.H5_uncultured.sludge.bacterium.H5
- Chloroflexi_Dehalococcoidia_MSBL5_uncultured.Chloroflexi.bacterium_uncultured.Chloroflexi.bacterium_uncultured.Chloroflexi.bacterium
- Chloroflexi_Dehalococcoidia_uncultured.Chloroflexi.bacterium_uncultured.Chloroflexi.bacterium_uncultured.Chloroflexi.bacterium_uncultured.Chloroflexi.bacterium
- Crenarchaeota_Bathyarchaeia_uncultured.methanogenic.archaeon_uncultured.methanogenic.archaeon_uncultured.methanogenic.archaeon_uncultured.methanogenic.archaeon

- Cyanobacteria_Sericytochromatia_uncultured.prokaryote_uncultured.prokaryote_uncultured.prokaryote_uncultured.prokaryote
- Dependentiae_Babeliae_Babeliales_Babeliaceae_Ambiguous_taxa_Ambiguous_taxa
- Elusimicrobia_Elusimicrobia_Lineage.IV_Ambiguous_taxa_Ambiguous_taxa_Ambiguous_taxa
- Elusimicrobia_Lineage.IIa_uncultured.Termite.group.1.bacterium_uncultured.Termite.group.1.bacterium_uncultured.Termite.group.1.bacterium_uncultured.Termite.group.1.bacterium
- Euryarchaeota_Methanomicrobia_ANME.1_ANME.1a_uncultured.ANME.1.euryarchaeote_uncultured.ANME.1.euryarchaeote
- Euryarchaeota_Methanomicrobia_Methanomicrobiales_Methanomicrobiaceae_uncultured_uncultured.Methanomicrobia.archaeon

- Euryarchaeota_Thermococci_Methanofastidiosales_Methanofastidiosaceae_Candidatus.Methanofastidiosum_uncultured.archaeon.WCHA1.57
- Firmicutes_Clostridia_Clostridiales_Defluviitaleaceae_Defluviitaleaceae.UCG.011_microbial.mat.metagenome
- Firmicutes_Clostridia_Clostridiales_Family.XI_Anaerococcus_uncultured.organism
- Firmicutes_Clostridia_Clostridiales_Family.XII_Acidaminobacter_uncultured.soil.bacterium
- Firmicutes_Clostridia_Clostridiales_Lachnospiraceae_Natranaerovirga_uncultured.bacterium
- Firmicutes_Clostridia_Clostridiales_Ruminococcaceae_Ruminococcaceae.UCG.010_uncultured.bacterium
- Gemmatimonadetes_Gemmatimonadetes_Gemmatimonadales_Gemmatimonadaceae_uncultured_Acidobacteria.bacterium.13_2_20CM_56_17
- Omnitrophicaeota_Ambiguous_taxa_Ambiguous_taxa_Ambiguous_taxa_Ambiguous_taxa_Ambiguous_taxa
- Omnitrophicaeota_Omnitrophia_Omnitrophales_Omnitrophaceae_Candidatus.Omnitrophus_uncultured.planctomycete
- Patescibacteria_ABY1_Candidatus.Falkowbacteria_uncultured.prokaryote_uncultured.prokaryote_uncultured.prokaryote
- Patescibacteria_ABY1_Candidatus.Magasanikbacteria_uncultured.Parcubacteria.group.bacterium_uncultured.Parcubacteria.group.bacterium_uncultured.Parcubacteria.group.bacterium
- Patescibacteria_Gracilibacteria_Candidatus.Peribacteria_uncultured.bacterium_uncultured.bacterium_uncultured.bacterium
- Patescibacteria_Gracilibacteria_uncultured.deep.sea.bacterium_uncultured.deep.sea.bacterium_uncultured.deep.sea.bacterium_uncultured.deep.sea.bacterium
- Patescibacteria_Parcubacteria_Candidatus.Azambacteria_Candidatus.Zambryskibacteria.bacterium.RIFCSPLOWO2_12_FULL_39_16_Candidatus.Zambryskibacteria.bacterium.RIFCSPLOWO2_12_FULL_39_16_Candidatus.Zambryskibacteria.bacterium.RIFCSPLOWO2_12_FULL_39_16
- Patescibacteria_Parcubacteria_Candidatus.Campbellbacteria_uncultured.bacterium_uncultured.bacterium_uncultured.bacterium
- Patescibacteria_Parcubacteria_Candidatus.Yanofskybacteria_metagenome_metagenome_metagenome
- Planctomycetes_Brocadiae_SM23.32_Planctomycetes.bacterium.SM23_32_Planctomycetes.bacterium.SM23_32_Planctomycetes.bacterium.SM23_32
- Planctomycetes_Phycisphaerae_Phycisphaerales_Phycisphaeraceae_I.8_uncultured.planctomycete
- Planctomycetes_Phycisphaerae_Pla1.lineage_uncultured.organism_uncultured.organism_uncultured.organism
- Planctomycetes_Planctomycetacia_Pirellulales_Pirellulaceae_Pirellula_bacterium.enrichment.culture.clone.B161.2011.
- Planctomycetes_vadinHA49_uncultured.sediment.bacterium_uncultured.sediment.bacterium_uncultured.sediment.bacterium_uncultured.sediment.bacterium
- Proteobacteria_Alphaproteobacteria_Micropepsales_Micropepsaceae_Rhizomicrobium_uncultured.bacterium
- Proteobacteria_Alphaproteobacteria_Rhizobiales_Hyphomicrobiaceae_uncultured.bacterium_uncultured.bacterium
- Proteobacteria_Alphaproteobacteria_Rickettsiales_Rickettsiaceae_Candidatus.Megaira_metagenome
- Proteobacteria_Alphaproteobacteria_Thalassobaculales_uncultured_uncultured.soil.bacterium_uncultured.soil.bacterium
- Proteobacteria_Deltaproteobacteria_Ambiguous_taxa_uncultured.delta.proteobacterium_uncultured.delta.proteobacterium_uncultured.delta.proteobacterium
- Proteobacteria_Deltaproteobacteria_Desulfobacterales_Desulfobacteraceae_Sva0081.sediment.group_delta.proteobacterium.enrichment.culture.clone.AP.FeEnrich12
- Proteobacteria_Deltaproteobacteria_Myxococcales_Nannocystaceae_Nannocystis_unidentified
- Proteobacteria_Deltaproteobacteria_Oligoflexales_Oligoflexaceae_uncultured_Ambiguous_taxa

