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Application of the Fine Sediment Biotic Index to Three Drain Tiled and Ditched Agricultural Systems in Southern Minnesota

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Application of the Fine Sediment Biotic Index to three drain tiled and ditched agricultural systems in southern Minnesota

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This thesis has been examined and approved by the following members of the student's committee.

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Committee Member

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Abstract

Artificial drainage is a common agricultural management technique in the United States used to remove excess water from poorly drained soils. Approximately 22.48 million hectares of crop land are drain-tiled in the United States, providing long-term economic benefits to farmers. However, artificial drainage increases sediment transport in agricultural watersheds, which can degrade aquatic systems, destroy habitats, and limit biological diversity. Biotic indices based on benthic macroinvertebrates are commonly used to assess surface water quality, but recent studies show potential in developing sediment biotic indices using benthic macroinvertebrates to estimate fine sediment in streams. The objective of this study was to initiate the development of a biological index that reflects the fine sediment conditions in ditch systems utilizing subsurface drainage in southern Minnesota. Macroinvertebrates, sediment cores, and suspended sediments were collected from agricultural ditches in 2021 and 2022 during severe drought conditions. Fine sediments stored on a streambed ranged between 0.716–100.6 g/m2 in 2021 and decreased to a range of 0.0762–34.28 g/m2 in 2022. Total Suspended Solids decreased from an average of 18.6 mg/L in 2021 to 8.9 mg/L in 2022. The TITAN analyses identified 10 indicator families in 2021 and 5 in 2022, with *Heptageniidae* being the only family appearing in both years. Calculated FSBI scores ranged from 19-31 and 5-13 for 2021 and 2022, respectively. This project serves as a foundation for future research on the development of fine sediment indices in this region, and demonstrates that the FSBI approach may be a useful tool for assessing stream ecosystem health in Minnesota.

Introduction

Artificial drainage is an agricultural management technique utilized in the United States to remove excess water from poorly drained soils (Fausey, 2005; Sands, 2018; University of Minnesota Extension, 2018; Ghane, 2018). These drainage systems can be surface or subsurface (also referred to as tile-drained or drain-tiled) and are used for redirecting water to create favorable agricultural conditions. Figure 1 presents major areas in the United States that rely on subsurface drainage. Approximately 22.48 million ha of crop-land are drain-tiled in the United States, providing a long-term economic benefit to farmers by increasing productivity, crop-yield, and enabling earlier planting and harvesting (USDA National Agricultural Statistics Service, 2017A; Fausey, 2005; Hofstrand, 2010). It also decreases the year-to-year variability of crop yields (Brown *et al*., 1998). A 35-year study indicated that increased subsurface drainage reduced fieldwork days up to two weeks while increasing corn yields by 24 bushels per acre (Kladivco, 2020).

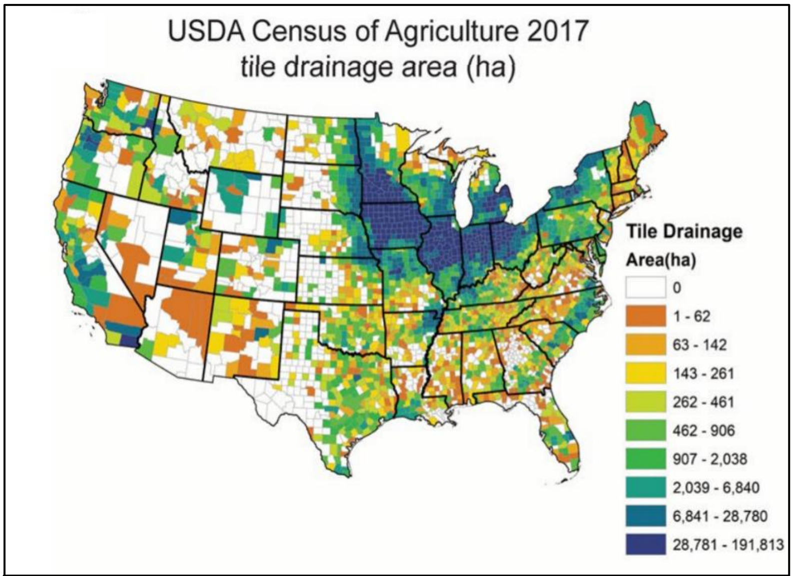

Figure 1: County-level subsurface drainage estimates (ha) from USDA 2017 Census of Agriculture.

In the Midwest, most productive soils are in regions that were predominantly wetlands, increasing the prevalence of artificial drainage. Figure 2 shows the estimated distribution of subsurface drainage for twelve Midwest States (Nakagaki & Wieczorek, 2016). Approximately 83.8% of the drain-tiling spatial extent (18.79 million ha) is concentrated in six Midwestern states: Minnesota, Iowa, Illinois, Indiana, Ohio, and Michigan (Valayamkunnath *et al*., 2020; USDA National Agricultural Statistics Service, 2017A).

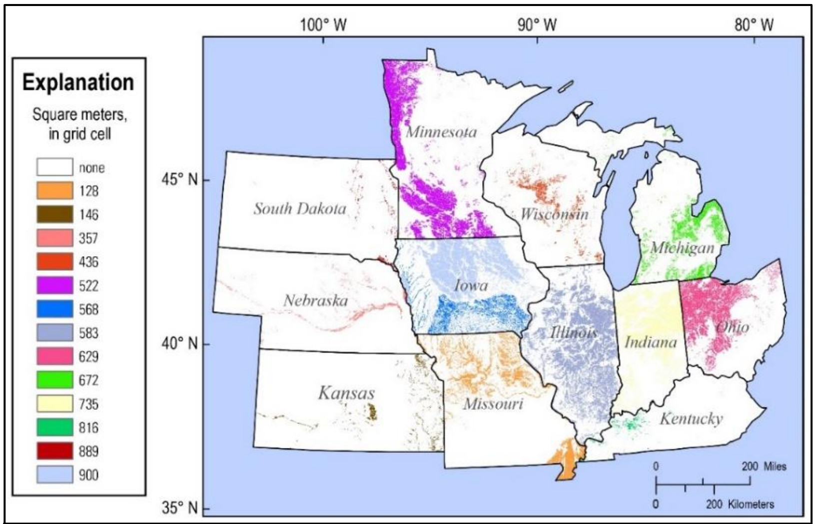

Figure 2: Estimates of subsurface tile drainage in twelve Midwestern states (Nakagaki & Wieczorek, 2016).

Minnesota has over 41,000 miles of ditches and channelized streams draining 3.27 million ha of tiled agricultural land (MPCA, 2014; Valayamkunnath *et al*., 2020). Minnesota ranks fifth in the United States for overall agricultural production, dedicating 51% of the total land area to agriculture and producing 9.5 million dollars in crop profits in 2017 (Ye, 2019). This has resulted in approximately 40.26 million tons (± 2.76 million tons) of soil lost from Minnesotan cropland in 2017 (U.S. Department of Agriculture, 2017). Much of this sediment is deposited into the Minnesota River, which receives 2,700 tons of suspended sediment per day (Minnesota River Basin Data Center, 2004).

Agriculture is a primary source of fine sediment deposits in streams and ditches (Lamba *et al*., 2015). Subsurface drainage contributes to this erosion problem by increasing sediment transport in agricultural watersheds that eventually flow into streams or rivers (Coelho *et al*., 2020; Blann *et al*., 2009; Golmohammadi *et al*., 2017; Kelly *et al*., 2017). Approximately 90% of the sediment in the Minnesota River is fine particles (<2mm) and sediment in drainage ditches is virtually all fine material, likely from intense agricultural operations (MPCA, 1994). Excess fine sediment can result in increased total suspended solids and turbidity, which can degrade the aquatic system, destroy habitats, and limit biological diversity (Minnesota River Basin Data Center, 2004; Jones *et al*., 2012; Naden *et al*., 2016).

The Minnesota River and associated drainage ditches are likely to be negatively impacted by fine sediment accumulation from agricultural operations, necessitating the development of tools to assess the ecological health of these ecosystems. One such tool is a biotic index. A biotic index is used to determine environmental quality of a system based on the presence or absence of pollution sensitive and/or tolerant organisms. Biotic indices are used to assess surface water quality (Carter *et al*., 2007; Bellan, 2008; Fedor & Spellerberg, 2013). Benthic macroinvertebrates are the most commonly used group of organisms for biomonitoring because: (1) they are found in a variety of aquatic environments (USEPA, 2013, Bonada *et al*., 2006;), (2) they are relatively easy to collect and identify (Barbour *et al*., 1999; De Pauw *et al*., 2006) (3) they have a large number of species with a wide range of responses to environmental stressors (USEPA, 2013, Bonada *et al*., 2006) (4) they have limited mobility which facilitates spatial analyses of pollutants and/or disturbance effects (Barbour *et al*., 1999; De Pauw *et al*., 2006) and (5) some have relatively long life cycles, which facilitates research on changes over time caused by perturbation (De Pauw *et al*., 2006).

Biotic indices are used to assess the presence of organic pollution; however, recent studies show potential in indices using benthic macroinvertebrates to estimate fine sediment in streams. The development of sediment biotic indices for macroinvertebrates varies in its methods and definitions of fine sediments. Relyea *et al*. (2012) used benthic macroinvertebrate and sediment data from 1,134 streams in 16 western United States ecoregions to develop a Fine Sediment Biotic Index (FSBI). This FSBI categorizes benthic macroinvertebrates according to their tolerance to fine sediment (<2mm). Relyea also used the presence of certain benthic macroinvertebrates to predict the percent fine sediment in freshwater systems. Turley *et al*. (2016) applied the FSBI concept to develop a sediment-macroinvertebrate specific family biomonitoring profile for temperate rivers and streams in the United Kingdom. Hubler *et al*. (2016) developed a Biological Sediment Tolerance Index assessing fine sediments (<0.06 mm) impacts on macroinvertebrates in Oregon streams. Gieswein *et al*. (2019) developed a macroinvertebrate biomonitoring tool to assess fine sediments (<0.06 mm and between 0.06 to 2 mm) impacts in small mountain streams in Germany. Gieswein's (2019) research is one of the first indices specific to a certain stream-type based on macroinvertebrate responses to deposition of sediments. There are no sediment biotic indices applicable to streams in the Midwest.

The objective of this research is to initiate the development of a macroinvertebrate biological index that reflects the fine sediment conditions in ditch systems utilizing subsurface drainage in southern Minnesota. The FSBI can assess impacts of fine sediment resulting from agriculture-related drainage manipulation. It is anticipated that this index will be used as a stand-alone tool to estimate fine sediment in a drain-tiled ditch system, as well as alongside traditional bioassessment procedures, with potential for expansion and fine-tuning for consistent use in the field.

Literature Review

Agriculture in the United States contributed $136.1 billion to the country's gross domestic product in 2019 (USDA Economic Research Service, 2021). Direct on-farm employment makes up approximately 2.6 million jobs, or 1.4% of total U.S. employment (USDA Economic Research Service, 2021). The United States produced 13.7 billion bushels of corn, harvested from an estimated 81.5 million acres in 2019 (USDA National Agricultural Statistics Service, 2020). In addition to providing food for the U.S. market, agricultural exports generated $177 billion in 2021 (USDA Foreign Agricultural Service, 2022). Soybeans were the predominant export product, producing $27.37 billion (USDA Foreign Agricultural Service, 2022).

According to the USDA, the Midwest region (127 million acres) represents one of the most intense areas of agricultural production in the world (USDA Climate Hubs, n.d.). This region had total crop sales over $92 billion in 2017 (USDA National Agricultural Statistics Service, 2017B). Seventy-five percent of the agricultural land in this region is dedicated to growing corn and soybeans (USDCH , n.d.). In 2021, these two crops accounted for half of all U.S. cash crop revenue at $121.2 billion dollars (USDA Economic Research Service, 2023).

In the Midwest, most productive soils are located in regions that were predominantly wetlands. Wetlands are defined as areas where soil is either saturated with water or water is near the surface of the soil for all or most of the year (U.S. EPA, 2015). Wetlands are characterized by hydric soils, or soils that developed anaerobic conditions resulting from the constant saturation of water (USDA NRCS, n.d.-b). They provide essential environmental services, including water storage, water filtration (specifically sediments & nutrients), and hosting biologically productive ecosystems (U.S. Environmental Protection Agency, 2002; Lenhart *et al*., 2016; Maalim & Melesse, 2013). By the 1980's, at least 85% of wetlands in the midwestern United States had been drained and converted into cropland with subsurface tile drainage (Yuhas, 1999).

Wetlands converted to crop land in the United States have been heavily modified by the installation of artificial drainage to increase agricultural production. The first drainage systems in the United States (typically made of clay, concrete, or wood) were installed in the mid-1800s on the east coast, and was adopted in the Midwest in the late 1800s (Sands, 2018; Hitz & Cruse, 2008; Blann *et al*., 2009). There are two main categories of artificial drainage: surface and subsurface drainage. Surface drainage utilizes sloping land to move water towards open ditches or drains (referred to as surface inlets), which collect water before it filters through the soil (Brouwer *et al*., 1985; USDA Natural Resources Conservation Service, 2001). They are often used in areas where water pools on a surface of the field (Wright, 2018; University of Vermont Extension, 2019). Subsurface drainage focuses on water table management (USDA Natural Resources Conservation Service, 2001;

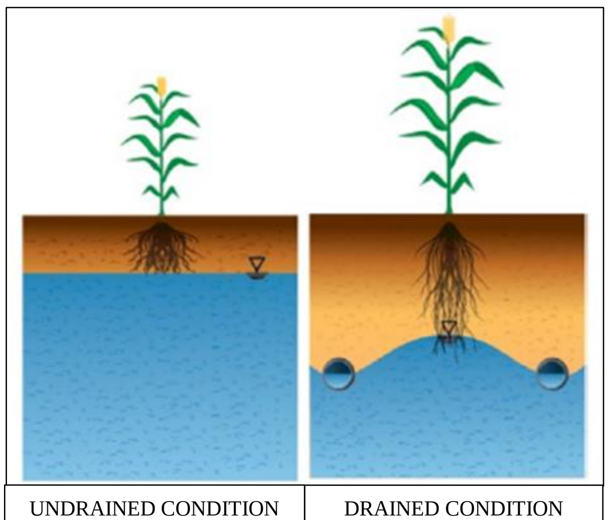

Figure 3: Crop root development for drained and undrained conditions, sourced from Ghane, 2018.

University of Vermont Extension, 2019). As seen in Figure 3, subsurface drainage systems use semi-permeable sloped pipes that lower the water table by transporting water away from the rootzone of crops and into drainage ditches (Ghane, 2018; UoME, 2018; University of Vermont Extension, 2019). It is not uncommon to see a combination of surface and subsurface drainage being used, where surface drains act as a conduit to subsurface systems (USDA Natural Resources Conservation Service, 2001; University of Vermont Extension, 2019).

In 2020, Minnesota ranked fifth in the country for both crops and total agricultural production, amassing $8.85 billion and $16.7 billion respectively (Minnesota Department of Employment & Economic Development, 2022). The state produces high-valued agricultural products, including corn, soybeans, and hogs, as well as ranking number one in sugar beets grown for processing (Minnesota Department of Employment & Economic Development, 2022). Minnesota's agricultural significance lends itself to hosting major food companies, like General Mills & Hormel (Minnesota Department of Employment & Economic Development, 2022). Approximately 47% of the land in Minnesota is utilized for agriculture, tallying about 25

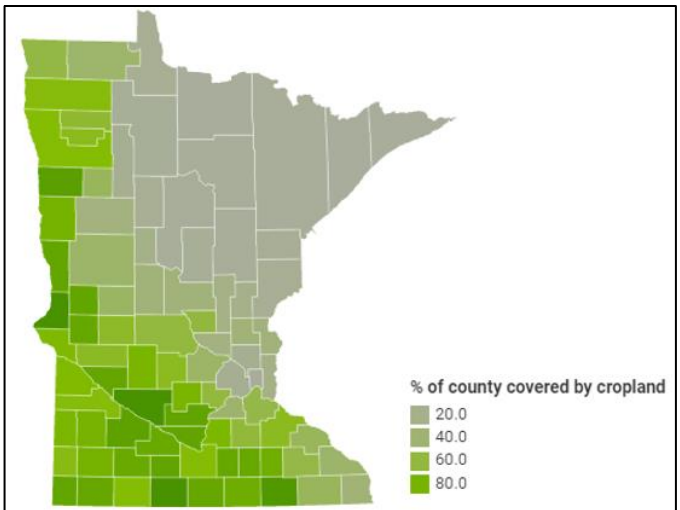

Figure 4: Distribution of cropland by county in Minnesota (data sourced from 2017 USDA Census of Agriculture, visualization produced by Choi, 2019).

million acres (USDA National Agricultural Statistics Service, 2017A). Figure 4 shows the distribution of cropland in Minnesota by county.

