The Role of Macrophytes in Nutrient Retention in Swedish Two-Staged Ditches

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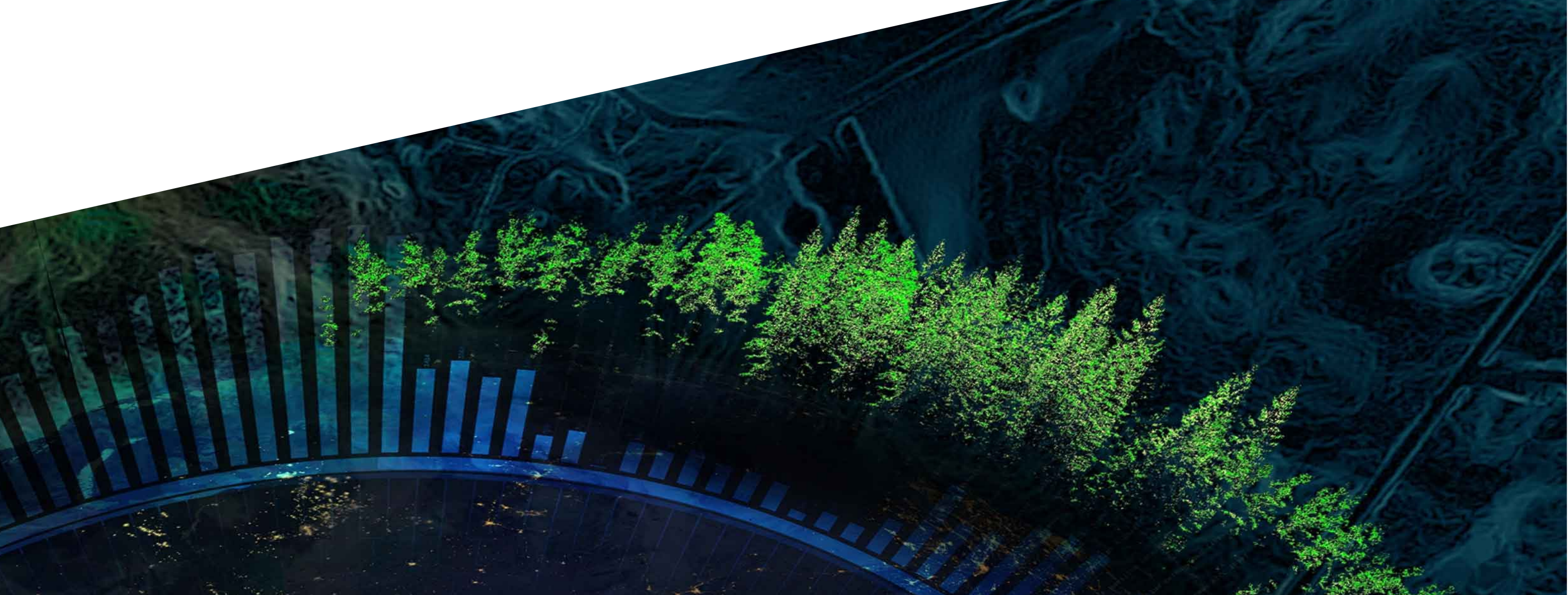

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Abstract

Eutrophication continues to be a persistent threat to the health of ecosystems globally and in the Baltic Sea region, causing a loss in ecosystems and industry profits. Measures have been researched and implemented to stop the spread of excess nutrients from agricultural sources, but innovative, cost-effective nutrient mitigation strategies are still needed to reach water quality targets.

Redesigning traditional trapezoidal ditches has been researched to offer alternative, sustainable methods of inducing water purification and biological physiochemical activity nearby agricultural fields. The two-stage ditch is a design that increases water retention by adding a flood plain buffer. This design encourages nutrient and carbon processing, including denitrification, sedimentation of particulate phosphate and sorption of phosphate among other processes. But generally, the role of ditch vegetation, particularly macrophytes, in promoting these processes is not well understood. Therefore, to improve this understanding of the role of macrophytes in ditch remediation, relationships between macrophyte diversity and abundance and water quality in two-stage ditches were studied.

This study was conducted in nine agricultural catchments where environmental factors (stream nutrient content, soil type, stream flow, land use, etc.) were investigated to explain macrophyte abundance and diversity during the summer growing period. An Ecological Quality Ratio was determined for each site and related to macrophyte diversity and water chemistry through the historical trends in total nitrogen and total phosphorus concentrations in each ditch. Macrophyte species that were most abundant at each site were then sampled for their total nitrogen and total phosphorus content and compared with the measured nutrient loads in their respective two-stage ditches.

The results showed that macrophyte communities commonly associated with eutrophic and mesotrophic aquatic environments, such as agricultural ditches, can be used as indicators for stream ecological quality that complement water chemistry measurements. Prevalence of certain macrophytes varied depending on the environmental characteristics, such as: ditch location and soil type and texture, but were heavily influenced by nitrogen and phosphorus availability. The total nitrogen concentrations in June of 2022 strongly negatively correlated with the macrophyte diversity recorded at that same time. The species identified for their nutrient retention capacity were: *Typha sp., Phragmites australis, Epilobium hirsutum,* and *Alisma plantago-aquatica.* Analysis of the August stream sample nutrient uptake data implies that species abundance and biomass affect water quality by decreasing nutrient availability in the water column and in turn are affected by water quality. Thus, harvesting them prior to decay is suggested as a remediation practice with potential for sustainable nutrient recycling, especially in two-stage ditches.

Keywords: Two-stage ditch, Eutrophication, Macrophytes, Ecological quality, Water Framework Directive, Streams, *Typha*, *Phragmites australis*

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Abbreviations

ALG	Cover of Green Filamentous Algae
BMP	Best Management Practice
BSAP	Baltic Sea Action Plan
CA	Correspondence Analysis
DCA	Detrended Correspondence Analysis
DO	Dissolved Oxygen
EQR	Ecological Quality Ratio
EU	European Union
HELCOM	The Baltic Marine Environment Protection Commission
N	Nitrogen
NFG	Number of Functional Groups
NH_3	Ammonia
NH_4^+	Ammonium
NMDS	Non-Metric Multidimensional Scaling
NO_3^-	Nitrate
NTAXA	Number of Macrophyte Taxa
P	Phosphorus
PO_4^{3-}	Phosphate
Q50	Median Flow
RMNI	River Macrophyte Nutrient Index
SD	Two-Stage Ditch
SDup	Two-Stage Ditch Upstream
SDmd	Two-stage Ditch Midstream
SDdn	Two-stage Ditch Downstream
SPC	Specific Conductance
TD	Traditional Trapezoidal Ditch
TP	Total Phosphorus
UKTAG	United Kingdom Technical Advisory Group
WFD	Water Framework Directive

1. Introduction

1.1 Background

Eutrophication continues to be a persistent threat to the health of aquatic ecosystems such as the Baltic Sea, causing a loss in ecosystem services and industry profits. Widespread hypoxic conditions alongside eutrophication by the excess of phosphorus and nitrogen fertilizers have over a long time plagued inland waterways and coastal receiving waters (Fölster et al. 2014). It is for this reason that surface water monitoring programs have been established in Sweden more than 50 years ago. Measures have been researched and implemented to stop the spread of excess nutrients at sources and delivery points, but innovative, cost-effective nutrient mitigation strategies are still needed to reach water quality targets.

One effective method is to modify agricultural ditches by adding a floodplain, known as a two-stage ditch. Macrophytes found on this floodplain and in the ditch's channel have the potential to retain nutrients and facilitate biogeochemical activity (Hallberg et al. 2022). Research such as those conducted by Greenway (2003) and Mebane et al. (2021) have already shown the remediating capabilities of certain emergent, surface, free floating, and submerged plants.

Current initiatives to reduce phosphorus (P) loads in catchments have in part been successful due to the implementation of best management practices (BMPs) at both nonpoint sources and transport (Jarvie et al., 2013). However, Jarvie et al. (2013) allege that stability and improvement of ecological status, have not been observably affected by these BMPs in many catchments studied in both Europe and North America. Thus, a holistic approach to evaluate the ecological status, such as the Ecological Quality Ratio (EQR), is suggested to play a stronger role in assessing BMPs success. EQR is a weighted measure of macrophyte diversity and hydromorphology. Here, its use is to assess the ecological status of waters more holistically, as observing nutrient reductions on its own can be misleading in the assessment of ecological status of water. As historic nutrient loads have lasting effects on macrophyte composition, diversity and hydromorphology must both be considered.

1.2 Aim and Objectives

The general aim of the study was to investigate the relationship between macrophyte diversity and biomass in two-stage ditches and water chemistry, to determine to what extent macrophytes have the capacity for nutrient retention and stream water quality remediation.

The first objective was to ascertain the variation and presence of macrophyte species within and between ditches and uncover which were most abundant. A macrophyte survey was conducted in nine two-stage ditches twice, first in June at the beginning of macrophyte growth and then again in August when macrophytes reach their maturity, to compare variability in presence and biomass. The second objective was to establish a relationship between macrophyte diversity and water quality, using commonly used indices: the Shannon Index, the Equitability Index and EQR. The third objective was to assess macrophyte impact on water quality by their uptake capacity of Total Nitrogen (TN) and Total Phosphorus (TP) for bioremediation. Analyzing sampled macrophytes biomass from the August excursion for their accumulated nutrient content, uptake was compared to the nutrient load of TN and TP transported through the ditches during the growing period.

It was hypothesized that stream water quality improves in areas of high macrophyte diversity and abundance, and that EQR can be established in Swedish two-stage ditches using their present macrophytes for the assessment of water quality. The specific questions this report answers are:

- what macrophyte species are present in the nine selected two-stage ditches and what factors drive their variation;
- can simple metrics for macrophyte diversity correlate and explain water quality patterns; and
- which of the present macrophyte species have the highest nutrient uptake capacity and could be used in stream remediation?