- Proteobacteria_Deltaproteobacteria_Oligoflexales_Oligoflexaceae_uncultured_uncultured.sediment.bacterium
- Proteobacteria_Deltaproteobacteria_Syntrophobacterales_Syntrophaceae_Syntrophus_Syntrophus.sp..PtaU1.Bin005
- Proteobacteria_Gammaproteobacteria_AT.s2.59_uncultured.marine.bacterium_uncultured.marine.bacterium_uncultured.marine.bacterium
- Spirochaetes_Spirochaetia_Spirochaetales_Spirochaetaceae_Spirochaeta.2_uncultured.Spirochaetales.bacterium
- Tenericutes_Mollicutes_Mycoplasmatales_Mycoplasmataceae_Mycoplasma_Oncorhynchus.mykiss..rainbow.trout.
- Thaumarchaeota_Nitrososphaeria_Nitrososphaerales_Nitrososphaeraceae_uncultured.euryarchaeote_uncultured.euryarchaeote
- Verrucomicrobia_Verrucomicrobiae_Verrucomicrobiales_Verrucomicrobiaceae_Prosthecobacter_Prosthecobacter.fluviatilis
- WS1_uncultured.candidate.division.WS1.bacterium_uncultured.candidate.division.WS1.bacterium_uncultured.candidate.division.WS1.bacterium_uncultured.candidate.division.WS1.bacterium_uncultured.candidate.division.WS1.bacterium
- Zixibacteria_bacterium.enrichment.culture.clone.Anammox_55_bacterium.enrichment.culture.clone.Anammox_55_bacterium.enrichment.culture.clone.Anammox_55_bacterium.enrichment.culture.clone.Anammox_55_bacterium.enrichment.culture.clone.Anammox_55