Wetlands cover 12.2 million acres of Minnesota's land; this is the 2nd highest state acreage of wetlands in the United States (Kloiber *et al*., 2019). Minnesota is also part of the Prairie Pothole Region, seen in Figure 5. This region consists of depressional wetlands formed by glacial movement that fill with snowmelt and rain during the spring (USEPA, 2023). The Prairie Pothole is one of the most productive and important wetland regions in the world, as it is home to more than 50% of North American migratory waterfowl (USEPA, 2023). Approximately 40-50% of the region's original prairie has been drained and altered for agricultural development (USEPA, 2023).

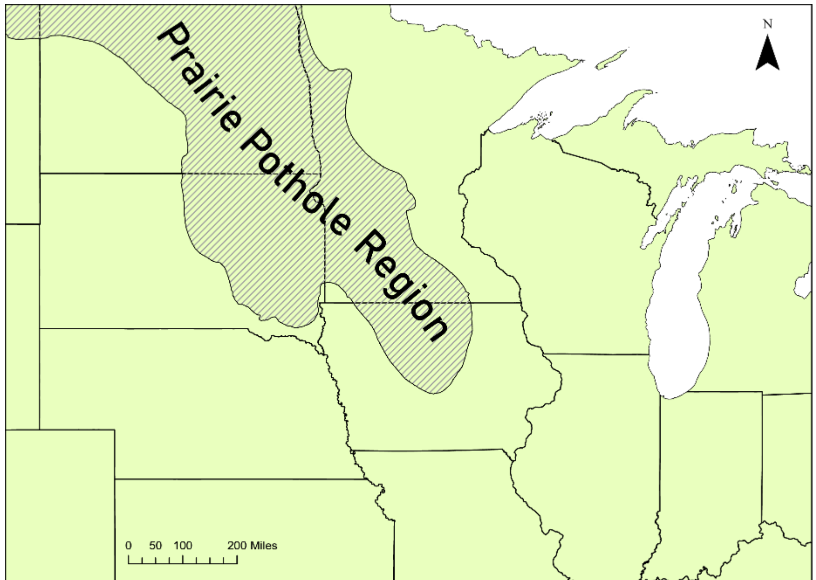

Figure 5: Map of Midwestern States and the Prairie Pothole Region (Data sourced from Mann, 1974).

Until the Wetland Conservation Act was passed in 1992, over half of all wetlands in Minnesota had been drained for artificial subsurface drainage, experiencing the largest loss of emergent wetland areas in the Midwest (Lenhart *et al*., 2016). Minnesota Drainage Law was first instated in 1858, introducing legislation specific to the drainage of land and establishing responsibilities of the individuals who plan to drain and redirect water (Chapter 128 – Lands, 1858). Drainage Law evolved over 130 years, until the Minnesota Public Drainage Manual was published in 1991 and refined in 2016 through a collaboration with the Minnesota Department of Natural Resources and the Board of Water and Soil Resources (MNBWSR, 2019).

There is no maintained inventory of agricultural land utilizing subsurface drainage in Minnesota, but it is estimated that 20-30% of agricultural soils in the Minnesota River Basin utilize it (Sands, 2018). Figure 6 provides an estimate on the extent of subsurface tile drainage in Minnesota, as well as three delineated drainage provinces defined by the Minnesota Groundwater Association (2018).

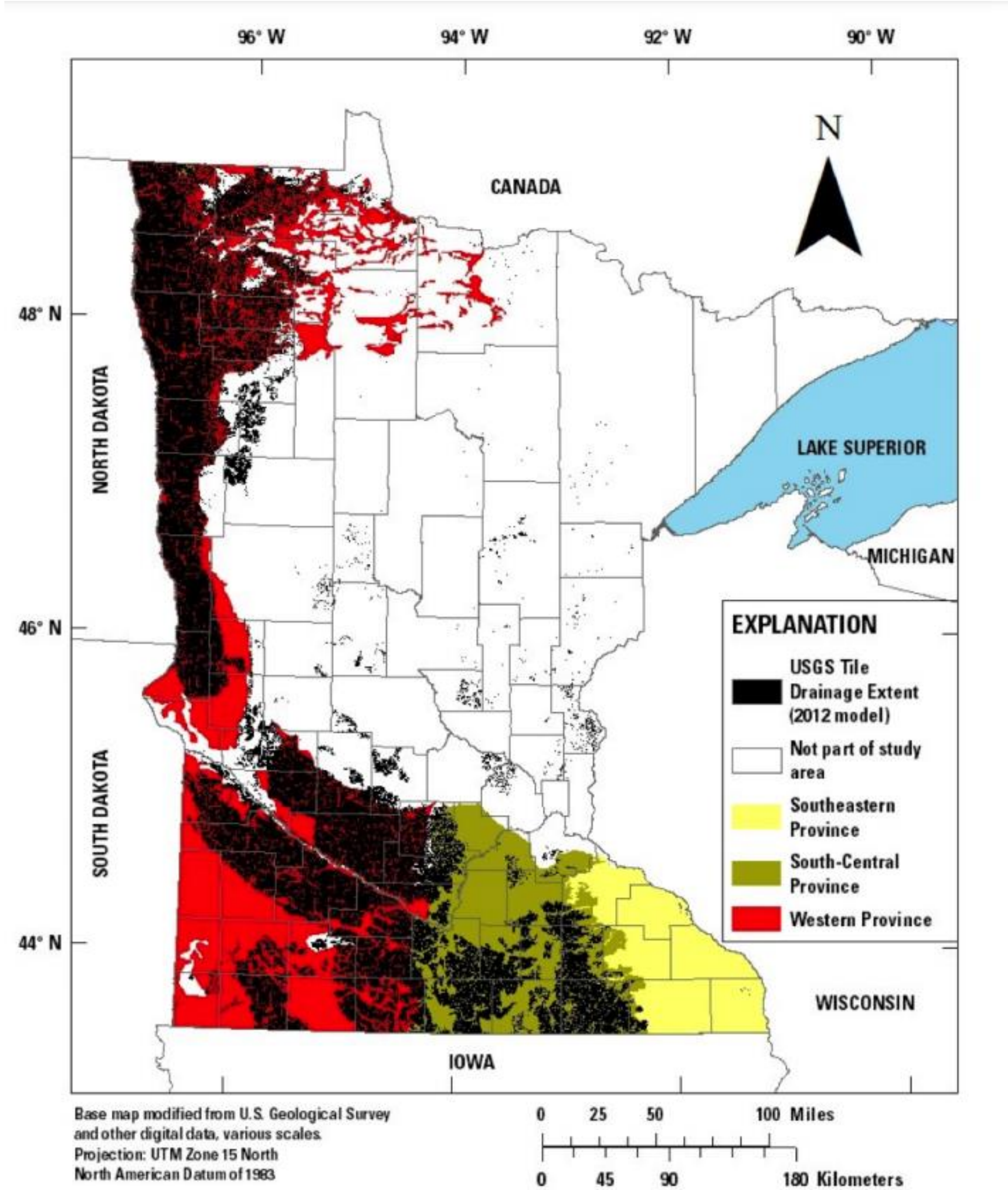

Figure 6: Three drainage provinces defined by Minnesota Groundwater Association, (2018), including 2012 USGS tile drainage model for the state of Minnesota.

Minnesota faces significant water erosion, due to agriculture. The current sheet & rill erosion rate on cultivated cropland in Minnesota is estimated to be 2.01 (± 0.14) tons per acre per year (USDA, 2020). Sheet erosion occurs as thin layers of topsoil are removed by rain and shallow flows of water, while rill erosion occurs as water erodes in the form of small channels down a slope (Al-Kaisi, 2000; Popa, 2016).

Materials being transported by water erosion can be classified according to size using the Udden-Wentworth Scale (Wentworth, 1922). This method of grain-size classification uses ranges of grain diameter to group particles into eight major groups, seen below in Table I.

Table I: Grain-Size Class Scaling Table, adapted from Wentworth, 1922.

Grain-Size Class	**Size**
Gravel	>2 mm
Very-Coarse Sand	2 to 1 mm
Coarse Sand	1 to 0.5 mm
Medium Sand	0.5 to 0.25 mm
Fine Sand	0.25 to 0.125 mm
Very Fine Sand	0.125 to 0.06 mm
Silt	0.06 to 0.004 mm
Clay	<0.004 mm

Sand

2 to 0.06 mm

Erosion from agriculture in Minnesota commonly consists of particles fine sand-sized or smaller (MPCA, 1994; Zhao, 2011). The use of artificial drainage in agricultural land significantly increases the erosion of fine sediments into streams and ditches (Coelho *et al*., 2020; Blann *et al*., 2009; Schottler *et al*., 2013; Golmohammadi *et al*., 2017). These ditches enhance internal drainage, increasing sediment transport and becoming significant sources of fine sediment in the watershed (Maalim & Melesse, 2013; Coelho *et al*., 2020; Golmohammadi *et al*., 2017). Approximately 90% of the sediment in the Minnesota River is fine particles and sediment in drainage ditches is virtually all fine material (MPCA, 1994).

Total Suspended Solids (TSS) is the quantification of suspended materials within the water column (MPCA, 2022; UW-Madison Division of Extension, 2019). Consisting of both mineral and organic material, increased sediment loads from agriculture can degrade streams and damage aquatic ecosystems by increasing the total suspended solids and turbidity, scattering light and preventing it from reaching aquatic plants, as well as clogging gills, smothering habitats, and disrupting feeding activities of benthic macroinvertebrates (MPCA, 2022; Parkhill & Gulliver, 2002; UW-Madison Division of Extension, 2019). Suspended particles can also serve as mechanisms for transporting pollutants through a watershed (Rügner *et al*., 2013).

In 2012, the Minnesota Pollution Control Agency started to develop a Total Maximum Daily Load (TMDL) for turbidity in the Minnesota and Greater Blue Earth River (MPCA, 2019). After continuous debate, it was replaced with a state-mandated TMDL standard for Total Suspended Solids in 2014. The TSS TMDL is region-specific.

Located in the South River Region, the Minnesota River has a 65 mg/L TSS standard that cannot be exceeded more than 10% of the time from April 1 through September 30 (MPCA, 2020). The average total suspended solids yield determined from 2007 to 2016 for regions in Minnesota are presented in Figure 7 (Watershed Pollutant Load Monitoring Network, 2019). When comparing Figure 6 & 7, note the increased total suspended solids yield in the drain-tiled regions.

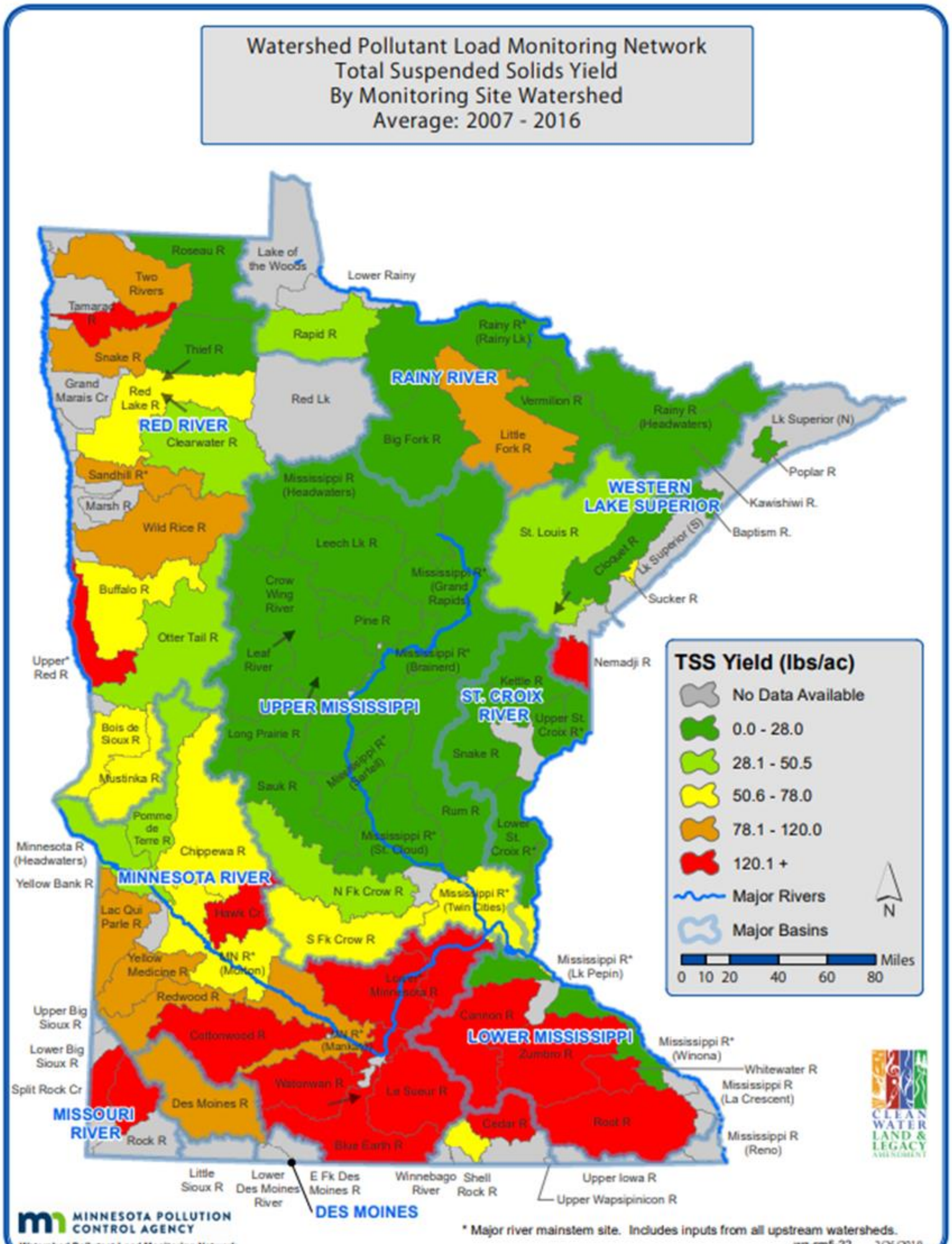

Figure 7: Average total suspended solids yield determined from 2007 to 2016 (Watershed Pollutant Load Monitoring Network, 2019).

As required by the Clean Water Act of 1972, the United States Environmental Protection Agency (US EPA) developed the National Aquatic Resources Survey (NARS) to assess surface waters for degradation and overall stream health (Buss *et al*., 2015). This led to many waterbodies that needed to be inventoried and evaluated. In response, the EPA introduced Rapid Bioassessment Protocols (RBP) in 1989 (Plafkin, 1989; Carter *et al*., 2006). These protocols used the presence of certain fish and benthic macroinvertebrates to indicate overall water quality until 1999, in which periphyton were added and benthic macroinvertebrate methods were refined (Plafkin, 1989; Barbour, 1999).

Biotic indices are often used to assess surface water quality by assigning pollution tolerance values to organisms that act as biological indicators (Bellan, 2008; Hawkins & Carlisle, 2021). The presence or absence of these organisms works as a proxy for biological degradation on varying temporal scales (Buss *et al*., 2015). The concept of a biotic index was initially developed on organisms' tolerance to reduced oxygen conditions resulting from organic pollution, but has since been expanded into other realms, such as macroinvertebrate responses to inorganic pollutants and using microfauna to gauge activated-sludge treatment plant performance (Madoni, 1994; Relyea *et al*., 2012; Hawkins & Carlisle, 2021).