2. Literature Review

2.1 Eutrophication

Eutrophication is the overabundance of nutrients (nitrogen (N) and P) into water bodies (seas, lakes, rivers, streams, etc.), causing blooms of sessile and planktonic algae from eutrophic conditions to produce adverse effects in the aquatic ecosystem (EEA, 2016). Of nitrogenous and phosphoric nutrients, excess amounts of ammonia (NH_3), ammonium (NH_4^+), nitrate (NO_3^-), and phosphates (containing PO_4^{3-}) are of the most concern to ecosystem health (USGS, 2019). In different amounts each of these nutrients can cause health effects that further impact the community. Ammonia when mixed with water forms ammonium hydroxide which then splits into an ammonium and hydroxide ion acidifying the environment making it toxic to fish and other sensitive organisms (Sannö et al., 2003). This usually occurs when ammonium ions from deposited soil enter water in which ammonium nitrifies into nitrate. Phosphates can end up in water by multiple methods such as through household or industrial sewage and is not typically from agricultural land, though phosphate attached to soil particles could erode into water (EEA, 2016). The overabundance of these nutrients promotes the growth of algae that when decomposed by bacteria consumes dissolved oxygen, killing fish. Mats of algae growing on the water surface may also cause shading, limiting the ability for submerged macrophytes to photosynthesize.

2.1.1 Dangers to the Baltic Sea

As presented in chapter 20 of the Coasts and Estuaries text, a large percentage of nutrient input to the Baltic Sea derives from agriculture, wastewater treatment plants, aquaculture, storm discharge, and runoff (Heiskanen et al., 2019). Algal blooms caused by anthropogenic activities are noted to have appeared as early as the mid-1900s. As phytoplankton biomass accumulated and an increase of organic matter sedimented, changes to the ecosystem would encourage phosphorus internal loading from dead organic material. What would incur was an endless loop of internal nutrient loading that promotes the growth of cyanobacteria further facilitating anoxic conditions. Cyanobacteria, commonly found in freshwater and not as commonly in marine water, in high production has the potential to harm human health through the release of potent toxins (USGS, 2019).

Through the European Union (EU), measures have been implemented to reduce nutrient pollution in water bodies including the Nitrates Directive (1991), Urban Waste Water Treatment Directive (1991), Water Framework Directive (2000), and Rural Development Programme (2000). However, success has varied over the years with new obstacles and stresses hindering progress every year. To map the ecological status of the Baltic Sea over a 350-year period between 1850 and 2200, a research study was conducted to model eutrophication changes over time following scenarios in which the reduction targets in the Baltic Sea Action Plan (BSAP) were met (Murray, 2019). There results found that of the several sub-basins and transition zones that make up the Baltic Sea, it could take until 2090 for a majority to achieve a passable status void of eutrophication. Furthermore, good status for the Baltic Proper and Bothnian Sea were not expected to be achieved before 2200. The Gulf of Riga and Bothnian Bay were unlikely to meet the targets of the BSAP. However, these scenarios did not take climate change into account which continues to challenge measures intended to remediate ecosystems impacted by human activity. Despite setbacks, through socio-economic and ecological policy and action plans a great effort has been made by the EU and The Baltic Marine Environment Protection Commission (HELCOM) to curb nutrient loading trends (Heiskanen et al., 2019).

2.2 Swedish Macrophytes Geography

With much environmental variation across the Swedish streams and rivers, a wide array of macrophyte species inhabit these reaches. Of these habitats, the riparian zones are of the most species rich. (Rydin et al., 1999) The riparian zone is the terrestrial boundary between a river or stream and surrounding land. It is crucial to the influences of biodiversity and landscape ecology of inland water ecosystems. (Wittmann, 2022) All rivers and streams similarly deposit eroded material as well as organic and inorganic matter. Soil fertility and productivity increase in riparian ecosystems when nutrient rich, waterborne sediments are deposited. (Rydin et al., 1999). More varied vegetation is often commonly found within this zone than in the river or stream. The *Salix* species (commonly known as willow) dominate in the riparian forest among other shrub vegetation. Though permanently flooded parts of rivers and streams support various types of macrophytes, emergent vascular plants with long shoots classified as helophytes are the most common, especially in slower moving water reaches composed of fine sediments. Macrophytes are defined as aquatic vegetation including the likes of algae visible to the naked eye, bryophytes, charophytes, and vascular plants growing in or around water (Lindegarth et al., 2016). Helophytes, that reach from the sediment to above the water's surface, such as *Equisetum fluviatile*, are commonly found in both Swedish lakes and rivers (Rydin et al., 1999). An excerpt from the *Swedish Plant Geography* text further states that, "*Phragmites australis* and *Eleocharis palustris* [though] not common in the large rivers [do] often grow abundantly in small streams."

Moving towards macrophyte compositions in Swedish lakes, nutrient availability directly affects species composition of macrophytes. This is especially the case of phosphorus and nitrogen in lake water and bed sediments. Eutrophic and hypertrophic levels of nutrient loads have the means to provide macrophytes with optimal conditions to populate an entire area. (Rydin et al., 1999) However, even in lakes with enough nutrients to encourage substantial plant growth, submerged macrophytes are susceptible to limited growth in places of high turbidity. Although unfavorable conditions in lakes may hamper lake macrophytes from forming flowers and seed production, vegetative reproduction (or asexual reproduction) allows for species to populate. *Elodea canadensis*, an invasive pondweed introduced to Sweden from Canada, has been able to vastly spread across Sweden and Europe, despite only the female plant having been introduced. During winter, seed production cannot continue undisturbed, thus some species have developed winter tolerant buds that separate from the plant and rest on the sediment in waiting for the spring season. In reference to seed distribution, the *Swedish Plant Geography* text mentions that, "depth and sediment texture influence species distribution as well as species composition and regulates the number of seeds produced by an individual of a given species."

In places of lower altitudes by the Swedish coast, especially in the southern more agricultural regions, are reports of higher nutrient and electrolyte concentrations than what is found in oligotrophic lakes. *Typha angustifolia*, *Schoenoplectus lacustris* and *Phragmites australis*, fringe on eutrophic lakes, as plants needing a nutrient rich environment to thrive in. (Rydin et al., 1999) In many cases you will find species exclusive to eutrophic environments, with some only rarely appearing in nutrient poor habitats. These include emergent species such as: *Carex pseudocyperus*, *Cicuta virosa*, *Ranunculus lingua*, *Rumex hydrolapathum*, and *Sparganium erectum*. In addition, free floating species such as *Lemna minor, Hydrocharis morsus-ranae*, *Ricciocarpus natans*, and *Sirodela polyrrhizea* are often found between the emergent macrophytes.

What is not known is how diverse macrophytes in Swedish agricultural drainage ditches are, nor of a list of species typically found in them, much less in two-stage ditches. Patterns seen in other Swedish water bodies may occur similarly or drastically different across the studied environments. At this time there is no present literature to assist on this specific matter, therefore the above text on lake and river habitats was supplemented where appropriate for this study.

2.3 Macrophyte Nutrient Retention

The extent to which nutrient rich water is remediated, with respect to plant activity, heavily relies on the types of macrophytes present. Species of emergent, submerged, surface floating and free floating macrophytes each have a threshold in nutrient uptake availability, which can be pressured by the presence of excess nutrients (Mebane et al., 2021). In identifying these thresholds and the capabilities of certain macrophytes to remediate eutrophicated waters, many studies have researched both nutrient uptake in plants and how nutrients are stored within plant biomass (Greenway, 2003; Mebane et al., 2021). Research in wetlands in Queensland, Australia looking at the remediation capabilities of emergent, submerged, and floating macrophytes, and algae, saw that emergent macrophytes were most capable at amassing and storing nutrients per unit area of wetland (Greenway, 2003). This was due to their larger biomass, despite having a generally lower nutrient uptake of the group. Emergent macrophyte also had the added benefit of only being capable of removing nutrients from the sediment. All 63 endemic and 14 introduced species were reported to have removed nutrients from the water column. This suggests that a range of species was necessary for amplifying nutrient removal in wetlands.

Nutrient pollution remains a threat, however, as the death and decay of macrophyte species can release captured nutrients back into the environment (Barko & Smart, 1981). In Barko and Smart's work in *Aquatic Botany* (1981) though N and P may seep out in negligible quantities, significant amounts may excrete from the sediment where once growing emergent macrophytes have decayed. It is then important to identify and remove plants before death for remediation to properly take place in a water body. Pertaining to stream macrophytes, it was not known the extent to which they retain nutrients in agriculturally impacted streams. The remedial capabilities of macrophytes found in two-stage ditches were investigated, using the above literature to supplement the results of the research.

2.4 Ecological Quality Ratio

In this paper, EQR is a proxy for long-term water quality. Higher levels of EQR are observed in streams and catchments with lower anthropogenic impacts from agricultural land use (van de Bund & Solimini, 2007). The lower EQR value indicates on the other hand poorer biodiversity and/or higher environmental disturbance in the assessed area. EQR is classified as follows: bad (<0.2), poor (0.2-0.4), moderate (0.4-0.6), good (0.6-0.8), and high (0.8-1). (WFD-UKTAG, 2014).
EQR is calculated using a combination of macrophyte taxon abundance, hydromorphology and alkalinity data. This has previously been calculated in

streams in the UK, using United Kingdom Technical Advisory Group's (UKTAG) River LEAFPACS2 tool. This tool is a method for classifying macrophytes in river waterbodies in accordance with the Water Framework Directive (WFD) requirements (WFD-UKTAG, 2014). This is conducted by first identifying the macrophytes within a testing site and their coverage. Species cover % along 100 m survey lengths is classified as 1-9, corresponding to <0.1%, 0.1-1%, 1-2.5%, 2.5-5%, 5-10%, 10-25%, 25-50%, 50-75%, and >75% cover (Holmes et al., 1999). From these taxon cover classes four metrics are derived for further calculation of the overall ecological quality ratio (EQR). These metrics are river macrophyte nutrient index (RMNI), number of macrophyte taxa (NTAXA), number of functional groups (NFG), and cover of green filamentous algae (ALG). Raw EQR values are then calculated for each metric based on reference values, provided by the tool, and observed data collected from the field survey. Reference values and background data for specific taxa were obtained from the first River LEAFPACS iteration (Willby et al., 2012). These macrophytes are used in the tool as indicators for ecological quality as they are influenced by certain pressures including increased nutrient load in water, acidification, hydromorphological changes, etc. Once generated, the raw EQR values of EQR_{RMNI}, EQR_{NTAXA}, EQR_{NFG}, and EQR_{ALG} combine to make the LEAFPACS parameter, or $EQR_{LEAFPACS}$. Reference value calculations differ for the UK between rivers in Great Britain (England, Scotland, and Wales) and Northern Ireland. As Scotland is most like Sweden latitudinally and is not as influenced by coastal waters as Northern Ireland is, this project uses the calculations designated for Great Britain. The River LEAFPACS2 tool has been used in the development of an index for responses to hydrological changes in the Thames Basin, UK, by plant communities (Westwood et al., 2021), and a study utilizing the earlier LEAFPACS iteration for analyzing survey datasets in UK lakes for the classification filamentous algae in the ecological assessment of lakes (Kelly et al., 2016). In both cases results were conclusive within their respective usage of LEAFPACS and LEAFPACS2, thus adapting the tool for use in Swedish streams may be beneficial in the ecological assessment of two-stage ditches.