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- Acidobacteria_Blastocatellia..Subgroup.4._11.24_uncultured.Acidobacteriales.bacterium_uncultured.Acidobacteriales.bacterium_uncultured.Acidobacteriales.bacterium
- Acidobacteria_Blastocatellia..Subgroup.4._Blastocatellales_Blastocatellaceae_Stenotrophobacter_uncultured.Acidobacterium.sp.
- Acidobacteria_Blastocatellia..Subgroup.4._Blastocatellales_Blastocatellaceae_Stenotrophobacter_uncultured.soil.bacterium
- Acidobacteria_Fischerbacteria_Candidatus.Fischerbacteria.bacterium.RBG_13_37_8_Candidatus.Fischerbacteria.bacterium.RBG_13_37_8_Candidatus.Fischerbacteria.bacterium.RBG_13_37_8_Candidatus.Fischerbacteria.bacterium.RBG_13_37_8
- Actinobacteria_Acidimicrobiia_Microtrichales_Microtrichaceae_uncultured_Acidimicrobidae.bacterium.YM18.244
- Actinobacteria_Thermoleophilia_Gaiellales_Gaiellaceae_Gaiella_Gaiella.occulta
- Bacteroidetes_Bacteroidia_Bacteroidales_Bacteroidetes.vadinHA17_uncultured.bacterium_uncultured.bacterium
- Bacteroidetes_Bacteroidia_Bacteroidales_Dysgonomonadaceae_Candidatus.Armantifilum_Coptotermes.gestroi
- Bacteroidetes_Bacteroidia_Chitinophagales_37.13_uncultured.prokaryote_uncultured.prokaryote
- Bacteroidetes_Bacteroidia_Sphingobacteriales_S15A.MN91_uncultured.Bacteroidetes.bacterium_uncultured.Bacteroidetes.bacterium
- Bacteroidetes_Bacteroidia_Sphingobacteriales_s74e.6049_uncultured.bacterium_uncultured.bacterium
- Bacteroidetes_Ignavibacteria_Ignavibacteriales_BSN166_uncultured.bacterium_uncultured.bacterium
- Chlamydiae_Chlamydiae_Chlamydiales_Parachlamydiaceae_Candidatus.Mesochlamydia_Candidatus.Mesochlamydia.elodeae
- Chlamydiae_Chlamydiae_Chlamydiales_Simkaniaceae_Candidatus.Fritschea_Candidatus.Syngnamydia.venezia
- Chlamydiae_Chlamydiae_Chlamydiales_Simkaniaceae_uncultured_metagenome