Benthic macroinvertebrates are frequently used as bioindicators of stream health. They are not only easily found in a variety of environments, but they have limited mobility and are good indicators of localized conditions, specifically concerning anthropogenic disturbance (Barbour, 1999; Huff *et al*., 2008). Sampling benthic macroinvertebrates is useful in conditions with a time or financial constraint, as they react very predictably to disturbance through their associated tolerance levels (US EPA, 2013). In a healthy waterbody, there will be higher degrees of diversity and abundance of benthic macroinvertebrates; however, in a waterbody that may be impaired, you will see a lack of pollution-sensitive taxa. In a biotic index, benthic macroinvertebrates are given a pollution-tolerance value to calculate an overall score for the waterbody.

The major controls of macroinvertebrate distribution are water temperature, dissolved oxygen, available habitat, and water flow. Water temperature is one of the primary factors, altering life-cycle phases and timing of important events, like egg laying, egg hatching, larval development, and emergence (Dallas & Ross-Gillespie, 2015; Bonacina *et al*., 2022). Macroinvertebrates possess individual thermal tolerances and preferences. Temperature-induced stress can cause changes in behavior (Bruno *et al.,* 2012), physiology (Zimmerman & Wissing, 1978), reproductive strategies (Everall *et al*., 2015), and their susceptibility to predators (Smolinský & Gvoždík, 2014; Śniegula, Golab & Johansson, 2019).

Changes in water temperature can also influence dissolved oxygen (DO) levels (Bonacina *et al*., 2022). Benthic macroinvertebrates have varying tolerances to low levels of dissolved oxygen. When DO levels fall below a certain threshold, macroinvertebrates can experience oxygen stress, which can affect their behavior and metabolism. These thresholds are dependent upon the organism. Some species of midges can survive in as little as 0.5 mg/L DO, while mayflies exhibit lethal effects in the same conditions (Connelly et al., 2004; Cummins and Merritt, 2008; Croijmans *et al*., 2021). The presence of submerged vegetation not only provides habitat for macroinvertebrates, but photosynthesis that occurs during the day supplements dissolved oxygen levels, as well (Caraco *et al.*, 2006).

Habitat type is a crucial factor controlling macroinvertebrate diversity. More specifically, macroinvertebrates have preferences for sediment composition (grain-size). Burrowing taxa, like burrowing mayflies or crayfish, prefer soft-bottomed with finer sediments, as opposed to stony-cased caddisflies, which prefer gravel and coarse sand (Bouchard, 2004; Smith *et al.*, 2019). Other macroinvertebrates, like damselflies and dragonflies, prefer woody or vegetated areas (Bouchard, 2004; Smith *et al.*, 2019). Water flow can alter sediment regimes, which in turn affect benthic macroinvertebrates (Hershey *et al*., 2010; Merritt *et al*., 2019). Low water velocities may lead to sediments settling out of the water column, an accumulation of organic matter, and reduced oxygen levels (Allan *et al.*, 2021). Shallow waters may support more diverse communities, particularly in areas with a lot of vegetation (Balian *et al*., 2008).

Traditionally, macroinvertebrate indices are used to gauge stream health with an organic pollution focus. However, fine sediment has become a problem for these organisms. Negative impacts of excessive deposition of fine sediments on benthic macroinvertebrates include: (1) altering substrate composition, making it unsuitable for certain taxa (Wood & Armitage, 1997; Harrison *et al*., 2007); (2) increasing the downstream drift of sediment-sensitive species (Harrison *et al*., 2007; Gomi *et al*. 2010; Larsen & Ormerod, 2010; O'Callaghan *et al*., 2015); (3) Clogging respiratory structures, hindering oxygen exchange (Harrison *et al*., 2007; Bilotta & Brazier, 2008; Jones *et al*., 2012); and (4) affecting feeding activities due to increased turbidity or burial of the streambed (Harrison *et al*., 2007; Izagirre *et al*., 2009; Murphy *et al*., 2011). Fortunately, benthic macroinvertebrates might react to inorganic fine sediment deposition similarly to organic pollution. This has led to the development and application of several fine-sediment biotic indices (FSBIs) in different locations around the globe.

Most of the existing sediment biotic indices are intended for use in specific regions and lack consistency in the definition of fine sediments. Relyea *et al*. (2012) looks at the Pacific Northwest. This study produced sediment indices across level III ecoregions using consolidated macroinvertebrate and sediment data from other studies, where "fine sediments" was defined as less than 2 mm. All sediment data used was obtained via the Wolman-Pebble Count, which influenced the decision to choose 2 mm as the threshold, as it's the smallest size consistently measured using that method of grainsize analysis (Relyea *et al*., 2012).

Hubler *et al*. (2016) uses a similar approach in the development of an FSBI for Oregon streams. Fine sediments are collected in a visual-based method, classifying grainsize by median particle diameter across five randomized transects in a wetted width (Hubler *et al*., 2016). Fine sediments were defined as less than 0.06 mm, though particles of this size could not be individually differentiated. Combined visual and touch observations were used to distinguish fines from sand by rolling it between fingers and noting a lack of gritty texture. This index was heavily model-based and included rare taxa, unlike Relyea *et al*. (2016).

Turley *et al*. (2016) developed an FSBI for temperate rivers and streams in the United Kingdom. This paper highlights the importance of the confounding pressures associated with fine sediments and their documented effects on macroinvertebrate communities, such as increased transport of pollutants. They note that these interactions should be considered when utilizing a sediment biotic index. Fine sediment data were obtained through a visual inspection to estimate substrate composition, recording the percentage of bedrock, boulders and cobbles, pebbles and gravel, sand, and silt and clay (Turley *et al*., 2016). Fines were defined as less than 2 mm.

Gieswein *et al*. (2019) modifies the FSBI concept for a specific stream type: small, coarse substrate-dominated mountain streams. This index differs from the previous ones because it does not utilize all taxa available, only those deemed most reliable in estimating fine sediments. This is conducted using the Threshold Indicator Taxa Analysis (TITAN), which assigns taxa a directional response to the fine sediment stressor gradient and estimates purity with 500 bootstrap replicates at a desired reliability (for their index, the reliability value was set to 0.7). Fine sediment data were obtained using a remobilization technique that enables quantification of deposited sediment by mixing above the stream bed within an open-tube, syphoning out the water, drying the sample, and sieving. Fine sediments were defined as <2 mm, but were differentiated into sand (0.06 – 2mm) and silts & clays (<0.06mm) through dry sieving.

There is a lacking of fines sediment biotic indices specifically for areas experiencing high fine sediment loads, like agricultural drainage ditches. There are approximately 83,000 stream miles in Minnesota, half of which have been physically altered by humans through channelizing, ditching, or damming (MPCA, n.d.). Both Relyea *et al*. (2012) and Gieswein *et al*. (2019) serves as a foundation for the development of other stream-type specific indices, including this research.

Study Area & Sampling Sites

The Western Corn Belt Plains (WCBP) ecoregion is a major agricultural area in Minnesota (Figure 8). Fertile, moist soils make this one of the most productive areas of corn and soybeans in the world (U.S. EPA, 2013). In Minnesota, the tall grass prairies of the WCBP have been converted almost completely into agricultural land (U.S. EPA, 2013; Wiken *et al*., 2011). Over 80% of region is used for crops, and much of the remainder is forage for livestock (U.S. EPA, 2013; Wiken *et al*., 2011; U.S. EPA, 2000;). It has a humid continental climate, consisting of cold winters and hot summers, and average rainfall between 28 to 35 inches, as seen in Figure 8 (U.S. EPA, 2013; U.S. EPA, 2000; MNDNR, 2017). Intermittent and perennial streams are present throughout the area (Wiken *et al*., 2011; U.S EPA, 2000).

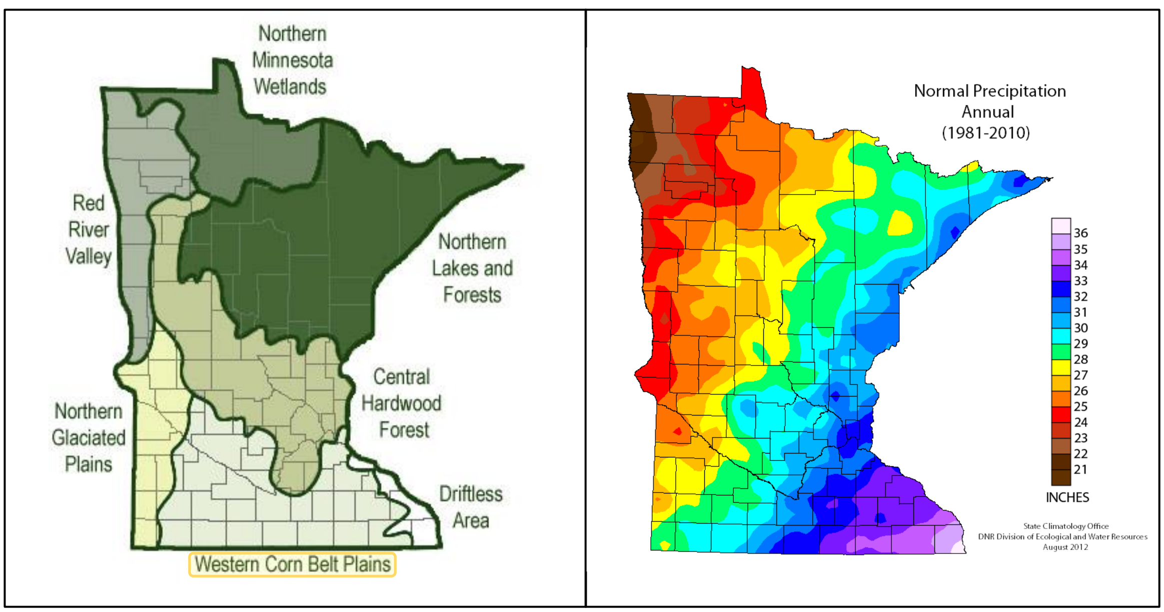

Figure 8: Left, Level III Ecoregions of Minnesota; Western Corn Belt Plains has been highlighted. (RMBEL, 2013). Right, Minnesota Average Annual Precipitation from 1981-2010 (MNDNR, 2017).

Eight sites in three drain-tiled ditch systems in the WCBP ecoregion were selected for this project. Table II lists each site, its associated watershed, and location.

Table II: Sites used to collect sediment and macroinvertebrate data during Summer of 2021 & 2022.

Site Name	Watershed	GPS Coordinates (DD)
County Ditch 27	Minneopa Creek	44.1127306, -94.2488132
County Ditch 56	Minneopa Creek	44.1013707, -94.2157919
Minneopa Cr into Lily Lake	Minneopa Creek	44.1243407, -94.2486972
Minneopa State Park Entrance	Minneopa Creek	44.1481140, -94.0958690
State Highway 119	Minneopa Creek	44.1303839, -94.3491406
Seven-Mile Creek B	Seven-Mile Creek	44.2984477, -9430794471
Seven-Mile Creek C	Seven-Mile Creek	44.3130789, -94.0514978
Beauford	Little Beauford Ditch	44.0176121, -93.9585097

The watersheds used in this project were chosen because they have been studied extensively over the past 30 years. Multiple studies were conducted within the Minneopa Creek watershed (Figure 9), including the Minnesota Pollution Control Agency's Clean Water Partnership program and the Minneopa Creek Watershed Plan, completed by the Legislative Commission on Minnesota Resources in cooperation with the Minnesota Department of Natural Resources, Blue Earth County Environmental Services and Mankato State University Water Resources Center (LCMR, 1997; Crystal Loon Mills Clean Water Partnership, n.d.). Seven-Mile Creek (Figure 10) serves as a reference stream for the Minnesota Pollution Control Agency and the Minnesota Department of Agriculture (Kuhner, 2001). Red Top Farm Demonstration Site is location within the Seven-Mile Creek watershed, serving as a ninety-acre research field studying the movement of water and agricultural chemicals through subsurface drainage systems (Brown Nicollet Cottonwood Water Quality Board, n.d.). Little Beauford Ditch (Figure

11) was one of the 10 minor watersheds included in the Minnesota River Assessment Project (MRAP; MPCA, 1994). From 1994 to 1999, this minor watershed was one of four Minnesota River Implementation Program Demonstration Watersheds (MRIP; Minnesota River Basin Data Center, 1994). This watershed was part of a longitudinal study to characterize fecal bacteria in rivers, as well as the Southern Minnesota Drain Tile Monitoring Project in 1991 and again in 2010 (Water Resources Center & Blue Earth River Basin Alliance, 2007; Southern Minnesota Tile monitoring project, n.d.)

Five sample sites are located in the Minneopa Creek Watershed. Minneopa Creek drains an 85.2 mi^2 watershed, flows through a ravine, and discharges into the Minnesota River after flowing through Minneopa State Park (MPCA, 2018). Approximately 87% of the watershed above Lily and Crystal Lake is cropland. This cropland is heavily tiled, and approximately 82% of the watershed drains into ditches. Ditch drainage areas and sampling sites in the Minneopa Creek Watershed are presented in Figure 9.

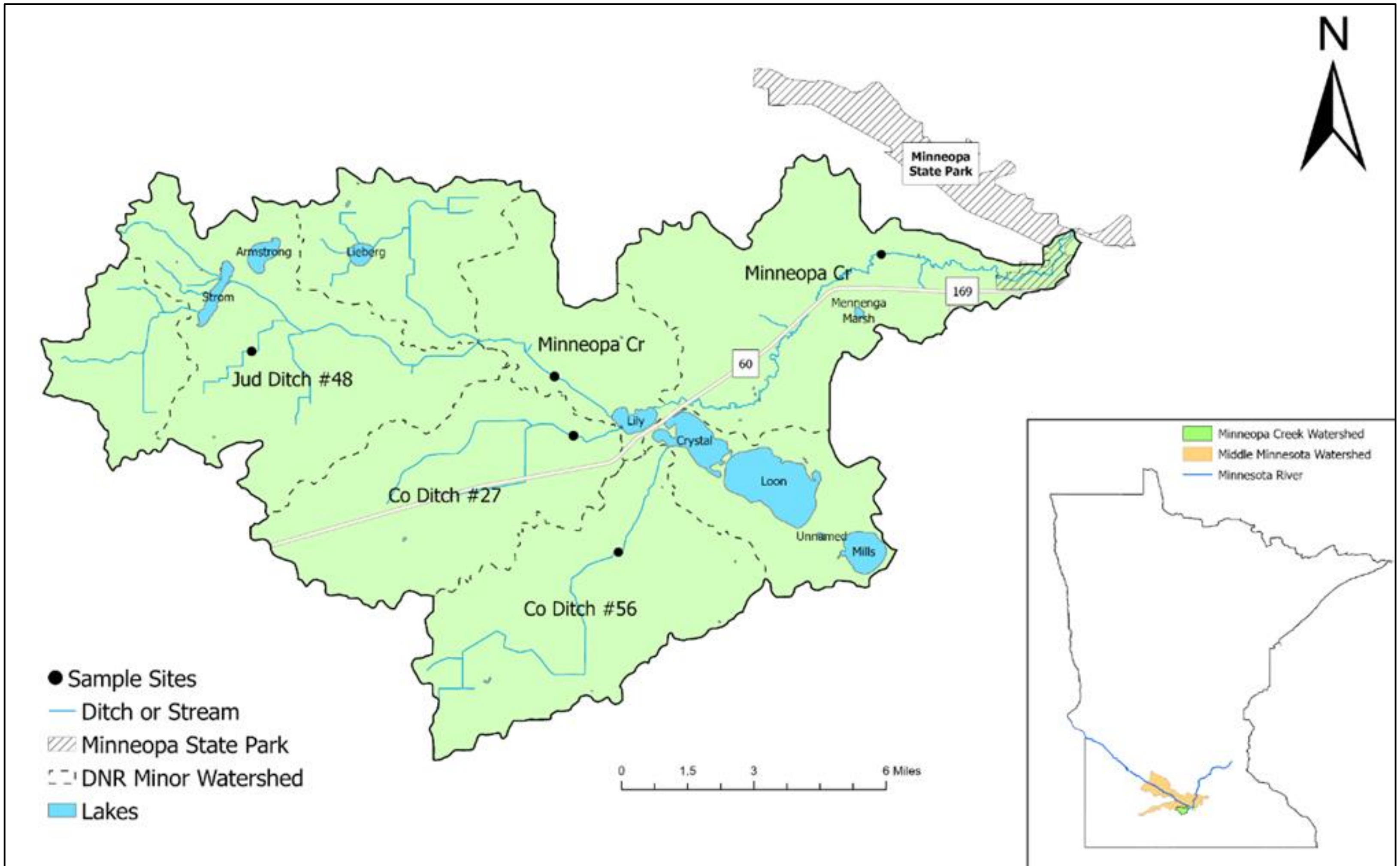

Figure 9: Minneopa Creek Watershed. Inset Minnesota map shows Minneopa Creek Watershed in green and the larger Middle Minnesota Watershed in orange.