2.5 Traditional vs Two-Stage Ditches

Ditching has been a land use practice since medieval Sweden for the removal of excess surface water (Jakobsson, 2013). With respect to agricultural land, drainage ditches had to be dug out deeply and flatly for water to quickly traverse. Outlets into other waterways were necessary to prevent water stagnation. Through this practice, agricultural drainage ditches were typically shaped like a trapezoid along the route towards an outfall. However, despite this design's advantage of evacuating surplus runoff, there are flaws that contribute to the transport of nutrients to larger water bodies. Firstly, the lack of a riparian zone limits biogeochemical reactions with sediment, such as the sorption of phosphates and the denitrification (Hallberg et al., 2022). Moreover, the rapid movement of water entering and exiting a drainage ditch limits the time at which nutrients can be retained within the ditch. A design that can both reduce the flow of water and promote biogeochemical activity would subvert the inefficiencies of the traditional trapezoidal design.

A two-stage ditch is drainage ditch dug with one or two terraces, or floodplains, intended to foster higher levels of bioactivity compared to that of the traditional ditch, as seen in Figure 1. By adding a riparian area, runoff with dissolved nutrients from agricultural land passing through has the potential for retention restraining eutrophic ecosystems from developing (Trentman et al., 2020).

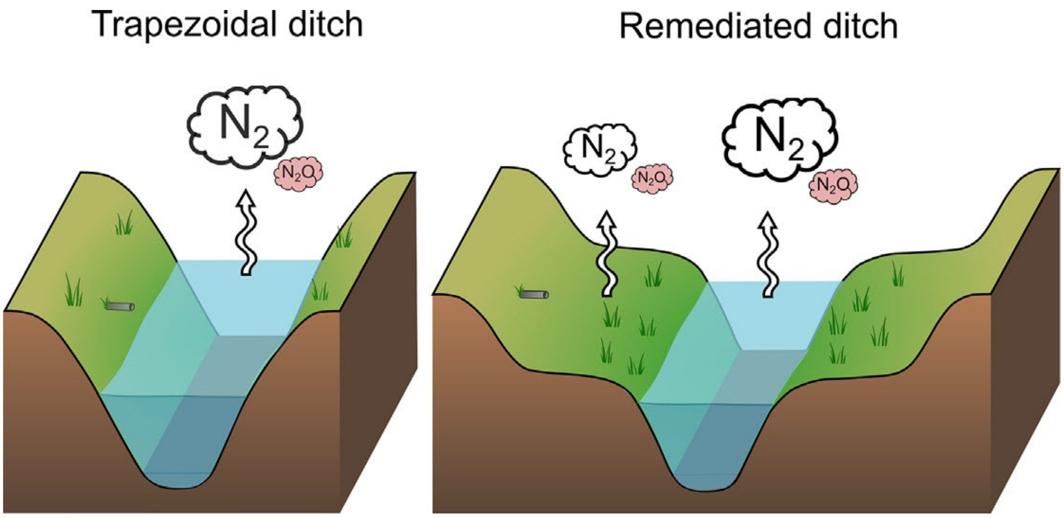

Figure 1. A diagram showing the structural differences of a traditional agricultural ditch and a two-stage ditch and their comparative effects on nitrification. Hallberg, L. (2022). Traditional vs Two-Stage. [illustration]. [2020-06-06] Used with the copyright holder's permission.

3. Methods and Materials

3.1 Site Descriptions and Catchment Details

The study was conducted within 9 agricultural headwater catchments with constructed two-stage ditches, located in the Central East Southern part of Sweden (Hallberg et al., 2022). Two-stage ditches were labeled as SD on the catchment maps while traditional ditches were TD. Study sites for two-stage ditches were referred to as SD1-SD10, respectively, and were regionally separated where SD1-SD4 were found in the Central East, and SD6-SD10 in the South (Figure 2). SD5 was not included in this study due to heavy dredging collapsing the terraces.

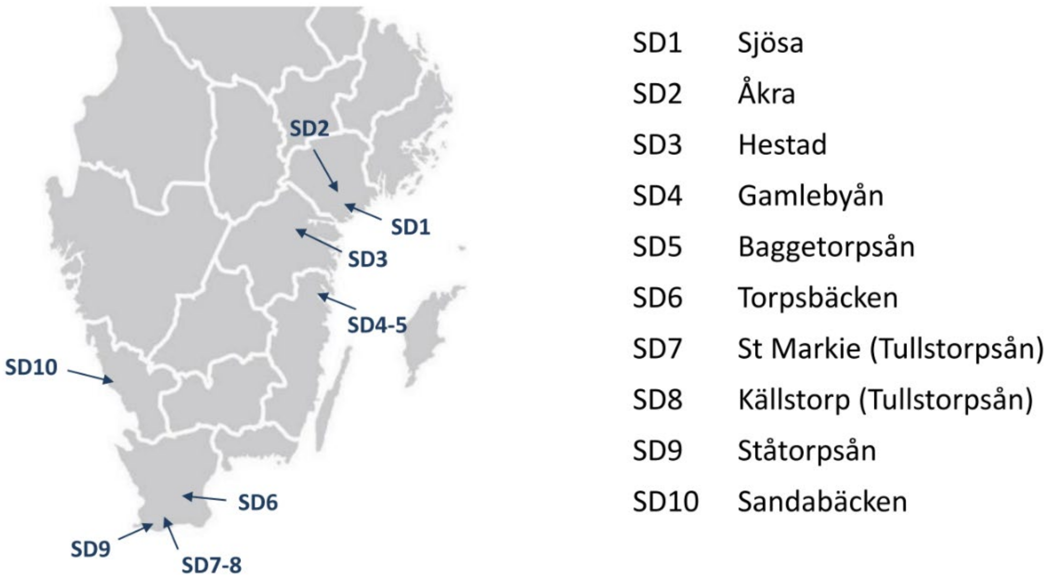

Figure 2. The locations of catchments with two-stage ditches in Sweden for this research project.

SD1 was a silty clay catchment 9.73 km^2 in area with only 16% agricultural land usage. SD2 was a catchment made up of silty clay loam, 7.91 km^2 in area with 27% agricultural land usage. With a catchment area of 7.11 km^2, SD3 was made up of 70% agricultural land with clay loam soil. SD4 had 35% agricultural land use, consisted of clay loam soils and was 8.12 km^2 in catchment area. At SD5, 38% of the clay loam soil was used for agriculture in the 16.32 km^2 area. Of the 13.09 km^2

area, SD6 had loam soil and was used primarily for agriculture (84% of land usage). SD7 comprised a 10.84 km^2 area of loam soil with 81% agricultural land use. At a size of 42.41 km^2, SD8 had a loam soil class, with 81% of the land being used for agriculture and was fed into from SD7's channel. 86% of the 31.02 km^2 SD9 catchment was agricultural land comprised of loam soils. Lastly, SD10's catchment had an area of 16.38 km^2, made up of sandy loam soils. 58% of SD10 was used for agriculture. Specific site details are found in the Appendix, Figures 18-26.

3.2 Surveying Methods

Research into macrophyte species that inhabit ditches in Sweden and their relation to nutrient removal remain underdeveloped. A lack of research methods for the surveying of ditch macrophytes resulted in the use of multiple established methodologies for lakes and rivers. Some references for the methodology were derived from the lake survey methods briefly mentioned in the Ecological Assessment of Swedish Water Bodies text, the UKTAG River Assessment Method and from work by the National Environmental Research Institute in Denmark. For this project, reference species of lake and river macrophyte were obtained from the "Ecological Assessment of Swedish Water Bodies" to help decide indicator species in ditches. These reference species were developed as lake indicators by their response to total phosphorus.

3.2.1 Field Surveying

For the field survey, vegetation identification and coverage were the focus. The presence of macrophytes was documented and their abundance quantified as a percentage within a plot. Presence refers to the existence of macrophyte species found at a site, while abundance is the amount of a present species.

Three plots for assessing macrophyte coverage and species identification were set up in the upstream, midstream, and downstream sections of each two-stage ditch for both terraces and streams, totaling 18 plots (9 on the terrace and 9 in the stream). Within each section, plots were assigned arbitrarily by placing markers as close to equidistant from each other in Google Earth as they could be marked. At each site the preset pins were followed until reaching the location directly over the pin. From there a corner of the terrace sample plot would get marked with a red stick. As the pegs were not tall enough to stay submerged in the streams, stream plots were designated from the banks a meter into the stream from their corresponding terrace spot. Plots were marked as 1m x 1m (1m^2) squares using a folding meter stick, each side measuring out to one meter. As the stream cross section for the plots seldom spanned two meters, 1m^2 plots were appropriate. Figure 3 shows the following design for the survey.