- Chloroflexi_Anaerolineae_RBG.13.54.9_uncultured.Anaerolineaceae.bacterium_uncultured.Anaerolineaceae.bacterium_uncultured.Anaerolineaceae.bacterium
- Chloroflexi_Anaerolineae_RBG.13.54.9_uncultured.bacterium.C2.12_uncultured.bacterium.C2.12_uncultured.bacterium.C2.12
- Chloroflexi_Anaerolineae_RBG.13.54.9_uncultured.soil.bacterium_uncultured.soil.bacterium_uncultured.soil.bacterium
- Chloroflexi_Anaerolineae_uncultured.Bellilinea.sp._uncultured.Bellilinea.sp._uncultured.Bellilinea.sp._uncultured.Bellilinea.sp.
- Chloroflexi_SHA.26_uncultured.Chloroflexi.bacterium_uncultured.Chloroflexi.bacterium_uncultured.Chloroflexi.bacterium_uncultured.Chloroflexi.bacterium
- Elusimicrobia_Lineage.IIa_actinobacterium.YJF1.30_actinobacterium.YJF1.30_actinobacterium.YJF1.30_actinobacterium.YJF1.30
- Elusimicrobia_Lineage.IIc_uncultured.bacterium_uncultured.bacterium_uncultured.bacterium_uncultured.bacterium
- Euryarchaeota_Methanomicrobia_Methanomicrobiales_Methanomicrobiaceae_uncultured_uncultured.euryarchaeote
- Firmicutes_Bacilli_Bacillales_Pasteuriaceae_Pasteuria_Daphnia.endosymbiotic.bacterium
- Firmicutes_Clostridia_Clostridiales_Clostridiaceae.1_Clostridium.sensu.stricto.19_Clostridium.cylindrosporum
- Firmicutes_Clostridia_Clostridiales_Family.XI_Sedimentibacter_uncultured.Clostridia.bacterium
- Firmicutes_Clostridia_Clostridiales_Lachnospiraceae_Cellulosilyticum_Cellulosilyticum.sp.
- Firmicutes_Clostridia_Clostridiales_Lachnospiraceae_Epulopiscium_uncultured.Lachnospiraceae.bacterium
- Firmicutes_Clostridia_Clostridiales_Syntrophomonadaceae_Syntrophomonas_Syntrophomonas.sp..enrichment.culture.clone.06.1235251.81
- Firmicutes_Clostridia_Clostridiales_TSAC18_Firmicutes.bacterium.RBG_13_65_8_Firmicutes.bacterium.RBG_13_65_8
- Margulisbacteria_uncultured.organism_uncultured.organism_uncultured.organism_uncultured.organism_uncultured.organism
- Omnitrophicaeota_Omnitrophia_uncultured.organism_uncultured.organism_uncultured.organism_uncultured.organism
- Omnitrophicaeota_uncultured.sediment.bacterium_uncultured.sediment.bacterium_uncultured.sediment.bacterium_uncultured.sediment.bacterium_uncultured.sediment.bacterium
- Patescibacteria_ABY1_Candidatus.Kerfeldbacteria_uncultured.bacterium_uncultured.bacterium_uncultured.bacterium
- Patescibacteria_ABY1_Candidatus.Komeilibacteria_Candidatus.Komeilibacteria.bacterium.RIFCSPHIGHO2_01_FULL_52_14_Candidatus.Komeilibacteria.bacterium.RIFCSPHIGHO2_01_FULL_52_14_Candidatus.Komeilibacteria.bacterium.RIFCSPHIGHO2_01_FULL_52_14
- Patescibacteria_Gracilibacteria_Candidatus.Abawacabacteria_uncultured.bacterium_uncultured.bacterium_uncultured.bacterium
- Planctomycetes_BD7.11_uncultured.Planctomycetales.bacterium_uncultured.Planctomycetales.bacterium_uncultured.Planctomycetales.bacterium_uncultured.Planctomycetales.bacterium
- Planctomycetes_Planctomycetacia_Gemmatales_Gemmataceae_Gemmata_Gemmata.like.str..CJuql4
- Planctomycetes_Planctomycetacia_Gemmatales_Gemmataceae_uncultured_planctomycete.WY69
- Proteobacteria_Alphaproteobacteria_Caulobacterales_Caulobacteraceae_Asticcacaulis_Asticcacaulis.sp..YBE204
- Proteobacteria_Alphaproteobacteria_Caulobacterales_Hyphomonadaceae_SWB02_uncultured.Caulobacterales.bacterium
- Proteobacteria_Alphaproteobacteria_Elsterales_uncultured_uncultured.Nitrosomonadales.bacterium_uncultured.Nitrosomonadales.bacterium