The Seven-Mile Creek is 6.1 miles long, drains 23,551 acres (38.6 square miles), and discharges into the Minnesota River (Nicollet Soil & Water Conservation District, n.d.). Cultivated lands comprise 86% of the watershed and approximately 6% is deciduous forest (Nicollet Soil & Water Conservation District, n.d.). Two ditch sites were sampled in the upper Seven-Mile Creek system (Figure 10).

Little Beauford Ditch is 7 square miles (5,600 acres). Approximately 81% of land is cultivated, 6% is used for pasture/grass/hay, and 4% is wetlands (Southern Minnesota Tile monitoring project, n.d.; Metropolitan Council, 2005; MPCA, 1994). Beauford Ditch has been referred to as an unnamed tributary to the Cobb River, and more recently as the Little Beauford Ditch (07020011-503). Since it appears on the Minnesota impaired waters listing as the Little Beauford Ditch, I will refer to it as such in my thesis (Metropolitan Council, 2005; MPCA, 2016). This minor watershed will be sampled at Highway 22 crossing (Figure 11).

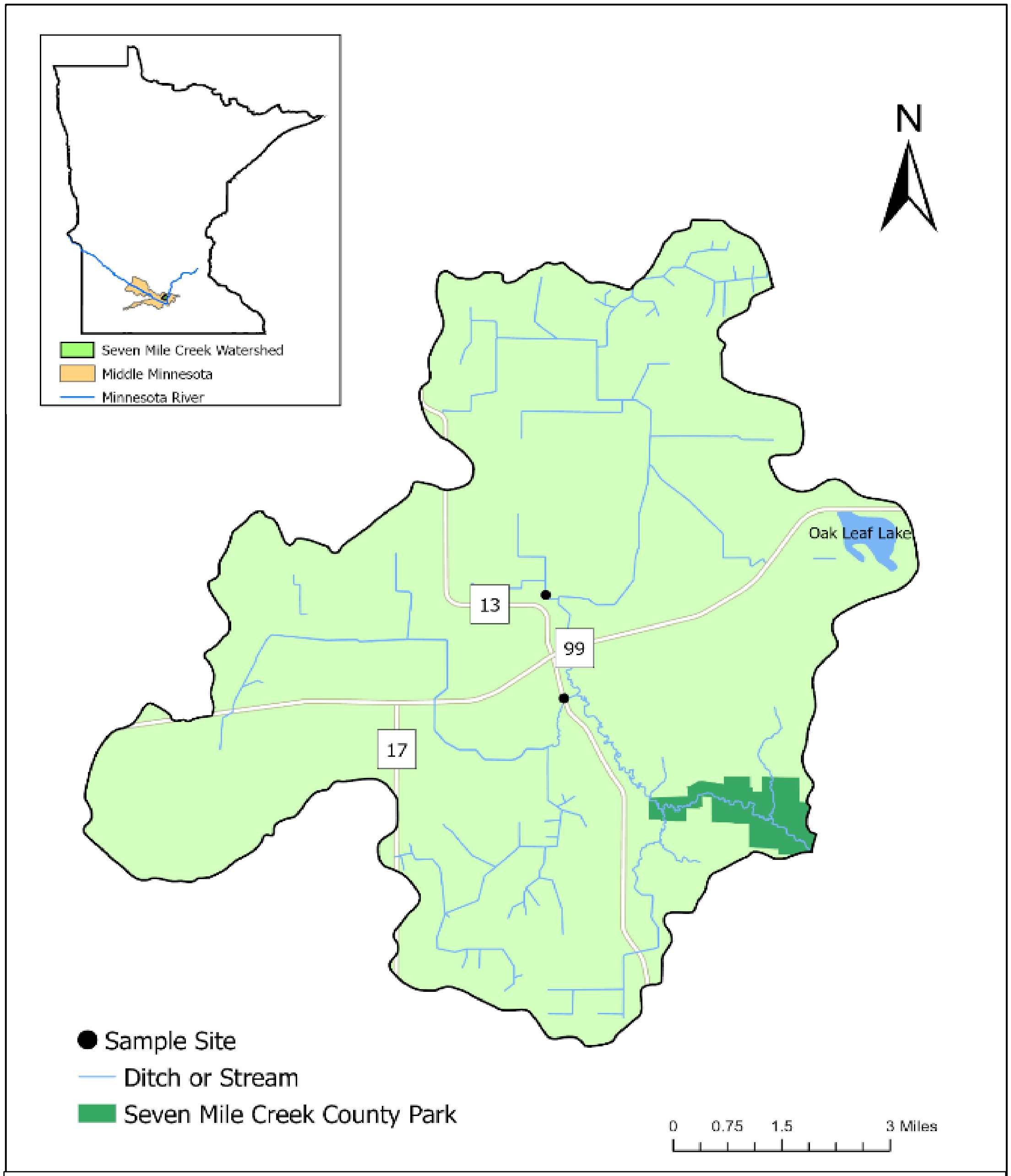

Figure 10: The Seven-Mile Creek Watershed. Sample sites represented by black circles.

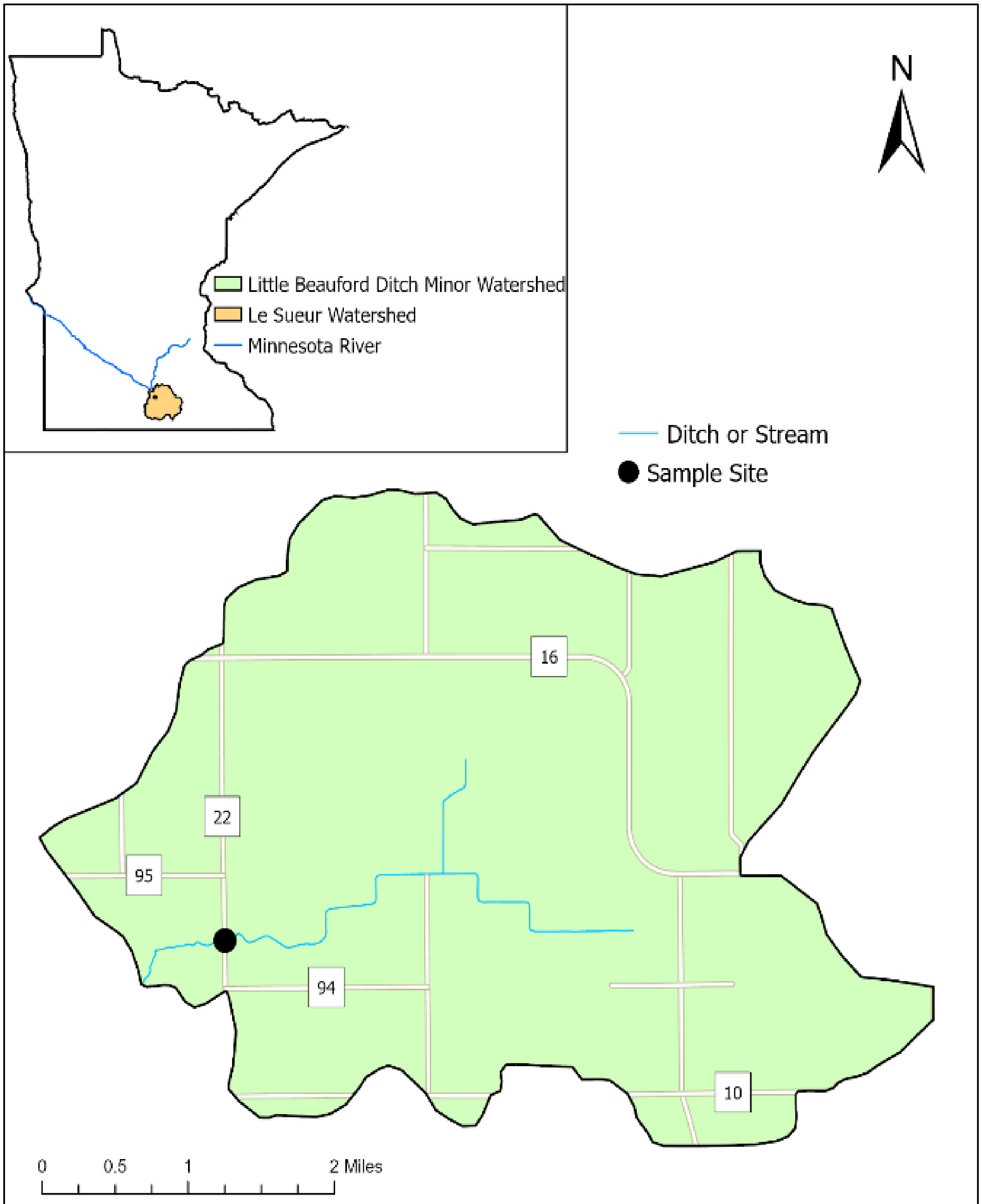

Figure 11: Little Beauford Ditch Minor Watershed (32073). Sample site represented by black circle.

Materials & Methods

I. *Water Quality*

On-site temperature was taken with a Hach Pocket Pro+ Multi 2, following the Hach procedures provided with the meter (Hach, 2023). Dissolved Oxygen was measured using a Modified Winkler drop count titration (protocol number in Table 3). Turbidity was measured using a secchi disk transparency tube (Figure 12).

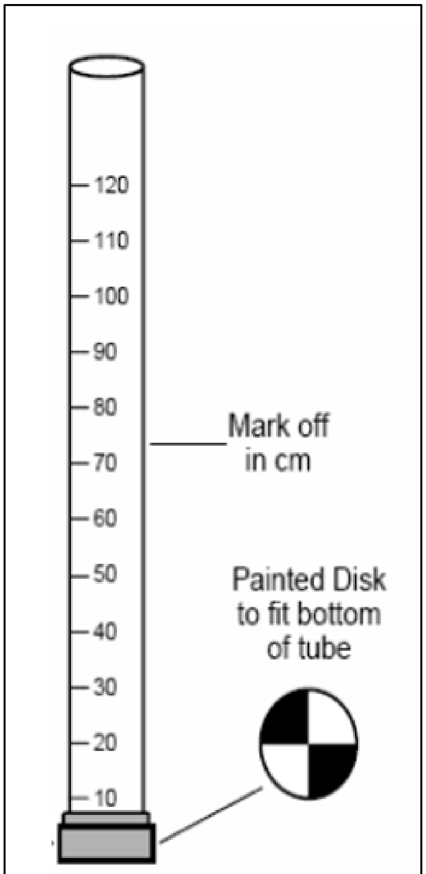

Figure 12: Transparency (or Turbidity) tube (NKU, n.d.).

Before macroinvertebrate and sediment samples were taken, a one-liter sample of water was obtained and taken back to the lab for water chemistry analysis (procedures in Table 3). Additional water quality data taken each trip can be found in Appendix A.

Standard quality control procedures including field duplicates, laboratory duplicates, instrument calibration checks, field and lab blanks were used in analysis of water samples. Duplicates for Dissolved Oxygen and the transparency tube were conducted, as well.

Table III: Methods used in measurement of Water Quality Parameters (Hach, n.d.; USEPA, 2001).

Procedure	**EPA Method**	**Range**	**Location Conducted**
Total Suspended Solids	EPA Method 1684 (EPA-821-R-01-015)	-	Lab
Dissolved Oxygen (146900 – Model OX-2P)	EPA 4500-O (B-F)-2016	1 - 20 mg/L O_2	Field

II. Sediments

Sediment cores were collected to determine grain size distribution for material greater than 2 mm, 2 mm- to 0.63 mm and below 0.63mm as well as percent organic matter (loss on ignition). Number of cores taken ranged from 1 to 3 according to the width across a ditch (i.e. narrow ditches. A 2-inch diameter PVC pipe was pushed 5 cm into the stream bed. A rubber plug was applied to the top of the pipe, creating a suction effect. The pipe was pulled upward and the bottom was covered with a hand to reduce sediment loss from the suction core. The rubber plug was removed and the core was deposited into a one-gallon plastic wide-mouth jar or 1.25-gallon screw top bucket for transport back to the lab.

Sediment stored on the streambed was obtained using the remobilization approach developed by Lambert and Walling (1988), refined by Collins & Walling (2007), and utilized in Gieswein *et al*. (2019). An open-ended, 6-inch PVC tube was pushed into the sediments, creating a seal to prevent sediment or water from escaping. Water depth inside the tube was recorded. A meter stick was inserted 5 inches above the stream bed in the tube and agitated for 30 seconds, except in low flow conditions where water was less than 5 inches. In this case, agitation occurred carefully at the top of the water. The water was promptly removed by immersing a small plastic bottle or jar and bailing the product into one gallon plastic wide-mouth jars or 1.25-gallon screw top buckets. Water was removed until there was no longer any remaining within the tube. This was conducted twice at each stream site, unless otherwise stated or stream width was limited. All samples were taken by the same two people to ensure consistency in the methodology.

Both methods underwent the same processing technique in the lab. Each sample was wet-sieved, using USGS size 10 (2mm) and USGS size 230 (0.06 mm) sieves. A sample was slowly added to the sieves and spread thin using a plastic scraper. Sediments were run through the sieve and rinsed until the sediments measuring less than 0.06mm ran clear. The resulting separated sediments were categorized in beakers (or buckets, if needed): >2mm, 2-0.06mm, and <0.06mm. Samples were put into a drying oven at 105℃ for two to three days. Dried sediments were measured and recorded.

Percent water, dry weight, and loss on ignition percent organic were determined for sediment core samples with EPA Method 1684 (EPA-821-R-01-015). Subsamples were taken from each particle-size range and ash-free dry mass was obtained. Subsamples were ashed in a muffle furnace at 550℃ for 1 hour. Samples were reweighed and recorded. The difference in mass before and after the muffle furnace represents the mass of organic matter burned away.

III. *Macroinvertebrates*

Habitats at each sampling site were described and recorded at the time of each sampling. Macroinvertebrates were collected last at all sites using D-frame dip nets simultaneously on both sides of the channel for one minute and thirty seconds by a team of two. Materials in the net were sorted in the field and deposited into plastic bottles with water. For samples particularly dense with vegetation, the entire contents of the net were brought back to the lab for easier sorting.

The Hess stream sampler was also used to collect macroinvertebrates in areas where the water depth was less than 25 cm and rocks were present. The Hess sampler is much more quantitative but is limited by water depth (Relyea *et al*., 2012; Tronstad *et al*., 2019). If there were no rocks or boulders present, the Hess stream bottom sampler was not used. Rocks were washed within the sampler to account for any organisms that might be more commonly found on them. Materials from the Hess stream bottom sampler were stored in separate bottles. Macroinvertebrates were transported live, extracted from the sample, preserved in a 3:1 ratio of water and 90% ethanol, then identified and sorted to Family level in the lab, using The Guide to Aquatic Invertebrates of the Upper Midwest as the primary dichotomous key, unless otherwise specified (Bouchard, 2004).

IV. *Statistical Analysis*

The Threshold Indicator Taxa Analysis, or TITAN, is a statistical analysis package in the programming language, R, that observes changes in taxa distributions along an environmental gradient (Gieswein *et al*., 2019; Baker and King, 2010). In development, TITAN correctly identified taxon and their environmental change-points in

99% of 500 unique versions of two simulated data sets (Baker & King, 2010). TITAN was used to find change-points for each taxon across the fine sediment gradient we sampled. Steps in the TITAN analysis are explained in Figure 13.