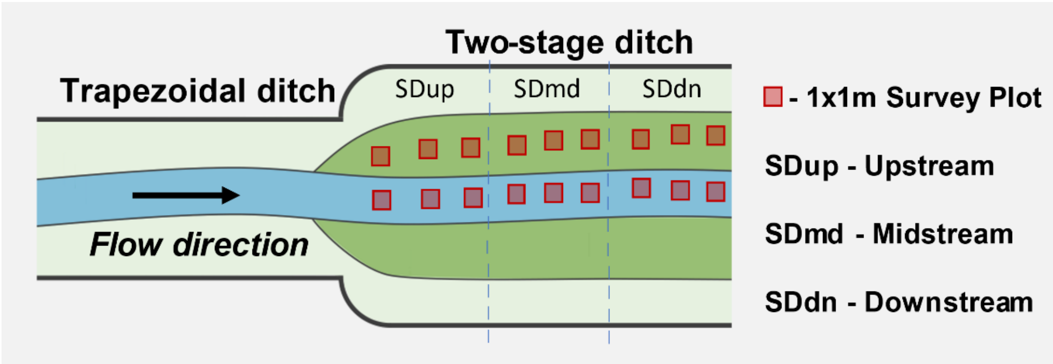

Figure 3. Survey design for macrophyte coverage in two-stage ditches. Nine plots were set in both the terrace and the stream (3 in the upstream, midstream, and downstream).

Within each plot, an estimate was recorded off observing the coverage of vegetation and organic litter by percentage. Total coverage was segmented into categories of present macrophyte, and further percentages were given to each identified species. These categories included: reed species, lower grasses, herbs, bryophytes, organic litter, bare soil, and woody vegetation for the terrace plots, and emergent macrophytes, floating macrophytes, submerged macrophytes, filamentous algae, and bare sediments for stream plots. For measuring algal coverage, I assumed coverage by area of algal spread, not by its depth.

Due to the difficult nature of identifying algae, and the constraints on time, all present algae were classified as either being unidentified filamentous algae, or brown algae. Grass was also difficult to identify the individual species, therefore presence would be marked down as either unidentified grass or their accurately identified species name. I used a digital dichotomous key application called PlantNet to identify the macrophytes in my plots (Affouard et al., 2020). Identification was made possible using the PlantNet tool that uses citizen science and AI deep learning to identify a picture of a plant with relative accuracy. It was decided that results with a percentage higher than 60% would be confirmed without question. In cases where percentage were between 30% and 60%, a more detailed inquiry of the application was used to get closer to a confidence of 60%. At times the specie with the highest percentage was still close in percent to the second highest specie possibility. Therefore, additional photos and a more detailed inquiry were provided to the application. In other instances, a species name could not be provided due to the closeness in percent the resultant choices were, thus the use of the genus name followed by "sp." was used instead.

Using the same plot locations from June's vegetation survey, vegetation coverage and identification was conducted again in August. Returning to each plot as marked on the gps and with a peg, coverage was recorded using the same tools as in June for a comparison of the two months.

3.2.2 Calculating Ecological Quality Ratios

To capture the effects of the two-stage ditches on EQR, the surveying area was modified from 100 meter stretches to shorter distances. Other data requirements for the overall calculation of EQR were alkalinity (mg $CaCO_3$ L^{-1}), altitude of source (m), distance from source (m), and slope (%) (WFD-UKTAG, 2014). The altitude of source is the altitude of the headwater spring in the catchment. This data was provided from previously collected measurements at each of the research catchments and two-stage ditch sites. Alkalinity values were only available for sites SD8 and SD9, so SD9's lower value of 450mg $CaCO_3$/L was used for each site as a standard.

In the River LEAFPACS2 excel tool, using scale C of the MTR Species Covers Value class scales, 1-9 was entered for all present macrophyte and algae. In cases where labelled species were not observed, the cell was left blank since confidence of no presence requires substantial inventory effort. Once all observed values were provided, the River LEAFPACS2 tool calculated RMNI, NTAXA, NFG, and ALG. Images of the tool during this process can be seen in Appendix, Figures 27 and 28. These metric values were then copied over from the River LEAFPACS2 survey calculator to the classification calculator, where EQR values were generated using additional site data metrics (slope (m/km), distance from source (km), altitude of source (m), and alkalinity ($CaCO_3$ mg/L)).

Due to the limitations of the LEAFPACS2 tool for Swedish waterbodies, some final EQRs could not be calculated automatically. Fitting each of the sites' reference EQR (adjusted RMNI EQR) and their final EQR values to a scatter plot, a trendline was set and the associated equation was used to solve for the missing final EQRs. To predict what a final EQR could look like for the missing sites, those scores were calculated using adjusted RMNI EQR, represented as x, and the generated equation to solve for y.

3.3 Sampling Methods

The sampling of fresh weight and dry weight biomass of the most abundant species found at each site follows the procedures for assessing biomass by the University of Idaho College of Natural Resources. The species chosen made up the three most abundant species identified in the June survey. Dry weight was then converted to biomass per area and compared among the instances of the same species in multiple catchments.

3.3.1 Field Sampling

To obtain the dry weight biomass of the three most abundant species for each ditch, samples were collected twice, once in the upstream and once in the downstream section. Samples were collected above sediment in square sections measuring 25 x 25 cm. In the channel, the three most dominant species at each site were collected just above the sediment. Samples were then bagged in their appropriately marked paper bag. As some previously dominant species in June were absent in the August survey, they could not be sampled. In total, 35 stream samples were collected and analyzed.

In taking fresh weight in lab, sediment was cleaned off the plant samples with tap water and blotted dry with a paper towel. Samples were then measured on a scale in the bag after weighing the bag and zeroing the scale to obtain the plant sample's weight. For dry weight, samples were placed side by side in a drying oven while still in their respective paper bags. At 60°C the samples dried in the oven, measuring weight every 24 hours until a consistent number could be recorded twice.

After drying, the samples were milled finely and analyzed for their total nitrogen and total phosphorous uptake. The data returned for TN and TP were then extrapolated across the length of entire streams or reaches.

Figure 4. Stream macrophyte sampling of Alisma plantago-aquatica at SD2.

3.4 Data Analyses

To assess the diversity of macrophytes per site, the Shannon Diversity Index and Shannon Equitability Index were calculated and compared. The Shannon Diversity Index is a measurement of species diversity used in ecology. (Konopinski, 2020) For this project it was used to measure the diversity of species for each two-stage ditch site.

$$H = -\Sigma pi * ln(pi)$$

To normalize the Shannon Diversity Index value between 0 and 1, the Shannon Equitability Index, which measures species evenness within a community, is divided by the natural log of total species (Ortiz-Burgos, 2016).

$$EH = H / ln(S)$$

To test if plant diversity and equitability differed between the two sampling times (June and August) a t-test was conducted. Rejecting the null hypothesis means that a statistical significance in evidence says that either species diversity and equitability is different between June and August, accepting the alternative. By contrast, if the null hypothesis fails to be rejected, significant statistical evidence was found to suggest that diversity or equitability is not different between the two months.

To test if the generated EQR values were negatively related to the nutrient concentration of stream water, a linear correlation was performed. A linear correlation was also used to measure the relationships between June diversity, equitability, and final EQR with the average nitrate nitrogen (NO_3-N) and phosphate phosphorus (PO_4-P) concentrations in stream water and average flow obtained from the two-stage ditch sites' June 2022 hydrochemical data. This short-term analysis was then repeated for August diversity, equitability, and final EQR results and the August 2022 hydrochemical data. In the test, an R-value is generated between -1 and 1 to identify the strength and magnitude of correlation between a pair (Williams et al. 2020). A value of -1 means a total negative correlation, 0 means no correlation, and +1 a total positive correlation. The p-value is also presented, where a value less than 0.05 means that the null hypothesis, true correlation is equal to 0, is rejected and that the alternative hypothesis, true correlation is not equal to 0, is accepted.

For visualization of the raw collected data, graphs were produced in R version 4.0.3 (RStudio Team, 2020) using the package ggplot2. For visualizing the relationship between species presence and their respective environmental factors detrended correspondence analysis (DCA) and a non-metric multidimensional scaling (NMDS) were used. Environmental factors refer to the soil type, stream turbidity, average flow, dissolved oxygen concentration, site location, agricultural land usage, pH concentration, stream slopage, and specific conductance data for each two-stage ditch collected in June of 2022. A DCA was chosen since detrending the bell shape curve produced by correspondence analysis (CA) of sampled data (Buttigieg & Ramette 2014:543-50).

"[NMDS] is an indirect gradient analysis approach which produces an ordination based on a distance or dissimilarity matrix…. NMDS attempts to represent, as closely as possible, the pairwise dissimilarity between objects in a low-dimensional space (Buttigieg & Ramette 2014:543-50)." The axises were arbitrary and ordination is related to a dissimilarity matrix.

The effects of water quality on macrophytes can be delayed and long-term. Thus, to account for potential time lags, long-term historic nutrient data was assessed and compared with current data collected from both field surveys in 2022. June and August EQR were linearly regressed against average concentrations of NO_3–N and PO_4–P in each two-stage ditch site across the summer and winter months of 2020 to 2022 to compare historical trends. Calculations for determining assimilated nutrient mass for the full reaches were conducted in Excel, by multiplying estimated nutrient uptake of plant species per area sampled by the distance of the two-stage ditch per study site. To normalize the data on uptake potential by the area sampled, as opposed to the full reach, plant dry weight was converted from grams to kilograms and multiplied by the total nitrogen and phosphorus concentrations per kilogram for each sample to find the amount of total nutrient uptake. The results were presented as a box and whisker plot comparing the nutrient uptake amount among each study site and between the sampled species.

Lastly, loads of total phosphorus and dissolved inorganic nitrgoen (DIN) in stream water between the 2022 April and August growth periods were calculated with the flow weighted average concentration method by their downstream concentrations and continuous flow (Elwan et al., 2018). The equation is expressed as

$$\mathrm{Load} = \mathrm{mQ_t}\left(\frac{\sum C_i * Q_i}{\sum Q_i}\right) \tag{1}$$

where C_i is contaminant concentration (mg L-^{1}) measured at the *i*th day, Q_i is flow mean daily flow (L s^{-1}) measured at the *i*th day, Q_t total flow of measured period (L time period^{-1}) and *m* is a unit conversion factor.

4. Results

As the focus of this project is on instream macrophytes, floodplain macrophyte data is presented in Appendix, Tables 6-23. Data on instream macrophyte percentage coverage is presented in Appendix, Tables 24-25.

4.1 Objective 1 – Macrophyte Diversity and Abundance Against Environmental Factors

Macrophytes were identified and documented for diversity using established plots, and percentage coverages were taken for macrophyte abundance during two surveys in June and August 2022. The results at each individual site were a normalized representation of the macrophyte coverage found across the upstream, midstream, and downstream locations. Plots typically contained 5-10 individual species of macrophytes with no more than 10 individuals present within a 1m^2 plot.