- Proteobacteria_Alphaproteobacteria_Paracaedibacterales_Paracaedibacteraceae_uncultured_uncultured.Rickettsiales.bacterium
- Proteobacteria_Alphaproteobacteria_Reyranellales_Reyranellaceae_Reyranella_uncultured.soil.bacterium
- Proteobacteria_Alphaproteobacteria_Rhizobiales_Devosiaceae_Devosia_Devosia.sp..R.43964
- Proteobacteria_Alphaproteobacteria_Rhizobiales_Hyphomicrobiaceae_Pedomicrobium_uncultured.alpha.proteobacterium
- Proteobacteria_Alphaproteobacteria_Rhizobiales_Rhizobiales.Incertae.Sedis_Phreatobacter_Alphaproteobacteria.bacterium
- Proteobacteria_Alphaproteobacteria_Rhodospirillales_uncultured_uncultured.Rhodospirillaceae.bacterium_uncultured.Rhodospirillaceae.bacterium
- Proteobacteria_Alphaproteobacteria_Rickettsiales_Midichloriaceae_Candidatus.Jidaibacter_uncultured.bacterium
- Proteobacteria_Alphaproteobacteria_Rickettsiales_Midichloriaceae_uncultured.Candidatus.Midichloria.sp._uncultured.Candidatus.Midichloria.sp.
- Proteobacteria_Alphaproteobacteria_Rickettsiales_Midichloriaceae_uncultured_uncultured.bacterium
- Proteobacteria_Alphaproteobacteria_Sphingomonadales_Sphingomonadaceae_Blastomonas_Sphingomonas.sp..OTSz_A_215
- Proteobacteria_Deltaproteobacteria_Desulfobacterales_Desulfobulbaceae_Desulforhopalus_Desulforhopalus.singaporensis
- Proteobacteria_Deltaproteobacteria_Myxococcales_mle1.27_uncultured.proteobacterium_uncultured.proteobacterium
- Proteobacteria_Gammaproteobacteria_Diplorickettsiales_Diplorickettsiaceae_uncultured_uncultured.bacterium
- Proteobacteria_Gammaproteobacteria_Gammaproteobacteria.Incertae.Sedis_Unknown.Family_uncultured.bacterium_uncultured.bacterium
- Proteobacteria_Gammaproteobacteria_JTB23_metagenome_metagenome_metagenome
- Proteobacteria_Gammaproteobacteria_uncultured_uncultured.bacterium_uncultured.bacterium_uncultured.bacterium
- Spirochaetes_Leptospirae_Leptospirales_Leptospiraceae_Leptonema_Ambiguous_taxa
- Spirochaetes_Spirochaetia_Spirochaetales_Spirochaetaceae_Spirochaeta.2_metagenome
- Thaumarchaeota_SCGC.AB.179.E04_Ambiguous_taxa_Ambiguous_taxa_Ambiguous_taxa_Ambiguous_taxa
- Verrucomicrobia_Verrucomicrobiae_Chthoniobacterales_Chthoniobacteraceae_Chthoniobacter_uncultured.Xiphinematobacteriaceae.bacterium
- Verrucomicrobia_Verrucomicrobiae_Opitutales_Opitutaceae_Lacunisphaera_bacterium.enrichment.culture.clone.auto123_4W
- Verrucomicrobia_Verrucomicrobiae_Verrucomicrobiales_Rubritaleaceae_Rubritalea_Rubritalea.sp..shu.10.MA45.1

RIVER CANARD TRIBUTARY

- Proteobacteria_Deltaproteobacteria_Oligoflexales_Oligoflexaceae_uncultured_uncultured.bacterium.Ak20.3
- Proteobacteria_Alphaproteobacteria_Micavibrionales_Micavibrionaceae_uncultured_metagenome
- Proteobacteria_Deltaproteobacteria_Oligoflexales_0319.6G20_uncultured.soil.bacterium_uncultured.soil.bacterium
- Deinococcus.Thermus_Deinococci_Deinococcales_Deinococcaceae_uncultured_uncultured.bacterium
- Fusobacteria_Fusobacteriia_Fusobacteriales_Leptotrichiaceae_uncultured_uncultured.microorganism
- Actinobacteria_Actinobacteria_Propionibacteriales_Propionibacteriaceae_uncultured_uncultured.Actinomycetales.bacterium