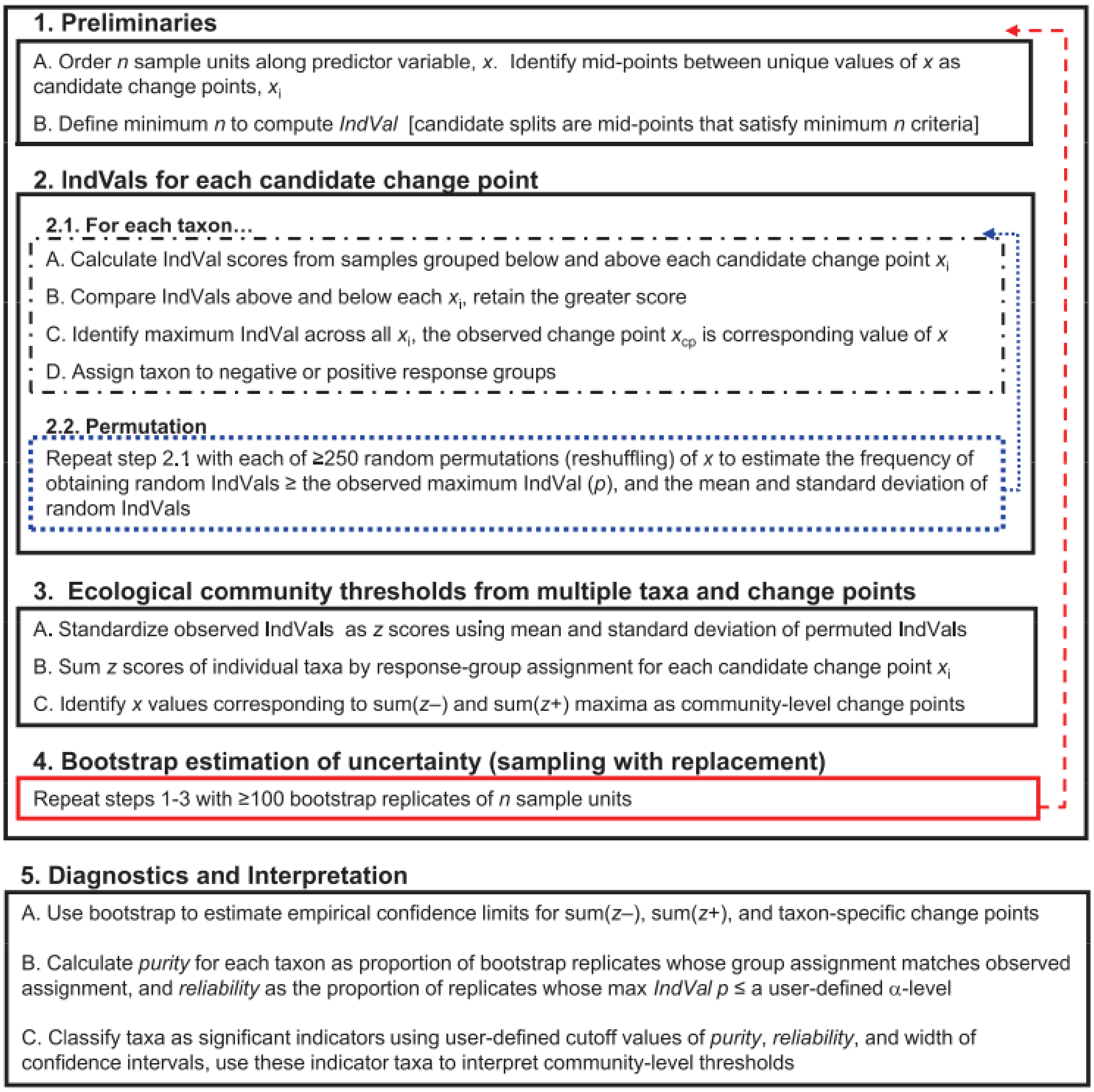

Figure 13: Flow chart of Threshold Indicator Taxa ANalysis (TITAN), where the predictor variable, *x*, is fine sediment (g/m^2; Baker & King, 2010).

a. Data Preparation

Data preparation and analyses for 2021 and 2022 were conducted separately, due to extreme weather conditions including drought and flooding, respectively. Each sample date and location were given a site code, i.e. the first sampling trip conducted at County Ditch 27 is CD27-1, the second is CD27-2, etc. These site codes were used to connect the sediment and taxa data during statistical analysis. Fine sediments stored on the stream bed (g/m^2) was calculated using the average mass of dried sediments obtained through the remobilization technique described in the sediment section above multiplied by average water depth within the PVC pipe. This calculation was done individually for each site and every trip.

TITAN requires that taxa occur at least three separate times over a sampling period, regardless of site. All macroinvertebrates that did not follow this rule were removed from the list, bringing the total taxa groups observed from 52 to 33 in 2021 and 40 to 22 in 2022. TITAN was provided both the fine sediment data for each sampling, along with the macroinvertebrate groups found there. TITAN analyses were run on all three categories of sediment: <2 mm, 2-0.06 mm, and <0.06mm. Baker & King (2010) recommend setting the reliability value to greater than or equal to 0.95, but this resulted in an output of no reliable taxa, so a reliability value of greater than or equal to 0.70 was used, utilizing Gieswein *et al*. (2019)'s approach. Code and raw data can be found in the Appendix B.

b. Index Development

Index development follows a similar method to the German Saprobic Index, outlined by Gieswein (original document is in German). The index uses a combination of three parameters:

- **Median mass** of fine sediment from all sampling sites taxon was observed at
- **Abundance Value**, established using abundance classes provided in Gieswein *et al*. (2019), sourced from the German Saprobic Index (Figure 14A)
- **Weighting Factor**, which is the Z-Score from the TITAN results

These three factors were calculated for each taxon, and the resulting values were entered into a formula (Figure 14B) to calculate the Fine Sediment Biotic Index. The sum of all taxa's median fine sediment masses is multiplied by the individual taxon's z-score and the abundance value, divided by the sum of all taxa z-scores multiplied by the abundance class.

(A)

$$f(n) = \begin{cases} 0 \text{ for } n = 0 \\ 1 \text{ for } 0 < n < 2.5 \\ 2 \text{ for } 2.5 \leq n < 10.5 \\ 3 \text{ for } 10.5 \leq n < 30.5 \\ 4 \text{ for } 30.5 \leq n < 100.5 \\ 5 \text{ for } 100.5 \leq n < 300.5 \\ 6 \text{ for } 300.5 \leq n < 1000.5 \\ 7 \text{ for } 1000.5 \leq n \end{cases}$$

(B)

$$DFSI = \frac{\sum_i med_i \cdot z_i \cdot f(n_i)}{\sum_i z_i \cdot f(n_i)}$$

i : each indicator taxon.

Figure 14: (A) Conversion of the abundance of each taxon into seven abundance classes. (Gieswein *et al*., 2019) (B) Formula used to calculate FSBI, where med is the median mass of fine sediment, z is the taxon's z-score, and f(n) refers to the abundance class established in 14A (Gieswein *et al*., 2019).

Results

Analyses for 2021 and 2022 have been conducted separately; each sample trip and its associated issue(s) can be seen in Table IV. In 2021, fine sediments stored on a streambed ranged between 0.716– 100.6 g/m^2 Sediments obtained through the coring method averaged 6.1% (±4.7) organic constituents. Total Suspended Solids ranged from <1 mg/L up to 185.2 mg/L, averaging 18.6 mg/L (± 40.1) across all sites. We see a decrease of 66.32 g/m^2 of the upper limit of the fine sediment range in 2022, ranging from 0.0762 – 34.28 g/m^2 . Percent organic matter increased, averaging 8.5% (±6.7). Total Suspended Solids for 2022 decreases to a range of <1 and 60 mg/L, averaging 8.9 mg/L (±12.8).

The TITAN analyses used 33 observed taxonomic groups in 2021 and 22 groups in 2022. All groups were identified to family-level, except for subclass Hirudinea (leeches) whose classification becomes complicated in the field beyond subclass. TITAN utilized the data provided, as well as supplemented the analysis with 250 random permutations and 500 bootstrap replicates to produce 10 indicator families for 2021 and 5 families for 2022 (reliability ≥0.7) (Table V).

Sediment categories 2-0.06 mm and <0.06 mm were used in TITAN analyses, but only yielded one or two predictors. Taxonomic family occurrences were plotted along the fine sediment gradient using sediment <2 mm to visualize changes in distribution as fine sediment increased (Figures 15 & 16). Families are grouped by increasing or decreasing z-scores, identifying changepoints within a 5-95% quantile range. The z-scores, along with the abundance value and median mass of fine sediment (g/m^2), were used to calculate the FSBI Score for each indicator group, which can be seen in Table VI. FSBI scores ranged from 19-31 and 5-13 for 2021 and 2022, respectively. The top three highest FSBI scores for 2021 included *Hydrophilidae* (Water Scavenger Beetles), *Belostomidae* (Giant Water Bugs), and *Aeshnidae* (Darner Dragonfly Nymphs). For 2022, we see *Heptageniidae* (Flat-Headed Mayflies) as a stand-alone family with the highest FSBI Number. *Heptageniidae* is the only family that appears in both the 2021 and 2022 indicator taxa.

Table IV: Sample sites and their associated sampling issues.

Date Sampled	Sites Visited	Macroinvertebrates Sampled?	Problem Sites/Issues
2021			
24-May	CD27, LILY, 119	All, but LILY	LILY - High Flow, Too Turbid
3-Jun	CD56	Y	-
4-Jun	SMB	Y	-
7-Jun	BFRD	Y	-
15-Jun	CD27, LILY, 119	Y	LILY - Mucky
16-Jun	BFRD	Y	-
22-Jun	SMB, SMC, CD56, PARK	Y	SMB - Low Flow, Mucky
8-Jul	CD27, LILY, 119, CD56	All, but 119	119 - New boulders added to ditch, unsafe for sampling
13-Jul	SMB, SMC, BFRD	Y	SMB - Low Flow, Mucky SMC - Mucky
20-Jul	CD27, LILY, 119, CD56, PARK	Y	CD27 - Low Dissolved Oxygen 119 - Low Flow, Mucky CD56 - Large Algal Bloom & Duckweed Problem
30-Jul	SMB, SMC, BFRD	BFRD Only	**Severe Drought -** SMB & SMC - Low Flow, all muck
NO SAMPLES FROM AUGUST - SEVERE DROUGHT, NO FLOW IN DITCHES			
11-Sep	CD27, 119, LILY, CD56, PARK	CD27 & LILY Only	CD27 - Mucky 119 - No Flow PARK - No Flow
2022			
1-Jun	BFRD	Y	BFRD - Low Dissolved Oxygen, High flow
6-Jun	CD27, LILY, 119, PARK, CD56	All, but CD56	CD56 - Flooded
7-Jun	SMB, SMC	Y	SMC - Fields surrounding site flooded, putting in drain-tile
21-Jun	CD27, LILY, 119, PARK, CD56	All, but CD56	119 - Reduced Sampling Time, too mucky to walk CD56 - Flooded, **Removing From Sample Sites for 2022**
23-Jun	SMB, SMC, BFRD	Y	SMB - No Flow, Mucky, Algal Bloom SMC - Low Flow
11-Jul	SMB, SMC	Y	SMB - VERY Low Dissolved Oxygen, Duckweed
19-Jul	CD27, LILY, 119, PARK	Y	-
26-Jul	SMB, SMC, BFRD	Y	SMB - No Flow, Mucky, White Mold/Fungus on Veg BFRD - Low Flow
NO SAMPLES FROM AUGUST OR SEPTEMBER - ADVISOR INJURY			

Table V: Indicator taxa (reliability ≥ 0.7) identified using a TITAN analysis along a fine sediment (<2 mm) environmental gradient. Fine sediment mass data consists of samples from each site taxon was observed at.

*Hirudinea is not a family, but a subclass.

Order	Taxonomic Family*	Frequency	Z-score	Reliability	Fine sediment mass (g/m^2)			
					Median	Mean	SD	Range
2021								
Trichoptera	Hydropsychidae	9	-4.78	0.930	8.90	14.4	15.9	0.716 - 46.2
Hemiptera	Belostomidae	14	4.22	0.758	21.9	33.9	29.8	2.99 - 100.6
Decapoda	Cambaridae	14	-3.61	0.894	12.2	14.6	13.7	0.716 - 48.2
-	Hirudinea*	20	-3.3	0.732	17.5	25.7	26.8	1.21 - 100.6
Odonata	Coenagrionidae	21	3.21	0.830	16.6	25.8	26.8	1.55-100.6
Coleoptera	Elmidae	8	-2.75	0.890	22.3	29.5	33.2	0.716-100.6
Coleoptera	Hydrophilidae	12	2.43	0.758	30.5	36.4	30.4	2.13 - 100.6
Odonata	Libellulidae	11	1.90	0.776	22.3	34.2	29.7	2.99-100.6
Ephemeroptera	Heptageniidae	10	-1.82	0.898	12.2	21.8	27.6	0.716-78.3
Odonata	Aeshnidae	10	1.7	0.786	21.9	37.9	32.5	10.3 - 100.6
2022								
Ephemeroptera	Heptageniidae	5	-6.89	0.898	0.0762	2.65	4.33	0.0762 - 10.1
Diptera	Chironomidae, Pale-form	8	3.14	0.808	6.88	11.1	10.3	0.0762 - 28.5
Coleoptera	Dytiscidae	14	2.57	0.948	9.31	12.8	9.59	0.653 - 34.3
Coleoptera	Haliplidae	14	2.97	0.878	9.88	12.7	9.93	1.13 - 34.3
-	Lymnaeidae	14	2.98	0.906	9.36	12.0	9.70	0.831 - 34.3

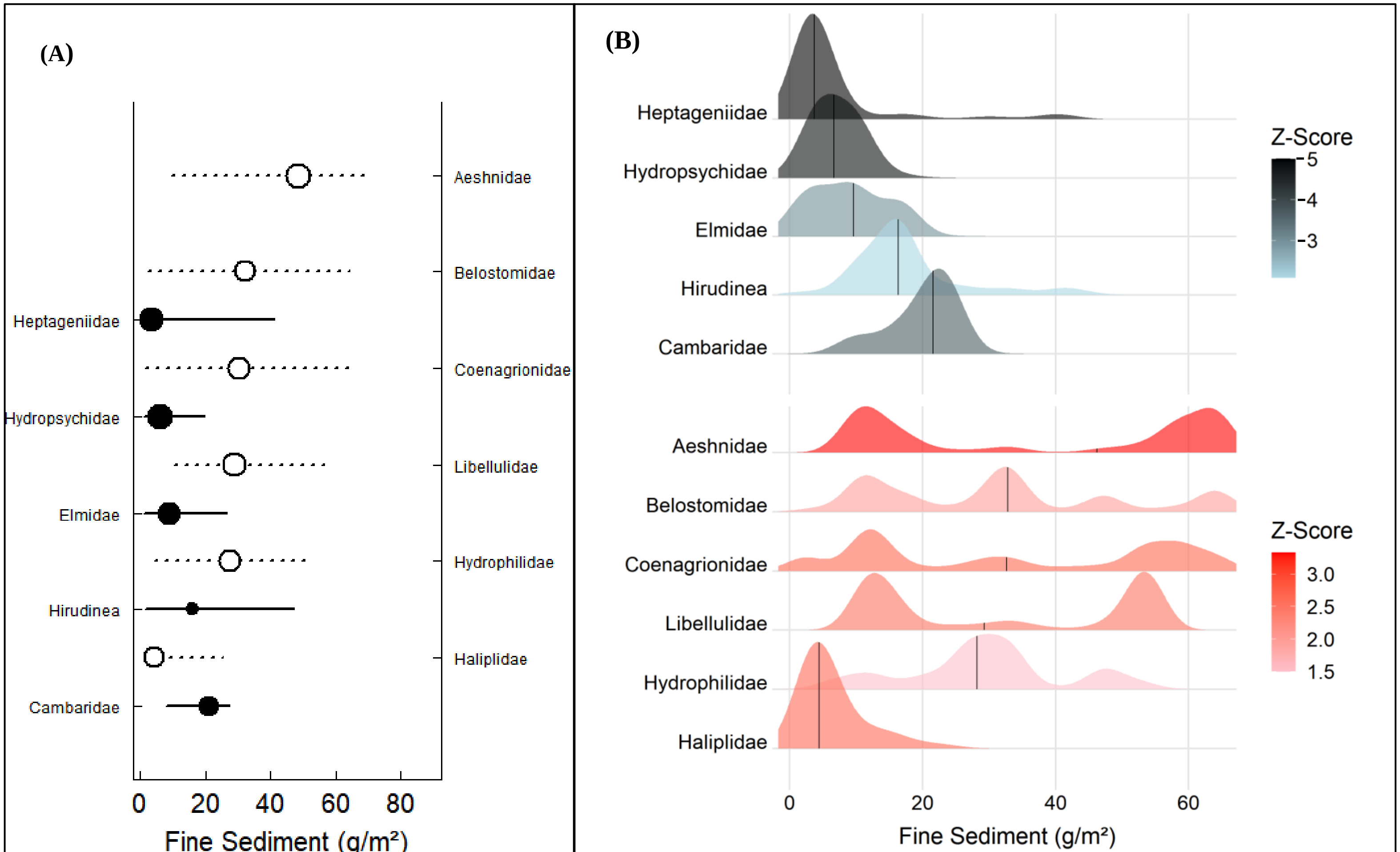

Figure 15: (A) Indicator taxonomic groups for 2021 plotted on fine sediment gradient. Groups plotted on the left (filled circles) are negative z-scores, while groups on the right (unfilled circles) are positive z-scores. Symbols are sized in proportion to z scores. This plot uses the median of the bootstrap replicates as the change point (location of the circle on the dotted or solid line). The lines represent 5-95% quantiles from the changepoint. **(B)** Indicator groups for 2021 plotted on fine sediment gradient. Negative z-scores are featured in the top half (blue/black) and positive z-scores are featured in the bottom half (pink/red).