In June, *Phragmites australis* was the most abundant species of the nine sites at 20.1% coverage, while algae made up the second most abundant species at 18.9%, and *Typha spp.* the third most with 17.9%. The diversity of macrophyte species averaged around 8 different species per site with SD2 (13 species) and SD9 (12 species) seeing the most diversity. SD10 was the least diverse stream with only three identifiable species found. There, the highest percentage coverage of any plant belonged to *Phragmites australis* at approximately 95.8% coverage. Weighing the environmental factors and nutrient content from site data with macrophyte presence and variation, the likely interrelations can be seen in the NMDS, Figures 5 and 6.

From interpreting the NMDS in Figure 5, the species *Alisma plantago-aquatica*, *Carex rostrata*, *Eleocharis palastris*, *Elodea*, *Galium palustre*, *Lemna* and *Veronica scutelleta* grow more on clay soils in turbid waters at a lower pH (a pH of less than 6). However, as the ordination of the species were related to abundance and not to presence, these species can still be present in different conditions. Instead, the NMDS would suggest that at the research sites, species were more abundant under these circumstances and may find these environmental conditions optimal for their growth and spread.

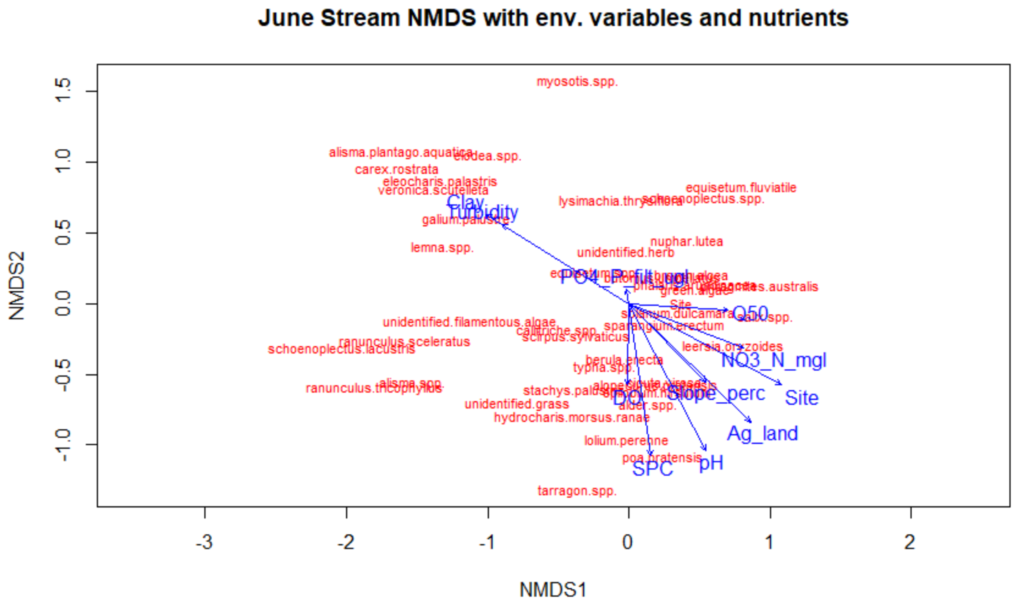

Figure 5. NMDS of stream macrophytes present in June showing their relation to environmental factors and nutrients (Missing SD3). The listed environmental factors were clay soil type (Clay), stream turbidity, (Turbidity), average flow (Q50), dissolved oxygen concentration (DO), site location (Site), agricultural land usage (Ag_land), pH concentration (pH), stream slopage (Slope_perc), and specific conductance (SPC). Vectors representing stream nutrient uptake were filtered phosphate in micrograms per liter (PO4_P_filt_ugl) and nitrate in miligrams per liter (NO3_N_mgl).

In Figure 6, each polygon in the NMDS represents a two-stage ditch site and species abundance. The species far away from these polygons do not indicate absence at those sites. Instead, its distance means an unlikelihood that that species would be found at a site. Of the investigated sites, SD2 saw the most instances of *Alisma plantago-aquatica*, *Elodea sp.*, *Carex rostrata*, *Eleocharis palustris*, *Galium palustre*, *Lemna sp.*, and *Myosotis sp.* SD1 had a larger concentration of *Ranunculus sceleratus*, *Ranunculus tricophyllus*, *Alisma sp.*, and *Schoenoplectus lacustris*. All other species were common amongst SD4, SD6, SD7, SD8, SD9, and SD10.

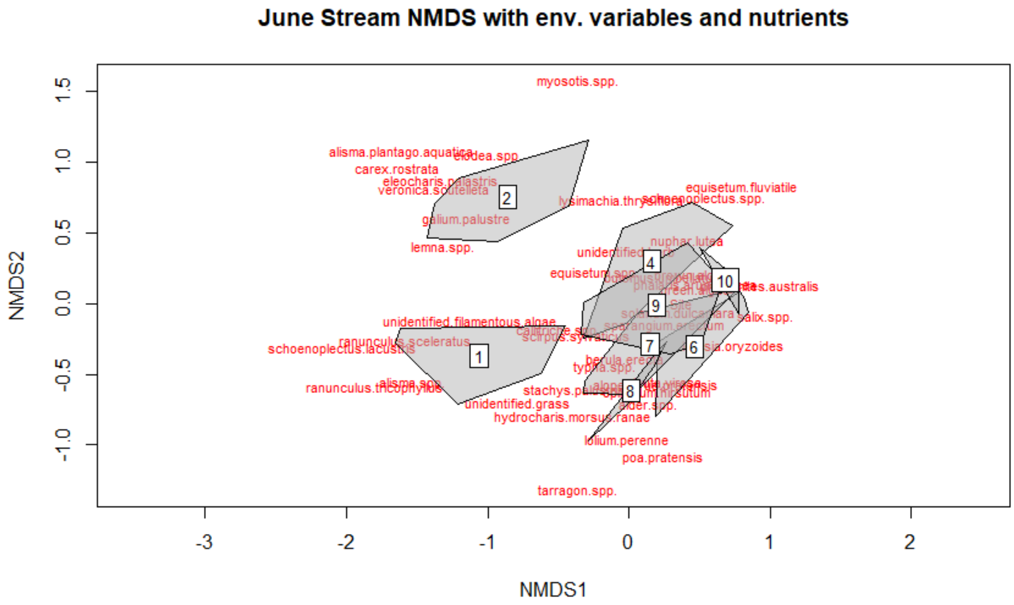

Figure 6. NMDS of stream macrophytes present in June and their abundance with respect to the sampled sites (Missing SD3).

Returning in August to the plots previously marked, macrophyte presence and percent coverage were again documented. The three most abundant stream macrophytes were *Phragmites australis* at 16.04% coverage, *Typha* with 15.96%, and unidentified grass with 11.3%. Like with the case with identifying algae, grass was difficult to properly identify under time constraints, and with the time allotted, only the family *Poaceae* could confidently be identified. *Phragmites australis* had very high coverage in SD6 and SD10. However, *Typha* was present at all sites, excluding SD2, and overshadowed *Phragmites australis*'s thin coverage in SD4, SD7 and SD9. *Typha* only follows *Phragmites australis* in count by a few digits. Furthermore, *Typha* was the only species in August to be seen at more than 5 of the study sites.

The results of the NMDS in Figure 7 show the August species as they relate to one another fitted against environmental factors. Species that were found more in clay soils and turbid waters not commonly found in areas of high agricultural land usage were *Alisma plantago-aquatica*, *Lemna sp.*, *Elodea nuttalli*, *Sparganium sp.*, *Schoenoplectus sp.*, *Equisetum sp.*, and *Hydrochaeris morsus-ranae*. Species typically found in areas of higher DO, SPC and pH were *Filipendula ulmaria*, *phalaris arundinacea*, other unidentified grasses, *unidentified bryophytes*, and *Equisetum sp*. Similarly, *Typha* appears to grow mostly in areas of high DO, but also in low nutrient environments. It is important to note that the ordination of each species does not particularly indicate the ability to grow in the prescence of other species, but instead that there was a tendency towards growing under certain environmental factors that may be seen by various species from multiple catchments. Hence the overlap of species seen in Figure 8.

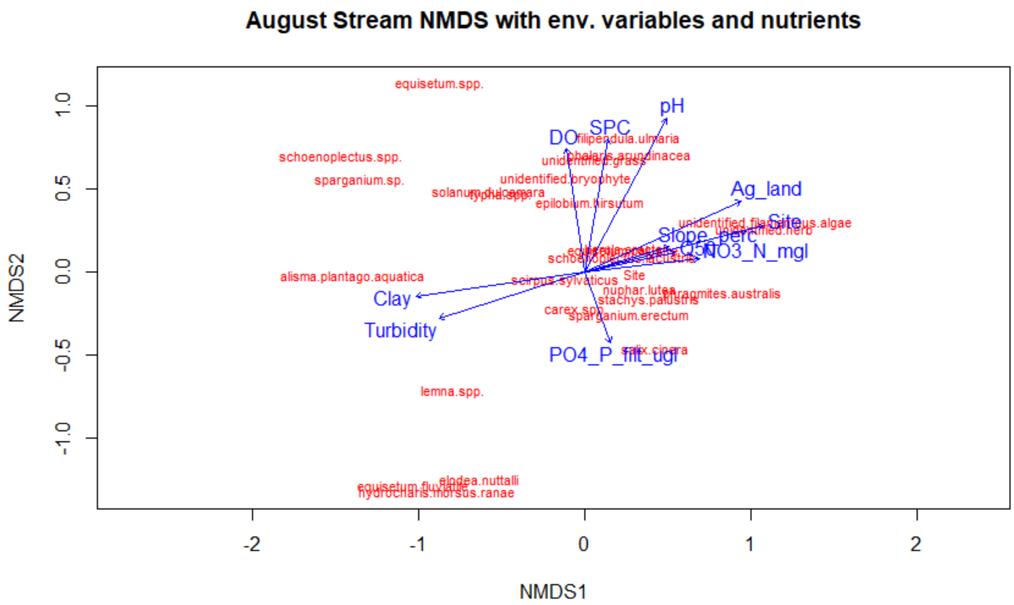

Figure 7. NMDS of stream macrophytes present in August showing their relation to environmental factors and nutrients (Missing SD3). The listed environmental factors were clay soil type (Clay), stream turbidity, (Turbidity), average flow (Q50), dissolved oxygen concentration (DO), site location (Site), agricultural land usage (Ag_land), pH concentration (pH), stream slopage (Slope_perc), and specific conductance (SPC). Vectors representing stream nutrient uptake were filtered phosphate in micrograms per liter (PO4_P_filt_ugl) and nitrate in miligrams per liter (NO3_N_mgl).