- Actinobacteria_Acidimicrobiia_Microtrichales_Ilumatobacteraceae_Ilumatobacter_uncultured.Actinomycetales.bacterium
- Actinobacteria_Actinobacteria_Micrococcales_Micrococcaceae_Kocuria_Kocuria.sp..KPA
- Firmicutes_Clostridia_Clostridiales_Family.XI_Finegoldia_Ambiguous_taxa
- Firmicutes_Clostridia_Clostridiales_Lachnospiraceae_Epulopiscium_Niameybacter.massiliensis
- Actinobacteria_Thermoleophilia_Solirubrobacterales_Solirubrobacteraceae_Conexibacter_uncultured.actinobacterium
- Chloroflexi_Dehalococcoidia_661239_uncultured.Dehalococcoides.sp._uncultured.Dehalococcoides.sp._uncultured.Dehalococcoides.sp.
- Zixibacteria_candidate.division.Zixibacteria.bacterium.SM1_73_candidate.division.Zixibacteria.bacterium.SM1_73_candidate.division.Zixibacteria.bacterium.SM1_73_candidate.division.Zixibacteria.bacterium.SM1_73_candidate.division.Zixibacteria.bacterium.SM1_73
- Bacteroidetes_Rhodothermia_Rhodothermales_Rhodothermaceae_uncultured_uncultured.organism
- Bacteroidetes_Ignavibacteria_Ignavibacteriales_SM1H02_Ambiguous_taxa_Ambiguous_taxa
- Verrucomicrobia_Verrucomicrobiae_Opitutales_Opitutaceae_IMCC26134_Verrucomicrobia.bacterium.IMCC26134
- Spirochaetes_V2072.189E03_uncultured.Firmicutes.bacterium_uncultured.Firmicutes.bacterium_uncultured.Firmicutes.bacterium_uncultured.Firmicutes.bacterium
- Planctomycetes_Phycisphaerae_Phycisphaerales_Phycisphaeraceae_SM1A02_uncultured.soil.bacterium
- Bacteroidetes_Bacteroidia_Chitinophagales_Saprospiraceae_Phaeodactylibacter_uncultured.Haliscomenobacter.sp.
- Acidobacteria_Thermoanaerobaculia_Thermoanaerobaculales_Thermoanaerobaculaceae_Subgroup.10_metagenome
- Proteobacteria_Deltaproteobacteria_RCP2.54_uncultured.soil.bacterium_uncultured.soil.bacterium_uncultured.soil.bacterium
- Bacteroidetes_Bacteroidia_Chitinophagales_Chitinophagaceae_Haoranjiania_uncultured.Bacteroidetes.bacterium
- Thaumarchaeota_Nitrososphaeria_Nitrososphaerales_Nitrososphaeraceae_Candidatus.Nitrocosmicus_uncultured.crenarchaeote
- Zixibacteria_candidate.division.Zixibacteria.bacterium.RBG_16_43_9_candidate.division.Zixibacteria.bacterium.RBG_16_43_9_candidate.division.Zixibacteria.bacterium.RBG_16_43_9_candidate.division.Zixibacteria.bacterium.RBG_16_43_9_candidate.division.Zixibacteria.bacterium.RBG_16_43_9
- Proteobacteria_Deltaproteobacteria_Oligoflexales_Oligoflexaceae_uncultured_uncultured.marine.microorganism
- Patescibacteria_Saccharimonadia_Saccharimonadales_metal.contaminated.soil.clone.K20.27_metal.contaminated.soil.clone.K20.27_metal.contaminated.soil.clone.K20.27
- Patescibacteria_Microgenomatia_Candidatus.Levybacteria_uncultured.soil.bacterium_uncultured.soil.bacterium_uncultured.soil.bacterium
- Patescibacteria_Parcubacteria_Candidatus.Kaiserbacteria_uncultured.organism_uncultured.organism_uncultured.organism
- Patescibacteria_Microgenomatia_Candidatus.Woykebacteria_Candidatus.Woykebacteria.bacterium.RBG_13_40_15_Candidatus.Woykebacteria.bacterium.RBG_13_40_15_Candidatus.Woykebacteria.bacterium.RBG_13_40_15
- TA06_uncultured.candidate.division.AC1.bacterium_uncultured.candidate.division.AC1.bacterium_uncultured.candidate.division.AC1.bacterium_uncultured.candidate.division.AC1.bacterium_uncultured.candidate.division.AC1.bacterium
- Dependentiae_Babeliae_Babeliales_Vermiphilaceae_metagenome_metagenome
- Spirochaetes_Spirochaetia_Spirochaetales_Spirochaetaceae_Candidatus.Marispirochaeta_Candidatus.Marispirochaeta.associata
- Spirochaetes_Spirochaetia_Spirochaetales_Spirochaetaceae_Salinispira_uncultured.bacterium

- Bacteroidetes_Bacteroidia_Flavobacteriales_Crocinitomicaceae_Fluviicola_uncultured.Cryomorphaceae.bacterium
- Bacteroidetes_Bacteroidia_Flavobacteriales_Flavobacteriaceae_Flavobacterium_Flavobacterium.sp..UCM.R36
- Bacteroidetes_Bacteroidia_Sphingobacteriales_NS11.12.marine.group_uncultured.Sphingobacterium.sp._uncultured.Sphingobacterium.sp.
- Bacteroidetes_Bacteroidia_Chitinophagales_Saprospiraceae_uncultured_uncultured.Cytophaga.sp.

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