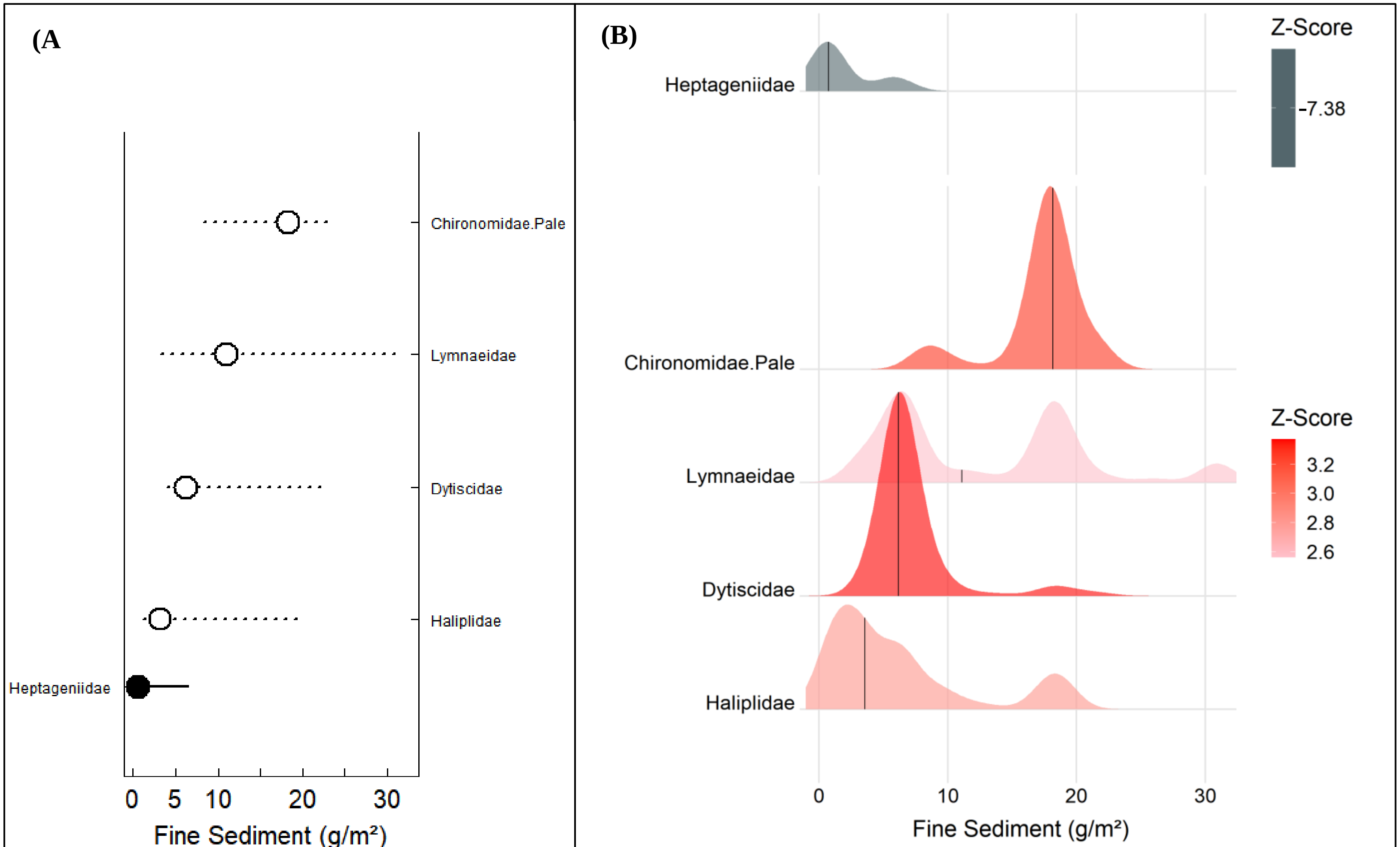

Figure 16: (A) Indicator taxonomic groups for 2022 plotted on fine sediment gradient. Groups plotted on the left (filled circles) are negative z-scores, while groups on the right (unfilled circles) are positive z-scores. Symbols are sized in proportion to z scores. This plot uses the median of the bootstrap replicates as the change point (location of the circle on the dotted or solid line). The lines represent 5-95% quantiles from the changepoint. **(B)** Indicator groups for 2022 plotted on fine sediment gradient. Negative z-scores are featured in the top half (blue/black) and positive z-scores are featured in the bottom half (pink/red).

Table VI: Indicator species identified by TITAN analysis (Baker & King, 2010) and associated FSBI calculation, separated by year.

*Hirudinea is not a family, but a subclass.

2021

Order	Taxonomic Family*	Common Name	Median Mass (g/m^2)	Abundance Value	Z-Score	FSBI Number
Coleoptera	Hydrophilidae	Water Scavenger Beetles	30.5	5	2.43	31
Hemiptera	Belostomidae	Giant Water Bugs	21.9	5	4.22	31
Odonata	Aeshnidae	Darner Dragonfly	21.9	4	1.70	25
Trichoptera	Hydropsychidae	Net-Spinning Caddisflies	8.9	4	-4.78	25
-	Hirudinea*	Leeches	17.5	4	-3.30	25
Coleoptera	Elmidae	Riffle Beetles	22.3	4	-2.75	25
Odonata	Libellulidae	Common Skimmer Dragonfly	22.3	4	1.90	25
Ephemeroptera	Heptageniidae	Flat-Headed Mayflies	12.2	4	-1.82	25
Odonata	Coenagrionidae	Narrow-Winged Damselfly	16.6	3	3.21	19
Decapoda	Cambaridae	Freshwater Crayfish	12.2	3	-3.61	19

2022

Order	Taxonomic Family*	Common Name	Median Mass (g/m^2)	Abundance Value	Z-Score	FSBI Number
Ephemeroptera	Heptageniidae	Flat-Headed Mayflies	0.0762	3	6.89	13
Diptera	Chironomidae, Pale	Non-biting Midges	6.881889	5	3.14	6
-	Lymnaeidae	Pond Snails	9.35736	4	2.98	6
Coleoptera	Haliplidae	Crawling Water Beetles	9.88314	5	2.97	6
Coleoptera	Dytiscidae	Predaceous Diving Beetles	9.314815	5	2.57	5

Discussion

Biotic indices that utilize benthic macroinvertebrates as indicators of fine sediment have continually been published over the past 11 years (Relyea *et al*., 2012; Extence *et al*., 2013; Murphy *et al*., 2015; Turley *et al*., 2016; Hubler *et al*., 2016; Doretto *et al*., 2018; Gieswein *et al*., 2019; Kim *et al*., 2019). The impact of fine sediments on macroinvertebrates is a relationship that will become important, especially in the rise of climate change (Chen *et al*., 2020; US EPA, 2016; Walling, 2009). The development of this Fine Sediment Biotic Index was originally meant for standard conditions in southern Minnesota. This data cannot be considered "standard conditions", due to extreme weather events including a drought in 2021 (MNDNR, 2022a) and late springtime flooding in 2022 (MNDNR, 2022b).

The sampling season for 2021 began in late May but was halted before August because drought conditions were affecting ditch flow rates. Figure 17 shows monthly precipitation deficits compounding during the onset of the drought. Note the rapid decline during from April-21 to July-21. Figure 18 compares drought conditions at the start

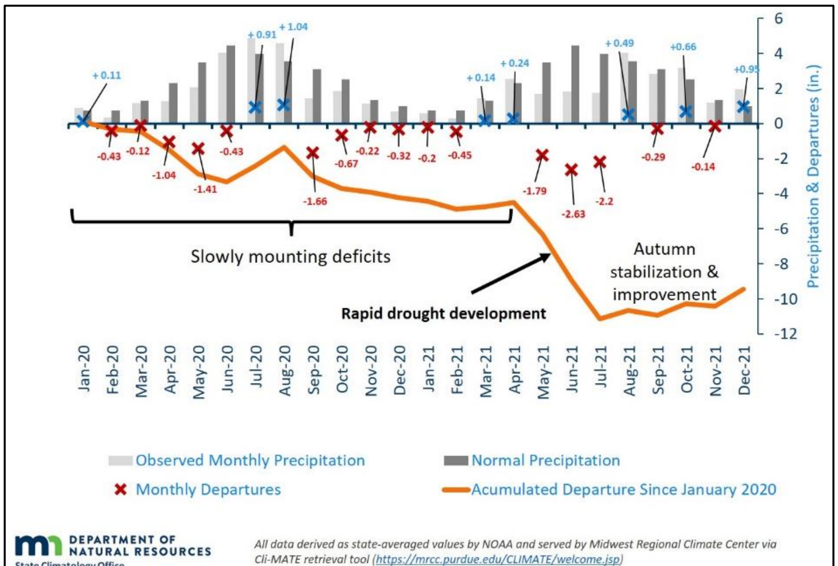

Figure 17: Monthly precipitation and departures from 1991-2020 "normals" for Minnesota from January 2020 to December 2021 (MNDNR, 2022a).

of the sampling season, where a majority of Blue Earth County was experiencing a moderate drought, and where the sampling season was paused, due to conditions worsening into Severe Drought status (University of Nebraska-Lincoln, 2021).

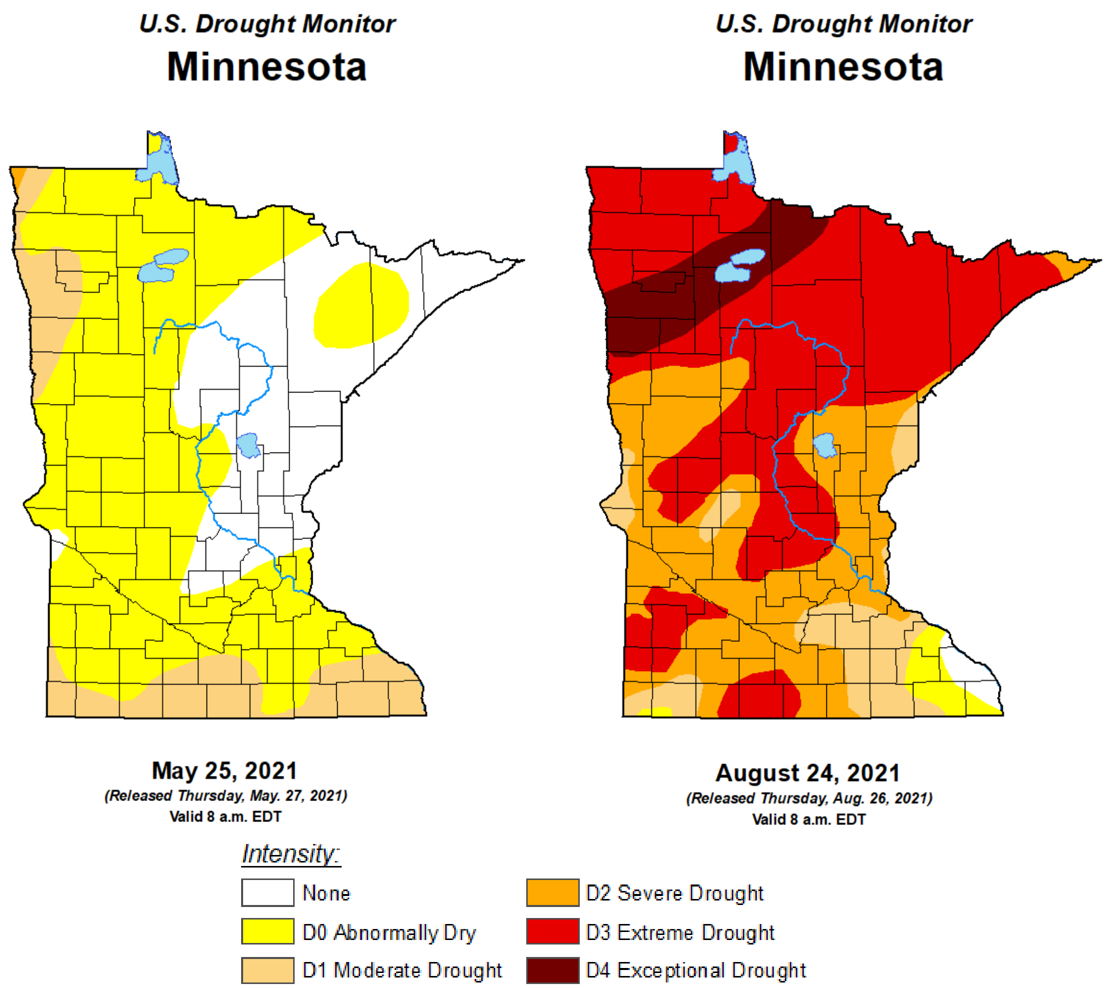

Figure 18: Changes in drought conditions in Minnesota from the end of May 2021 to August 2021 (University of Nebraska-Lincoln, 2021).

No samples were taken in August of 2021 and 2022, due to low or no flow. There was one sampling trip conducted in September of 2021. Drought conditions remained in effect, and the macroinvertebrate and sediment data followed the same trends as the samples taken earlier in the summer. These conditions were indicative of a bookend year, most of the state experiencing the worst drought in up to 30 years (MNDNR, 2022a). It was decided that keeping the September data could make the dataset used in TITAN more robust.

Fine sediment and reduced flow have been studied in tandem on multiple occasion. These studies yielded consistent results that suggest negative effects of reduced flow are as common as the negative effects of fine sediment deposition (Blöcher *et al*., 2020; Beermann *et al*., 2018; Elbrecht *et al*., 2016; Matthaei *et al*., 2010). Blöcher *et al*. (2020) investigated potential interactions between fine sediment grain size and flow velocity, noting that the effects of sedimentation on macroinvertebrates can be exacerbated by reduced flow velocity. Their analysis concluded sediment-sensitive species and invertebrate community metrics were negatively impacted by fine sediment, with worse impacts at reduced flow rates (Blöcher *et al*., 2020). While ditches generally experience slow velocities, this is something to consider when continuing the investigation into future sediment biotic indices, especially in drought conditions.

Minnesota experienced above average levels of precipitation in Spring of 2022, approximately 50% more than average precipitation from April 1st to May 31st (Minnesota DNR, 2022c; Figure 19). This resulted in a back-flooding event from Lake Crystal at the sample site County Ditch 56. Abnormal amounts of precipitation lead to scouring events. Scouring alters substrate composition, sediment transport, and vegetation, which in turn affects benthic macroinvertebrates (Gholizadeh, 2021; Foster *et al*., 2020; Calderon *et al*., 2017; Gibbins *et al*., 2005). The back-flooding was severe into June of 2022, inevitably resulting in the removal of County Ditch 56 from our sample sites for our 2022 dataset.