SD1 saw a large concentration of *Equisetum sp.*, *Schoenoplectus sp.*, *Sparganium sp.*, and *Alisma plantago-aquatica* (Figure 8). In SD2, *Alisma plantago-aquatica* was also common as well as *Lemna sp., Elodea nutalli*, *Equisetum fluviatile*, and *Hydrocharis morsus-ranae*. All other species were commonly found across the remaining sites.

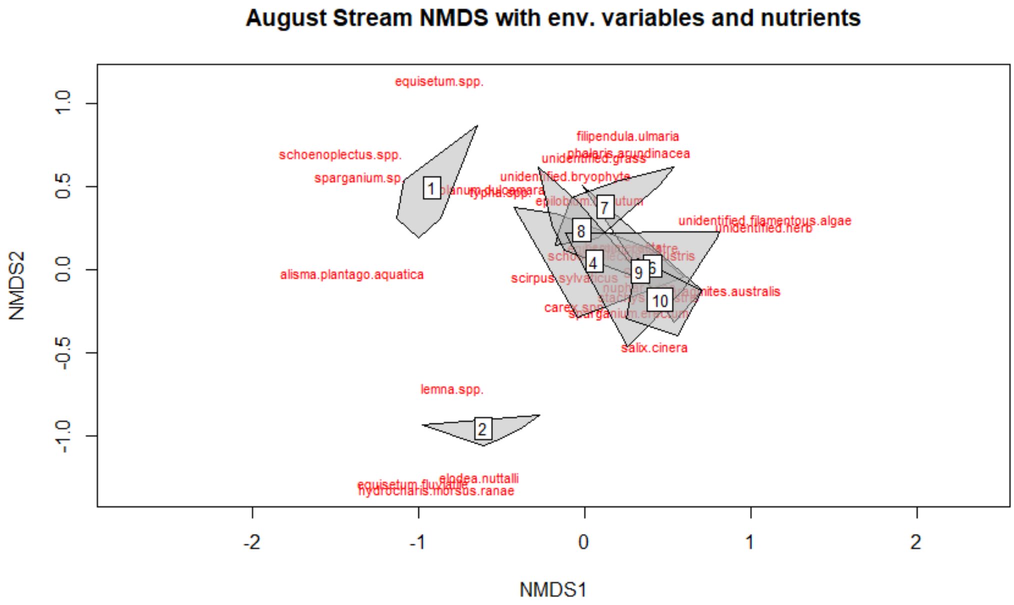

Figure 8. NMDS of stream macrophytes present in August and their abundance with respect to the sampled sites (Missing SD3).

4.2 Objective 2 – The Relationship Between Macrophyte Diversity and Water Quality

Continuing into the second objective, water quality represented by environmental factors (flow (Q50), NO3-N and PO4-P concentrations) obtained from the June and August surveys were compared against metrics for macrophyte diversity. Using the Shannon Diversity Index and Shannon Equitability Index, diversity and equitability of the research sites were identified and compared between the two survey months. The results provided numerical values depicting the quantitative similarities and variations between each site in June and August from each other.

The Shannon Diversity Index showed a varied representation of stream macrophytes over the course of June and August. In SD1, SD3, SD8, SD9, and SD10 species diversity increases over time, with the SD9 seeing the lowest increase and SD10 seeing the highest increase. In June, the Shannon Diversity Index for SD9 was 1.88, increasing only to 1.90 in August. SD10, however, increased from 0.20 in June to 1.06 in August. This being the case from *Phragmites australis* making up most of the coverage in SD10, being only one of three of the recorded stream species in June, and of 6 species in August. For SD2, SD4, SD6 and SD7, there was instead a decrease in diversity. The biggest decrease was seen between June and August of SD7 with a Shannon Diversity Index of 2.01 to 1.57. The smallest decrease was seen in SD4 from 1.76 in June to 1.71 in August.

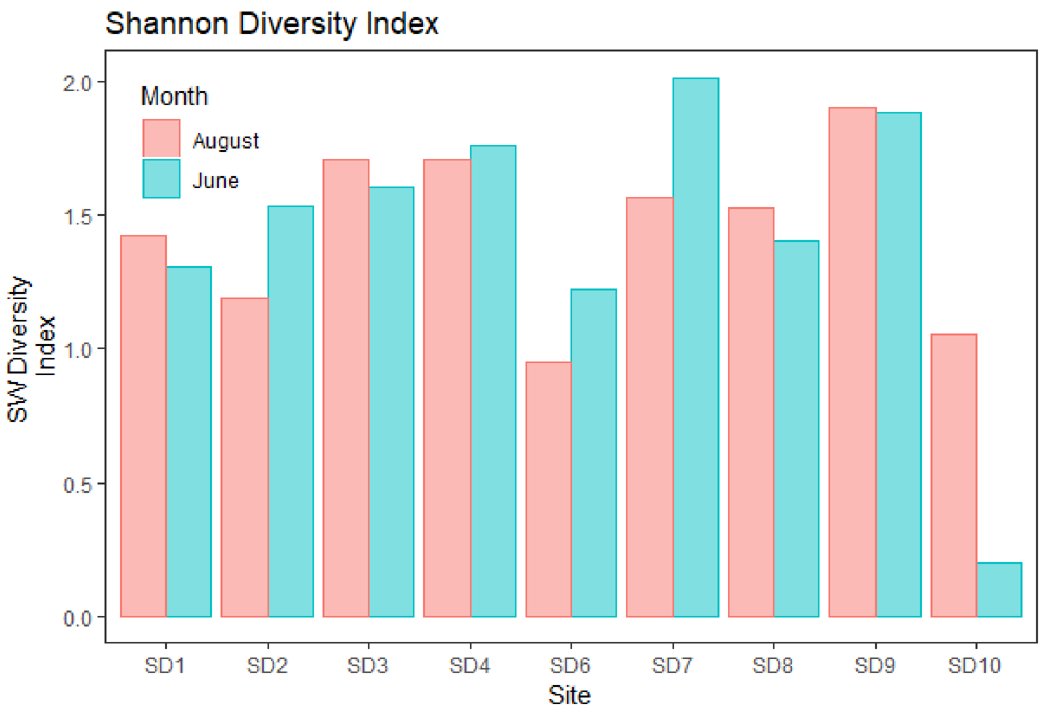

Figure 9. The Shannon Diversity Index was taken for each two-stage ditch site, compared with one another between June and August. A diversity value closer to 0 means a lack of diversity.

Equitability was shown to have increased in all catchments from June to August except for in SD4 and SD7. The largest increase was seen at SD10 with a Shannon Equitability Index of 0.07 in June and 0.18 in August. The smallest increase was at SD3 with values of 0.20 and 0.21 in June and August. SD4 and SD7, as with diversity, decreased in equitability with the larger decrease between June in August being about 0.007 in SD4 and 0.006 in SD7.

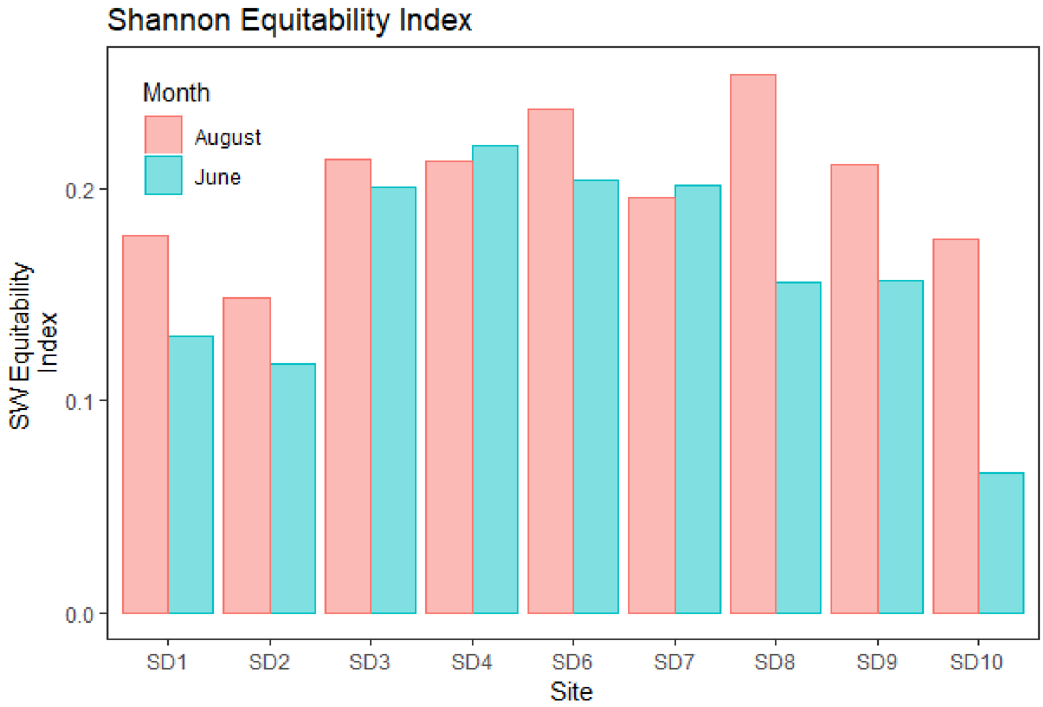

Figure 10. The Shannon Equitability Index compares each of the two-stage ditch sites between June and August. A diversity value closer to 1 means high similarity in abundance of the different species.

What was observed in the t-test for comparing the diversity means in June and August in Table 1 was a p-value greater than 0.05. Given this, there was no sufficient evidence to suggest that the means were much different between the two months.

Table 1. A t-test of the Shannon Diversity Index for species diversity between June and August.

t-Test: Paired Two Sample for Means		
Shannon Diversity Index		
	June	*August*
Mean	1.436745	1.448159
Variance	0.28409	0.103751
Observations	9	9
Pearson Correlation	0.704147	
Hypothesized Mean Difference	0	
df	8	
t Stat	-0.0896	
P(T<=t) one-tail	0.465405	
t Critical one-tail	1.859548	
P(T<=t) two-tail	**0.930809**	
t Critical two-tail	2.306004	

However, when testing the equitability of species, as seen in Table 2, the null hypothesis was rejected as a p-value of 0.02 was calculated. Thus, there was significant sufficient evidence to suggest that equitability means in the months June and August were different.

Table 2. A t-test of the Shannon Equitability Index for species evenness equitability between June and August.

t-Test: Paired Two Sample for Means		
Shannon Equitability Index		
	June	*August*
Mean	0.161574	0.203242
Variance	0.002546	0.001058
Observations	9	9
Pearson Correlation	0.579933	
Hypothesized Mean Difference	0	
df	8	
t Stat	-3.03155	
P(T<=t) one-tail	0.008136	
t Critical one-tail	1.859548	
P(T<=t) two-tail	**0.016271**	
t Critical two-tail	2.306004	

EQR as a metric using diversity to quantify water quality was calculated in the River LEAFPACS2 tool. The percentage coverage of each macrophyte species documented during the survey were weighted against one another and scaled using the MTR Species Covers Value class scale in the Excel tool, as described in the methods. After plugging in the observed values and required environmental parameters for each research site, the LEAFPACS2 tool provided the following June and August results. Over half of the tested sites in June were of less-than-good quality in accordance with the WFD. The worst results were for sites SD6, SD8 and SD10 resulting in the 0.148, 0.017, and 0.176 final EQR values seen in Table 3. In poor quality was SD1 (0.207) and SD3 (0.384). SD2 had a moderate class rating at 0.538, and SD4 (0.660), SD7 (0.659) and SD9 (0.604) were of good ecological quality.

Table 3. Final EQR for the 9 SD sites in June. A boundary class was determined from the results of the River LEAFPACS2. Boundary classes were represented as being either bad, poor, moderate, good, or high.

Water Body	SITE_ID	SAMPLE_ID	SAMPLE_DATE	**final_EQR**	***CLASS***
Sjösa	SD1		13-Jun-22	0.207	***Poor***
Åkra	SD2		13-Jun-22	0.538	***Moderate***
Hestad	SD3		13-Jun-22	0.384	***Poor***
Gamlebyån	SD4		14-Jun-22	0.660	***Good***
Torpsbäcken	SD6		14-Jun-22	0.148	***Bad***
St. Markie (Tullstorpsån)	SD7		15-Jun-22	0.659	***Good***
Källstorp (Tullstorpsån)	SD8		15-Jun-22	0.017	***Bad***
Ståtorpsån	SD9		15-Jun-22	0.604	***Good***
Sandabäcken	SD10		16-Jun-22	0.176	***Bad***

From the August survey, sites SD1 (0.364), SD2 (0.328), SD3 (0.295), SD7 (0.209), and SD8 (0.205) were of poor ecological quality, as seen in Table 4. SD6 and SD10 showed bad quality at 0.173 and 0.185, respectively, yet had both improved slightly compared to in June. SD9 (0.433) was the only site with a moderate class rating, while SD4 (0.693) was classified as good. Over the course of the growing season, ecological quality at SD2, SD3, SD7, and SD9 worsened with the sharpest decrease being that of SD7. SD8 improved the most out of the sites that did improve; SD1, SD4, SD6, SD8 and SD10.

Table 4. Final EQR for the 9 SD sites in August. A boundary class was determined from the results of the River LEAFPACS2. Boundary classes were represented as being either bad, poor, moderate, good, or high.

Water Body	SITE_ID	SAMPLE_ID	SAMPLE_DATE	final_EQR	CLASS
Sjösa	SD1		2-Aug-22	0.364	Poor
Åkra	SD2		2-Aug-22	0.328	Poor
Hestad	SD3		2-Aug-22	0.295	Poor
Gamlebyån	SD4		3-Aug-22	0.693	Good
Torpsbäcken	SD6		3-Aug-22	0.173	Bad
St. Markie (Tullstorpsån)	SD7		4-Aug-22	0.209	Poor
Källstorp (Tullstorpsån)	SD8		4-Aug-22	0.205	Poor
Ståtorpsån	SD9		4-Aug-22	0.433	Moderate
Sandabäcken	SD10		5-Aug-22	0.185	Bad

A correlation test was produced to find a relationship between flow, NO_3-N and PO_4-P concentrations, with diversity, equitability, and final EQR (the response variables) recorded from the month of June 2022. Only nitrate concentrations and diversity were significantly correlated. With an R-squared value of 0.69, and a multiple R-value of -0.83 seen in Table 5, macrophyte diversity and stream nitrate concentrations have a strong negative correlation. The linear regression for it is found in Appendix, Figure 36. The same correlation test was reproduced using the August 2022 data for flow, NO3-N and PO4-P concentrations, diversity, equitability and EQR, but no statistical significance was found in any of the correlations. That table is found in Appendix, Table 29.

Table 5. Correlated test between environmental factors (June Q50, and June 2022 NO3-N and PO4-P concentrations) and response variables (June Shannon Diversity, Shannon Equitability, and EQR). The test looked at stream values available for each SD site, excluding SD3.

	Response variable											
	Shannon Diversity				Shannon Equitability				EQR			
Factors	Multiple R	R Square	p	df	Multiple R	R Square	p	df	Multiple R	R Square	p	df
Q50	0.15	0.02	0.73	6	0.18	0.03	0.67	6	0.20	0.04	0.64	6
NO3-N	**0.83**	**0.69**	**0.01**	6	0.42	0.17	0.30	6	0.51	0.26	0.19	6
PO4-P	0.54	0.30	0.16	6	0.36	0.13	0.38	6	0.39	0.15	0.34	6

To account for potential time lags in the nutrient concentration data collected in the 2022 June and August surveys, a scatterplot was generated to find a relationship between June and August EQR and the average water quality concentrations from historical data between 2020-2022. EQR in August and NO_3-N averages across the summer months of 2020-2022 were significantly correlated in the linear regression seen in Figure 11. However, EQR in August was not correlated to PO_4-P concentrations in either winter or summer months. All other comparisons between the past summer and winter averages of NO_3-N and PO_4-P concentrations between the years 2020 and 2022 showed no correlation between EQR and stream nutrient concentrations (Appendix, Figure 29-35).

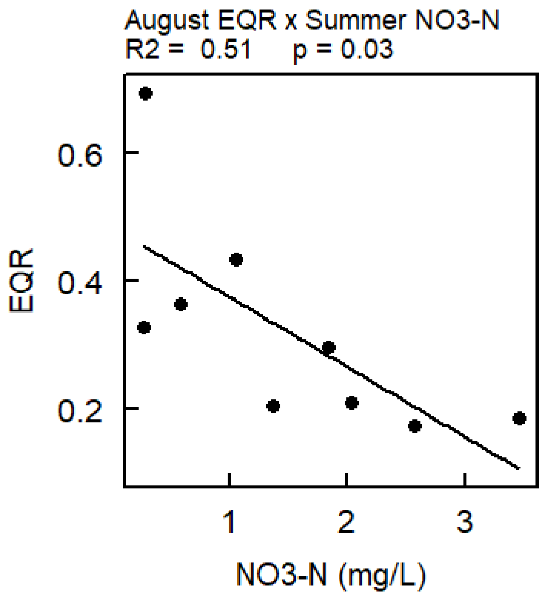

Figure 11. A scatterplot depicting the relationship between the August final EQR results and the average NO3-N concentration from the summers of 2020-2022 at all two-stage ditch sites.

4.3 Objective 3 – Most Suitable Bioremediators in Two-Stage Ditches

The last objective of the study was to ascertain which of the present macrophytes had the highest likely nutrient uptake capabilities for potential use in stream remediation. A sampling of the most dominant macrophytes was conducted during the August survey. Of the collected samples, SD10 accumulated the most biomass for a single species found within a 0.20 m^2 area in the upstream (4.68 kg m^2) and downstream (4.20 kg m^2), whereas SD2 had the least amount collected. Of all the macrophytes sampled, the *Elodea sp.* in SD2 had the least biomass per area at 0.12

kg m^2 in the upstream and 0.18 kg m^2 in the downstream. *Phragmites australis* and *Typha* were consistent in having the most biomass per area of the sample sites. By finding dry weight of the biomass collected, the samples were analyzed for their TN and TP concentrations. The full results of the biomass sampling are found in Appendix, Table 30.

TN mass in macrophytes was estimated for entire reaches of the two-stage ditches. The sum of all recorded macrophytes at each two-stage ditch site reveals that the highest mass of TN was in SD10 for the entire reach. SD8 and SD7 had the next highest uptake while SD1 and SD6 had the lowest recorded TN mass of the reaches. Total nitrogen uptake of sampled macrophytes was normalized to m^2 surface area. For every gram per square meter SD10 had the highest TN uptake by *Phragmites australis*, totaling 20.8 g/ m^2. From SD3 was the largest uptake recorded for *Typha sp.* and was the second largest TN uptake at 18.8 g/ m^2. With the least TN uptake of all species, *Elodea sp.* was recorded having 1.46 g/ m^2, closely passed by algae with 2.34 g/ m^2 and *Equisetum sp.* with 2.62 g/ m^2.