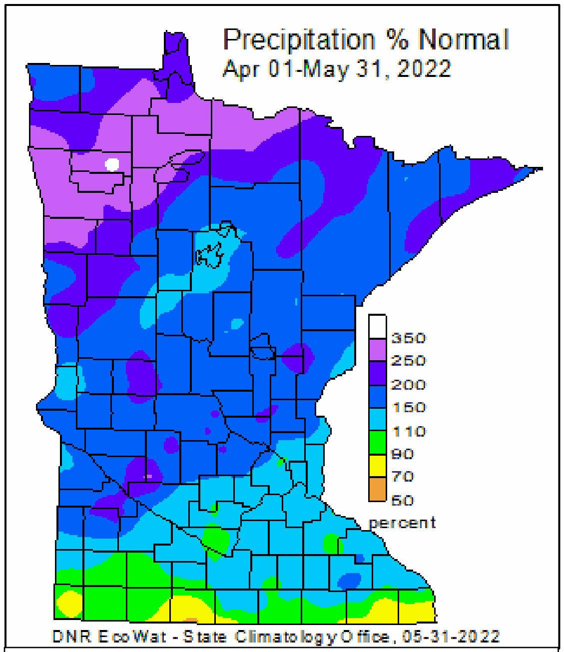

Figure 19: Percent of precipitation, compared to the normal quantity for the range of April 1, 2022 to May 31, 2022 (Minnesota DNR, 2022c).

Flooding conditions were followed by a rapid onset of abnormally dry/moderate drought conditions (Figure 20). Benthic macroinvertebrates would have faced tremendous pressure from low flow and low dissolved oxygen, making recolonization difficult after the flush and scour event. Water scorpions (family *Nepidae*) are notoriously elusive in collection, but over eleven were counted at one site in July 2022. Their emergence is speculated to be due to lack of a food resulting from the drought conditions. This pressure during a recolonization effort might be why we see less predictors overall for 2022.

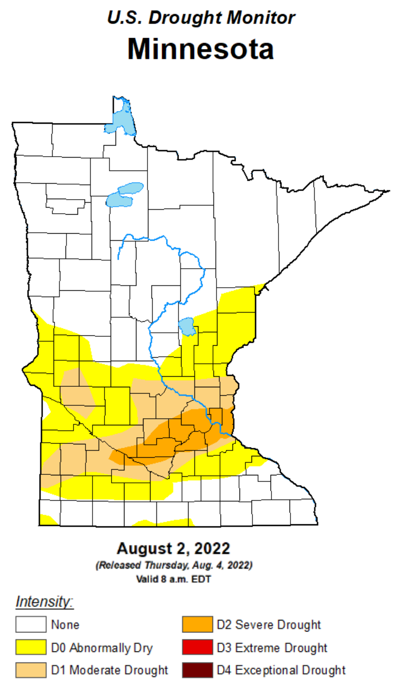

Figure 20: Drought conditions in August 2022 (University of Nebraska-Lincoln, 2021).

Total Suspended Solids yielded more resuspended material in 2021, compared to 2022, most likely a result of the scour event. At peak discharge during a flood, we typically see vast increases in TSS and turbidity (Figure 21; Rügner *et al*., 2013). The flush occurring could have altered

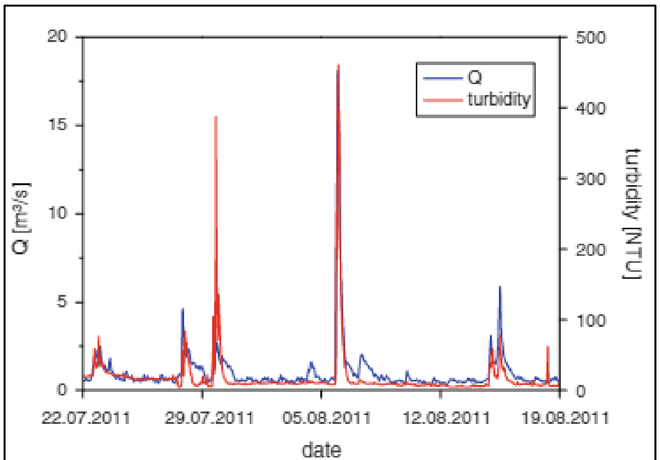

Figure 21: Relationship between flooding discharge (Q) and turbidity (NTU), determined by Rügner *et al*., 2013.

substrate composition through increased sediment transport and water flow (Rügner et al., 2013). Conditions post-scour resulted in substrate compositions with less fine sediment, therefore, decreasing resuspended materials in 2022.

The benthic macroinvertebrate indicator families differ between both years, only sharing one family: *Heptageniidae* (Flat-Headed Mayflies). Because we saw such vast ranges in deposited fine sediment and weather conditions across the two years, it is not unexpected to see variation across the indicator families. When compared against other papers looking at sediment sensitivities of benthic macroinvertebrates, genus and species-level identification is often used. As this was meant to be used as a rapid bioassessment tool, finding fine sediment sensitivities at a family level would be ideal for in-situ identification. However, studies show sensitivities may vary as low as species-level (Sandra *et al*., 2010; Govenor *et al*., 2019; Leitner *et al*., 2023). Each of these studies is conducted in different areas across the world, but they discuss similar trends in macroinvertebrate sediment data. We can see potential parallels, *Hydropsychidae* (Net-Spinning Caddisflies) acting as an indicator family in the 2021 TITAN Analysis also appears in both Sandra *et al*. (2010) and Leitner *et al*. (2023), specifically *Arctopsyche grandis* and the genus *Hydropsyche* as sediment-sensitive taxa, respectively. Additionally, *Epeorus longimanus*, part of the *Heptageniidae* (Flat-Headed Mayflies) family, is listed as a sediment-sensitive species in Sandra *et al*. (2010). Depending on the year, *Heptageniidae* in this FSBI can be looked at as less sensitive in drought conditions, but more sensitive in flood conditions. Sandra *et al*. observes a decrease in EPT and sediment-sensitive taxa as fine sediment increases.

Leitner *et al*. (2023) and Geiswein *et al*. (2019) both discuss the family *Elmidae*, and the lack of consistency in fine sediment sensitivities across the species within it. *Esolus angustatus* and *Limnius perrisi* were both considered sediment intolerant taxa, and *Oulimnius tuberculatus* was considered sediment tolerant. However, Leitner *et al*. identifies the genus *Elmis* as sediment intolerant, while Geiswein *et al*. declares *Elmis aenea* and *Elmis maugetii* as sediment tolerant. In our 2022 TITAN Analysis, *Haliplidae* (Crawling Water Beetles) and *Dytiscidae* (Predaceous Diving Beetles) appear as sediment-tolerant taxa, and *Hydrophilidae* (Water Scavenger Beetles) occur in the 2021 Analysis as sediment-intolerant. The diversity within Coleoptera and the variation in sediment sensitivities should be further explored, as there may be potential in specializing in a specific family for a sediment index.

Class Hirudinea (Leeches) continued to be consistent at sampling sites, even in areas at the highest range of deposited fine sediment. Hirudinea is known to be hardy in organically-polluted areas, but they seem to be excellent sediment-tolerant indicators, as well. Class Hirudinea was identified as an indicator taxon in our 2021 TITAN analysis, Geiswein *et al*. (2019), and Extence *et al*. (2013) (specifically genus *Erpobdella*). Although *Erpobdella* typically is found in a preferred substrate of solid gravel and boulders, Gieswein points out that one of their food sources, *Chironomidae* larvae (appearing as an indicator taxon in the 2022 TITAN Analysis), is associated with fine sediments because they can burrow (2019; Wood & Armitage, 1997).

The TITAN package recommends certain parameters be used when conducting a change-point analysis, including an organism occurrence rate of five, but an absolute minimum of 3. When working with limited datasets, increasing to an occurrence rate of five could eliminate a potential predictor. The package also recommends using a reliability of 0.95, but the same problem is encountered. Similar to Geiswein *et al*. (2019), a reliability of 0.70 was opted for. While sacrificing reliability, you gain more indicator taxa, which could result in increased ease of use in the field.

Overall, the TITAN Analysis provided us with 15 indicator taxa and their change-points over two years, but the FSBI scores are quite clumped together, making it difficult to form sensitivity groups. The fine sediment data had a large range, with macroinvertebrates occurring all throughout the range, making it difficult to pin down clear boundaries for Sensitivity Groups, i.e. Relyea *et al*.'s (2012) *Moderately Sensitive*, *Slightly Sensitive*, etc. The use of permutation and bootstrap replicates aided in the indicator taxa analysis, but more data is going to be needed before Sensitivity Groups can be formed and the FSBI can be functional.

Additionally, biotic indices are often validated using existing datasets representing the locations and conditions the index is intended for. Because this is a pilot study looking into macroinvertebrates and fine sediment within drainage ditches in southern Minnesota, there are little to no public datasets that have enough overlap specifically for high-sediment, slow-moving streams or the macroinvertebrates found in them. This index will require validation once more data is available to support it.

This concept, as well as the TITAN Analysis, has potential for use in other stream-type specific environments. A continuation of this drain-tiled ditch system study could marry the two extreme weather condition indices together with macroinvertebrates present in normal conditions. Alternatively, continued sampling in extreme weather events could expand and supplement the existing data used in this project. If possible, identifying macroinvertebrates to genus or species level may result in less conflicting sediment responses.

Conclusion

We have begun the development of a Fine Sediment Biotic Index (FSBI) for drain-tiled ditch systems in southern Minnesota using a Threshold Indicator Taxa Analysis. The study was limited by sample size due to extreme weather conditions, but serves as a foundation for future fine sediment indices in the Western Corn Belt Plains ecoregion and demonstrates that the FSBI approach may be a useful tool for assessing stream ecosystem health in Minnesota. Additional investigation into genus and species level interactions with fine sediment may improve FSBI.

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APPENDIX A

Table VII: Methods used in measurement of Water Quality Parameters.

Hach Procedure	**EPA Method**	**Range**	**Location Conducted**
Nitrate (Hach 10206)	40CFR 141	0.23 to 13.50 mg/L NO3–N	Lab
Phosphorous, Ortho (Hach 8048)	EPA 365.1	0.06 - 5.00 mg/L PO4	Lab
Total Phosphorous (Hach 8190)	EPA 365.1	0.06 - 3.50 mg/L PO_4	Lab
Escherichia coli (Hach 10029 Membrane Filtration with m-Coliblue24)	40 CFR 141.21	-	Lab
Dissolved Oxygen (146900 – Model OX-2P)	EPA 4500-O (B-F)-2016	1 - 20 mg/L O_2	Field

Table VIII: 2021 Field Water Quality Data by sample date and site.

Site Name	Sample Date	Dissolved Oxygen (mg/L)	pH	Temperature (℃)	Turbidity
Beauford	06.07.2021	10	8.34	25	74
Beauford	06.16.2021	10	8.48	23.5	>100
Beauford	07.13.2021	10	7.9	24.4	>100
Beauford	07.30.2021	9	7.79	22.5	22
Minneopa - 119	05.24.2021	10	8.38	25.4	>100
Minneopa - 119	06.15.2021	5	8.3	34.4	49
Minneopa - 119	07.08.2021	9	8.38	22.5	52
Minneopa - 119	07.20.2021	7	7.83	23.7	74
County Ditch 27	05.24.2021	14	8.28	22.2	NA*
County Ditch 27	06.15.2021	16	8.7	24.6	>100
County Ditch 27	07.08.2021	8	7.7	21	78
County Ditch 27	07.20.2021	4	7.66	21.6	>100
County Ditch 27	09.11.2021	7	7.74	18.9	24
County Ditch 56	06.03.2021	14	8.6	22.5	>100
County Ditch 56	06.22.2021	12	8.63	20.6	92
County Ditch 56	07.08.2021	10	8.29	22.8	77
County Ditch 56	07.20.2021	8	7.84	24.5	>100
County Ditch 56	09.11.2021	9	7.76	19.7	85.5
Minn Cr - Lily	06.15.2021	9	8.51	28.2	63
Minn Cr - Lily	07.08.2021	9	8.28	24.2	46
Minn Cr - Lily	07.20.2021	8	7.99	23.5	50
Minn Cr - Lily	09.11.2021	9	8.28	21.3	36.5
Minneopa Park	06.22.2021	8	8.28	23.5	49
Minneopa Park	07.20.2021	8	8.53	25.8	65
Seven-Mile B	06.04.2021	16	8.46	25.5	98
Seven-Mile B	06.22.2021	8	7.47	17.5	>100
Seven-Mile B	07.13.2021	4	7.64	23.3	34
Seven-Mile C	06.22.2021	13	8.29	18.6	28
Seven-Mile C	07.13.2021	6	7.97	23.7	NA*

*Not analyzed or lost

Table IX: 2021 Laboratory Water Quality Data by sample date and site. *E. Coli* samples exceeding Minnesota's Department of Health Standard for full body contact (235 colonies per 100 mL) are in red.

			units mg/L			colonies/100mL
Site Name	**Sample Date**	**N-NO3**	**TP**	**P-PO4**	**TSS**	**E.Coli**
Beauford	06.07.2021	7.7	0.37	0.21	<1	**TNTC****
Beauford	06.16.2021	6.1	0.98	0.15	<1	108
Beauford	07.13.2021	8.8	0.45	0.37	<1	128
Beauford	07.30.2021	3.1	0.51	0.19	12.2	168
Minneopa - 119	05.24.2021	15.8	0.21	0.12	<1	180
Minneopa - 119	06.15.2021	13.9	0.42	0.32	<1	204
Minneopa - 119	07.08.2021	10.4	0.83	NA*	27.0	**240**
Minneopa - 119	07.20.2021	2.0	0.36	0.31	32.6	120
County Ditch 27	05.24.2021	9.4	0.31	0.22	<1	68
County Ditch 27	06.15.2021	5.5	0.26	0.21	<1	132
County Ditch 27	07.08.2021	0.9	0.93	0.77	38.3	36
County Ditch 27	07.20.2021	0.5	>4.0	3.88	4.6	88
County Ditch 27	09.11.2021	0.8	1.08	0.52	32.7	**248**
County Ditch 56	06.03.2021	7.8	>4.0	0.32	<1	20
County Ditch 56	06.22.2021	2.7	0.83	0.67	<1	80
County Ditch 56	07.08.2021	3.0	1.67	1.15	2.6	48
County Ditch 56	07.20.2021	1.5	1.19	1.04	4.7	8
County Ditch 56	09.11.2021	3.1	0.88	0.56	2.0	28
Minn Cr - Lily	06.15.2021	6.0	0.3	0.21	<1	112
Minn Cr - Lily	07.08.2021	1.6	0.55	0.21	27.7	112
Minn Cr - Lily	07.20.2021	0.5	0.73	0.38	10.6	**TNTC****
Minn Cr - Lily	09.11.2021	0.9	0.6	0.46	1	**308**
Minneopa Park	06.22.2021	3.2	0.82	0.65	5.4	200
Minneopa Park	07.20.2021	1.9	0.7	0.52	3.6	140
Seven-Mile B	06.04.2021	11.2	0.24	0.08	<1	52
Seven-Mile B	06.22.2021	3.8	0.34	0.23	1	156
Seven-Mile B	07.13.2021	0.8	0.96	0.76	21.2	128
Seven-Mile C	06.22.2021	8.8	0.36	0.21	17.25	**TNTC****
Seven-Mile C	07.13.2021	1.9	0.89	0.42	40.3	**TNTC****

*Not analyzed or lost

**Too Numerous To Count

Table X: 2022 Field Water Quality Data by sample date and site.

Site Name	Sample Date	Dissolved Oxygen (mg/L)	pH	Temperature (℃)	Turbidity
Beauford	06.01.2022	5	8.15	18.2	75
Beauford	06.23.2022	10	7.96	21.1	>100
Beauford	07.11.2022	10	8.10	22.7	97
Beauford	07.26.2022	10	8.13	21.6	>100
Minneopa - 119	06.06.2022	10	7.65	16.2	29
Minneopa - 119	06.21.2022	5	7.57	21.8	48
Minneopa - 119	07.19.2022	5	7.69	23.4	40
County Ditch 27	06.06.2022	9	7.38	14.9	>100
County Ditch 27	06.21.2022	6	7.46	22.3	>100
County Ditch 27	07.19.2022	6	7.72	23.0	>100
Minn Cr - Lily	06.06.2022	11	7.78	18.3	29
Minn Cr - Lily	06.21.2022	8	7.47	23.8	>100
Minn Cr - Lily	07.19.2022	5	7.64	24.3	48
Minneopa Park	06.06.2022	10	8.08	20.2	56
Minneopa Park	06.21.2022	7	7.86	26.0	64
Minneopa Park	07.19.2022	8	8.47	26.4	62
Seven-Mile B	06.07.2022	6	7.26	16.8	>100
Seven-Mile B	06.23.2022	5	7.36	20.3	>100
Seven-Mile B	07.11.2022	1	6.32	22.6	>100
Seven-Mile B	07.26.2022	1	7.13	20.7	36
Seven-Mile C	06.07.2022	6	7.68	13.4	>100
Seven-Mile C	06.23.2022	8	7.65	18.2	41
Seven-Mile C	07.11.2022	11	8.02	21.8	52
Seven-Mile C	07.26.2022	4	7.73	20.5	30

*Not analyzed or lost

Table XI: 2022 Laboratory Water Quality Data by sample date and site. *E. Coli* samples exceeding Minnesota's Department of Health Standard for full body contact (235 colonies per 100 mL) are in red.

		units mg/L				colonies/100mL
Site Name	**Sample Date**	**N-NO3**	**TP**	**P-PO4**	**TSS**	**E.Coli**
Beauford	06.01.2022	10.2	0.31	0.17	6	40
Beauford	06.23.2022	12.6	0.46	0.28	9.4	192
Beauford	07.11.2022	12.0	0.55	0.42	5.5	224
Beauford	07.26.2022	8.3	0.3	0.20	2	**776**
Minneopa - 119	06.06.2022	22.7	0.57	0.33	18.2	144
Minneopa - 119	06.21.2022	16.7	0.66	0.42	11.6	**TNTC****
Minneopa - 119	07.19.2022	5.2	2.31	1.63	65	188
County Ditch 27	06.06.2022	11.4	0.34	0.15	2.4	152
County Ditch 27	06.21.2022	6.1	0.47	0.34	9.2	**484**
County Ditch 27	07.19.2022	3	1.86	0.30	10.3	104
Minn Cr - Lily	06.06.2022	8.5	0.36	0.10	5.0	76
Minn Cr - Lily	06.21.2022	5.6	0.18	0.09	60	**288**
Minn Cr - Lily	07.19.2022	4.3	1.26	0.78	20.3	60
Minneopa Park	06.06.2022	3.8	0.41	0.12	6.4	56
Minneopa Park	06.21.2022	2.6	0.73	0.52	1.6	**352**
Minneopa Park	07.19.2022	1.8	0.74	0.49	7.3	104
Seven-Mile B	06.07.2022	9.2	0.16	0.14	0.2	64
Seven-Mile B	06.23.2022	2.5	0.65	0.30	8.0	216
Seven-Mile B	07.11.2022	4.8	0.59	0.37	3.67	56
Seven-Mile B	07.26.2022	0.07	2.09	0.97	28.3	**TNTC****
Seven-Mile C	06.07.2022	9.8	0.18	0.11	<1	24
Seven-Mile C	06.23.2022	10.8	0.82	0.25	NA*	**512**
Seven-Mile C	07.11.2022	9.5	0.33	0.27	15.3	**268**
Seven-Mile C	07.26.2022	0.9	1.88	1.77	6	**268**

*Not analyzed or lost

**Too Numerous To Count

Table XII: Quality control samples and number of samples within 90-110% range in this 2-year study.

Quality Control Method	N-NO3	TP	P-PO4	*E. coli**	TSS*
Field Duplicate	23/26	15/26	18/28	6/11	6/16
Blanks	-	-	-	6/6	4/4
Standard Curve Check	41/41	28/29	42/42	-	-
Laboratory Duplicate	20/24	24/25	23/24	1/5	11/15
Percent Recovery	95-105%	95-100%	96-110%	-	-

*Only field duplicates, lab duplicates, and blanks conducted

APPENDIX B

```r
install.packages("tidyverse")
install.packages("TITAN2")
library(tidyverse)
library(TITAN2)

##2021 DATA STARTS HERE!!##

taxa2021together %>%
  select(-X) %>%
  drop_na() %>%
  remove_rownames  %>%
  column_to_rownames(var= "Site.Code") %>%
  rename(Hirudinea = Class.Hirudinea) %>%
  rename(Haliplidae = Halipildae) -> taxatogether2021fixed
#reading in taxa where adults, pupae, and larva/nymphs are counted together, plus making edits to neaten up

sediment_combined2021 <- read.csv("sediment_combined_2021.csv")
sediment_combined2021 %>%
  remove_rownames %>%
  column_to_rownames(var="Site.Code") -> sediment_combined2021_fixed
#reading in sediment data <2mm and cleaning

#FSBI for 2021
fsbi.double.combined.2021 <- titan(sediment_combined2021_fixed,taxatogether2021fixed,
                                   minSplt = 3,pur.cut = 0.7, rel.cut = 0.7)
plot_taxa_ridges(fsbi.double.combined.2021, xlabel = "Fine Sediment (g/m²)") -> doublecombined2021figure
plot_taxa(fsbi.double.combined.2021, xlabel = "Fine Sediment (g/m²)",z.med = TRUE) -> taxachangepoints_2021

#saving figures
ggsave("doublecombined2021figure2.png",doublecombined2021figure)
ggsave("taxachangepoints_2021.png", taxachangepoints_2021)

#####2022 Taxa Prep####

taxa2022together <- read.csv("taxa2022together.csv")

taxa2022together %>%
  drop_na() %>%
  remove_rownames  %>%
  column_to_rownames(var= "Site.Code") %>%
  rename(Hirudinea = Class.Hirudinea) -> taxatogether2022fixed
#reading in taxa where adults, pupae, and larva/nymphs are counted together, plus making edits to neaten up

#####reading in sediment data <2mm and cleaning#####

sediment_combined2022 <- read.csv("sediment_combined_2022.csv")
sediment_combined2022 %>%
  remove_rownames %>%
  column_to_rownames(var="Site.Code") -> sediment_combined2022_fixed

#FSBI for 2022
fsbi.doublecombined.2022 <- titan(sediment_combined2022_fixed,taxatogether2022fixed,
                             minSplt = 3,pur.cut = 0.7, rel.cut = 0.7)
#saving figures
plot_taxa_ridges(fsbi.doublecombined.2022, xlabel = "Fine Sediment (g/m²)") -> doublecombined2022figure
ggsave("doublecombined2022figure.png",doublecombined2022figure)

plot_taxa(fsbi.doublecombined.2022, xlabel = "Fine Sediment (g/m²)", z.med = TRUE)  -> taxachangepoints_2022
ggsave("taxachangepoints_2022.jpg", taxachangepoints_2022)
```

Figure 22: Screenshot of raw data from RStudio, showing the TITAN Analysis conducted for both 2021 and 2022.

	ienv.cp	zenv.cp	freq	maxgrp	IndVal	zscore	5%	10%	50%	90%	95%	purity	reliability	z.median	filter
Baetidae	1.844040	1.844040	8	1	52.07	2.27	1.381506	1.381506	5.967425	32.77743	63.27330	0.828	0.646	3.186788	0
Caenidae	1.844040	1.844040	20	2	68.94	1.46	1.844040	1.844040	25.919430	52.76748	53.69560	0.716	0.568	2.140222	0
Heptageniidae	2.563825	2.563825	10	1	87.25	4.78	2.101291	2.274265	2.994050	17.13983	41.21338	0.886	0.886	4.675645	1
Hydropsychidae	4.430725	9.631680	9	1	66.96	4.22	1.381506	1.381506	5.967425	12.16108	19.96704	0.978	0.926	5.044759	1
Lepidostomatidae	19.967042	19.967042	4	1	26.67	2.18	6.658305	9.631680	19.967042	22.06244	22.86254	0.890	0.422	2.309188	0
Leptoceridae	4.430725	4.430725	3	1	39.04	3.51	4.000500	4.000500	5.867400	28.38310	32.77743	0.838	0.596	3.524952	0
Chironomidae	7.404100	7.404100	22	1	71.53	1.02	5.867400	7.404100	17.006481	48.23968	53.69560	0.602	0.536	1.985338	0
Ephydridae	53.695600	53.695600	3	2	24.04	1.34	11.165840	11.165840	27.322780	53.69560	53.69560	0.754	0.298	1.859747	0
Simuliidae	17.468850	17.468850	4	2	26.67	1.98	16.675532	17.006481	17.468850	34.75511	52.66436	0.870	0.412	2.208269	0
Elmidae	1.844040	9.631680	8	1	53.88	3.21	1.381506	1.381506	8.094980	17.00648	19.50467	0.954	0.874	4.323065	1
Haliplidae	2.563825	2.563825	24	2	96.00	2.90	2.274265	2.563825	4.000500	16.30985	25.31618	0.858	0.748	2.758404	2
Dytiscidae	1.844040	1.844040	19	2	73.08	1.58	1.844040	1.844040	13.004463	47.20844	52.66436	0.764	0.608	2.169431	0
Hydrophilidae	47.208440	32.777430	12	2	46.32	1.70	2.563825	5.867400	27.322780	47.20844	63.55843	0.820	0.712	2.624461	2
Corixidae	1.844040	1.844040	24	2	75.39	1.26	1.381506	1.381506	15.126017	41.21338	41.21338	0.444	0.654	2.368744	0
Mesoveliidae	34.589060	17.468850	4	2	17.64	0.71	4.430725	5.967425	17.468850	32.77743	34.58906	0.622	0.198	1.504598	0
Belostomidae	64.063626	64.063626	14	2	68.42	1.90	2.563825	3.971544	30.541811	64.06363	64.06363	0.896	0.760	2.759097	2
Notonectidae	30.390681	30.390681	5	1	25.00	1.07	11.165840	11.165840	19.833692	30.52572	30.54181	0.518	0.188	1.619169	0
Veliidae	1.844040	1.844040	3	1	61.90	5.48	1.381506	1.381506	2.274265	25.31618	27.32278	0.874	0.556	4.493783	0
Gerridae	64.063626	64.063626	3	2	27.08	0.98	4.430725	4.430725	14.124902	68.97573	73.64133	0.394	0.380	2.778176	0
Nepidae	27.322780	17.006481	5	1	18.97	0.43	4.430725	8.025892	17.139831	73.64133	84.79018	0.586	0.228	1.588911	0
Aeshnidae	64.063626	64.063626	10	2	80.31	3.61	8.940800	9.631680	47.208440	68.72922	68.72922	0.880	0.794	3.336523	2
Calopterygidae	17.468850	17.468850	4	2	26.67	2.38	16.675532	17.126496	17.468850	59.15152	64.06363	0.928	0.476	2.498500	0
Coenagrionidae	64.063626	53.695600	21	2	78.57	2.43	1.381506	2.563825	30.458200	59.15152	64.06363	0.910	0.772	2.766867	2
Libellulidae	53.695600	53.695600	11	2	62.62	2.75	11.280521	11.315065	23.220782	53.69560	58.60771	0.902	0.786	3.021802	2
Gammarus	1.844040	1.844040	24	2	88.01	1.84	1.844040	1.844040	12.158345	27.33887	42.24462	0.498	0.470	1.736218	0
Asellidae	34.589060	34.589060	4	2	34.16	2.77	11.165840	12.158345	34.437930	39.55288	40.69677	0.850	0.604	3.024512	0
Cambaridae	23.220782	23.220782	14	1	65.88	3.30	8.898509	9.631680	21.929090	25.11425	36.45873	0.986	0.926	3.983785	1
Gordiidae	34.589060	23.220782	3	2	23.08	2.06	19.967042	20.722590	32.202310	53.69560	53.69560	0.956	0.490	2.938198	0
Planorbidae	17.468850	17.468850	17	2	58.33	1.28	2.079780	12.158345	20.722590	68.72922	73.64133	0.708	0.524	1.917675	0
Physidae	1.844040	1.844040	23	2	71.30	1.31	1.381506	1.381506	16.309848	47.25765	53.69560	0.630	0.568	2.027299	0
Fossaria	1.844040	1.844040	12	2	46.15	0.38	2.133600	2.563825	8.940800	27.32278	30.39068	0.424	0.384	1.553566	0
Hirudinea	16.309848	16.309848	20	1	59.86	1.82	1.554480	8.938260	16.309848	42.24462	47.20844	0.764	0.774	2.623688	1
Class.Bivalvia	53.695600	53.695600	5	2	24.17	0.74	1.381506	4.430725	25.316180	53.69560	53.69560	0.604	0.356	1.783764	0

Figure 23: Raw TITAN output for 2021 macroinvertebrate and sediment data.

	ienv.cp	zenv.cp	freq	maxgrp	IndVal	zscore	5%	10%	50%	90%	95%	purity	reliability	z.median	filter
Baetidae	0.478790	0.478790	5	1	57.19	2.82	0.152400	0.152400	1.341120	13.163550	18.166080	0.822	0.528	2.771443	0
Caenidae	11.766550	11.766550	3	1	21.43	1.08	3.423285	5.658485	8.273491	13.163550	13.163550	0.778	0.154	1.443241	0
Heptageniidae	0.478790	0.478790	5	1	98.25	6.52	0.478790	0.478790	0.746760	6.020117	6.949465	0.976	0.866	5.919932	1
Ceratopogonidae	22.985095	6.930784	3	2	20.00	0.91	6.416116	6.930784	16.562070	22.993731	27.973496	0.606	0.322	1.760754	0
Chironomidae..Red	3.423285	3.423285	11	2	61.11	1.77	3.228975	3.228975	11.034395	23.071455	26.167080	0.878	0.660	2.413912	0
Chironomidae..Other	18.166080	18.166080	8	2	64.06	3.42	8.215071	8.273491	18.166080	22.985095	22.985095	0.928	0.778	3.406314	2
Simuliidae	18.166080	18.166080	5	2	31.11	1.16	3.912219	6.569151	17.213580	22.985095	22.985095	0.742	0.398	1.737691	0
Stratiomyidae	3.423285	3.423285	6	2	33.33	1.01	3.228975	3.423285	8.215071	18.166080	22.659943	0.738	0.254	1.652326	0
Haliplidae	1.146810	1.146810	14	2	73.68	3.10	1.073150	1.146810	3.423285	18.594705	19.547205	0.982	0.862	3.282483	2
Dytiscidae	6.173153	6.173153	14	2	80.11	3.51	3.937953	6.020117	6.173153	19.118580	22.985095	0.994	0.964	3.536290	2
Hydrophilidae	15.289530	15.289530	10	1	51.92	2.14	0.878840	0.878840	11.766550	15.941040	15.941040	0.704	0.692	2.568404	0
Corixidae	3.423285	3.423285	10	2	49.15	0.81	3.423285	3.423285	6.569151	15.289530	22.642830	0.516	0.310	1.431808	0
Belostomidae	16.562070	16.562070	6	1	35.29	1.37	3.228975	3.423285	10.980420	17.322165	17.514570	0.672	0.318	1.662672	0
Gerridae	9.761220	9.161780	3	2	23.08	1.87	6.875329	8.814511	9.761220	19.118580	19.547205	0.828	0.230	1.957752	0
Aeshnidae	5.658485	16.562070	4	1	23.53	0.86	3.576320	5.658485	10.380980	16.562070	17.213580	0.476	0.224	1.669105	0
Coenagrionidae	11.766550	13.271500	7	1	39.14	1.49	1.146810	3.228975	11.766550	13.784580	18.166080	0.744	0.478	1.966561	0
Gammarus	3.423285	3.423285	12	2	60.33	1.98	3.228975	3.228975	4.333951	13.163550	19.568636	0.800	0.556	2.122520	0
Cambaridae	16.562070	16.562070	8	1	47.06	1.79	6.173153	6.930784	9.702800	17.514570	17.514570	0.922	0.618	2.546811	0
Planorbidae	0.878840	0.878840	12	2	60.00	1.61	0.878840	0.878840	7.667466	22.642830	22.985095	0.790	0.558	2.263468	0
Physidae	3.423285	3.423285	17	2	76.85	1.03	3.216624	3.423285	13.892530	27.818715	30.914340	0.766	0.360	1.489602	0
Fossaria	18.166080	18.166080	14	2	73.91	2.54	3.225292	3.423285	11.874500	30.914340	30.914340	0.988	0.906	3.353497	2
Hirudinea	0.878840	9.761220	10	2	44.87	1.57	0.878840	0.878840	9.761220	21.690330	26.080720	0.750	0.582	2.376638	0

Figure 24: Raw TITAN output for 2022 macroinvertebrate and sediment data.