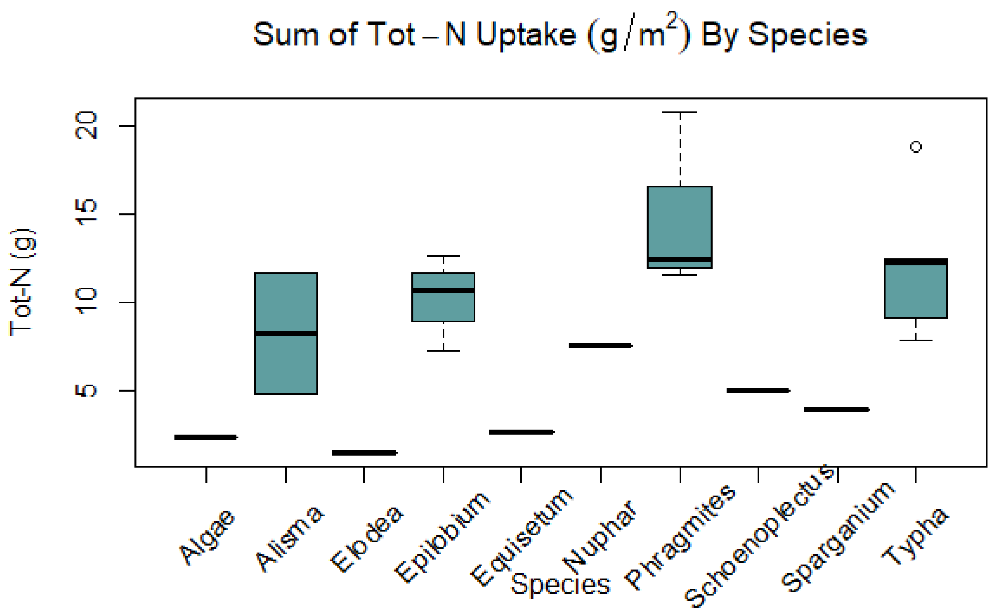

Figure 12. The sum of total nitrogen uptake in g/m2 normalized for each species sampled across all sites.

Across the reaches of all sites, *Phragmites australis* had the most TN uptake followed by *Tyhpa sp.* A large quantity of *Phragmites* uptake occurs in SD10 with an estimated 2.3 tonnes estimated across the entire reach as seen in Figure 13. *Epilobium hirsutum* had the next most uptake at a high of 0.42 tonnes, followed by *Typha* with a concentration of 0.36 tonnes. With the lowest TN uptake for the estimated reach, algae had the least with 0.024 tonnes, while *Elodea sp.* comes next as the second least mass at 0.030 tonnes.

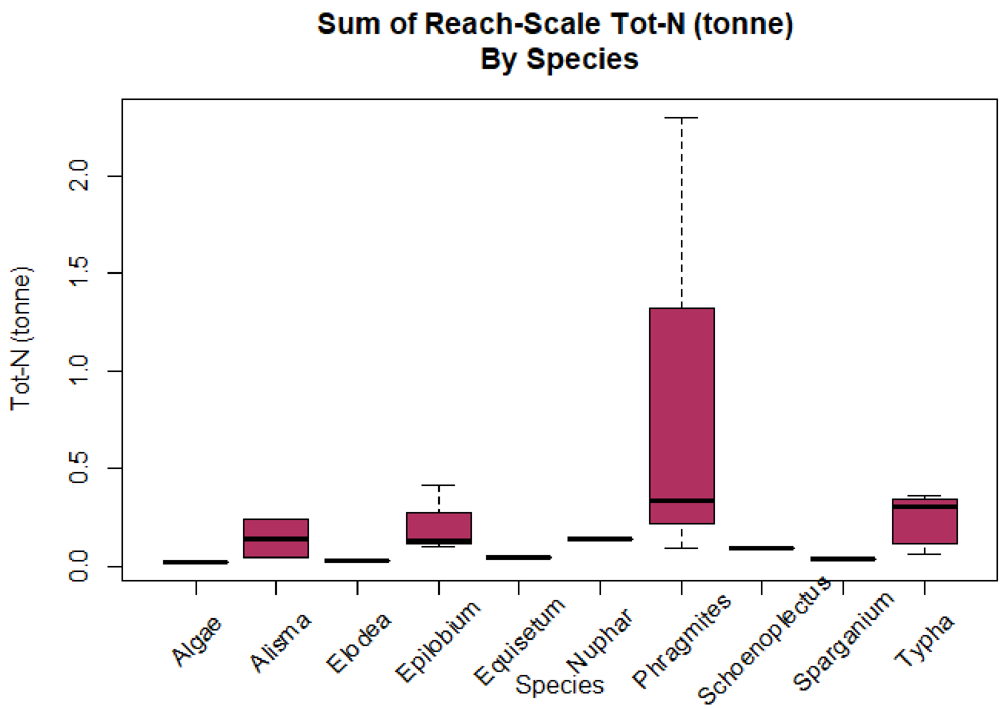

Figure 13. The sum of total nitrogen uptake in tonnes per species sampled across all sites.

For total phosphorus uptake in the reaches of each two-stage ditch site, SD10 was again observed to accumulate the most, with the sampled *Phragmites australis* species having a concentration of 0.23 tonnes, as observed in Figure 14. Likewise, SD7 and SD8 had the next highest concentrations of the nine sites at values of 0.078 tonnes and 0.075 tonnes, respectively. At the lower ends of phosphorus uptake were SD1, SD4, SD6 and SD9. Of these quantities, algae collected in SD9 had the lowest concentration of phosphorus at 0.005 tonnes. *Phragmites australis* had the largest range of phosphorus uptake, however due to its abundance in SD10. *Typha* and *Epilobium hirsutum* have the next most observed phosphorus uptake, making up the highest accumulation in SD7 and SD8.

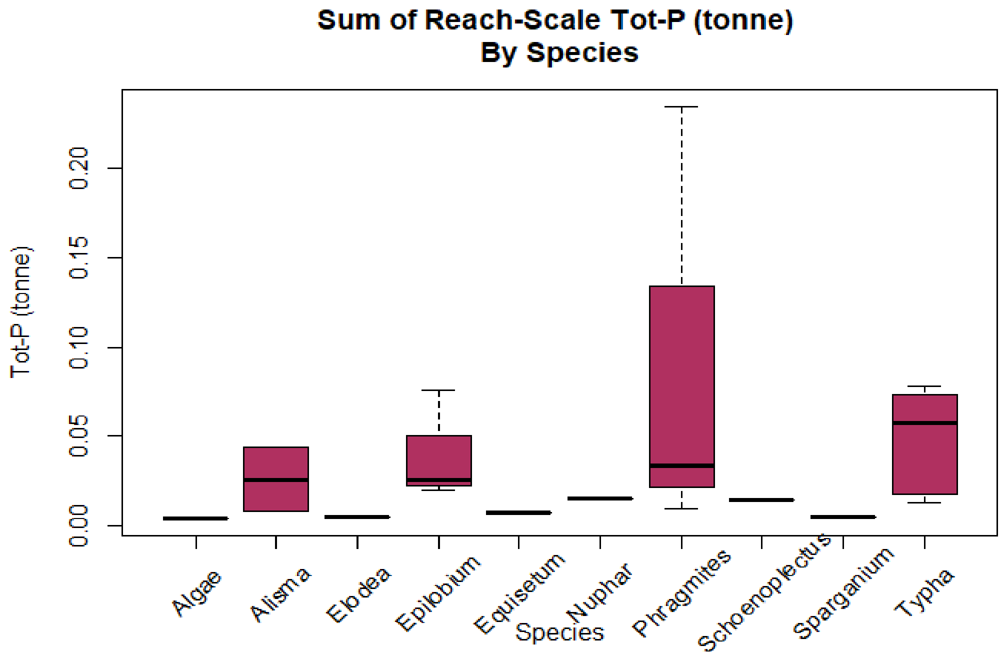

Figure 14. The sum of total phosphorus uptake in tonnes per species sampled across all sites.

SD3 had the most phosphorus uptake. SD4 had some of the lowest uptake observed in the sampling, however, SD2 had the lowest sampled estimated uptakes at 0.24 g/m^2 by *Elodea sp*, shown in Figure 15. Of the species, *Typha* had the highest phosphorus concentrations with a max of 3.59 g/m^2.

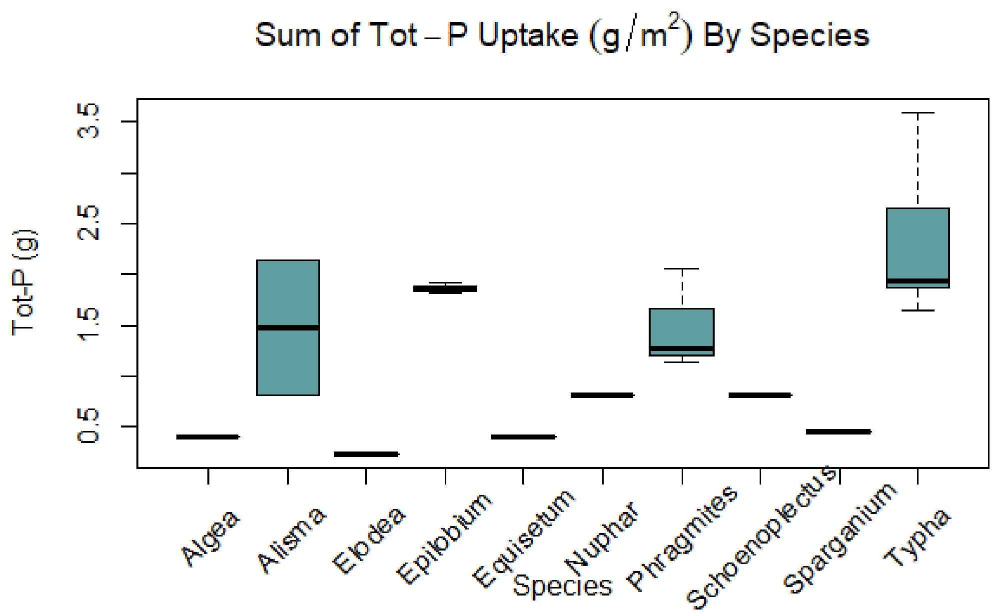

Figure 15. The average total phosphorus uptake in g/m2 per species sampled across all sites.

In comparing nutrient uptake and nutrient load, TN load far exceeded uptake by macrophytes at nearly every site as seen in Figure 16. Only at SD3 was nutrient uptake by macrophytes higher than TN load, where *Typha* was dominant. In comparing the relationship of total nitrogen concentrations in macrophyte uptake and environmental loads, a low positive correlation that was not significant was seen with a R-squared value of 0.1142 and a p value of 0.3737, observable in Appendix, Figure 41.

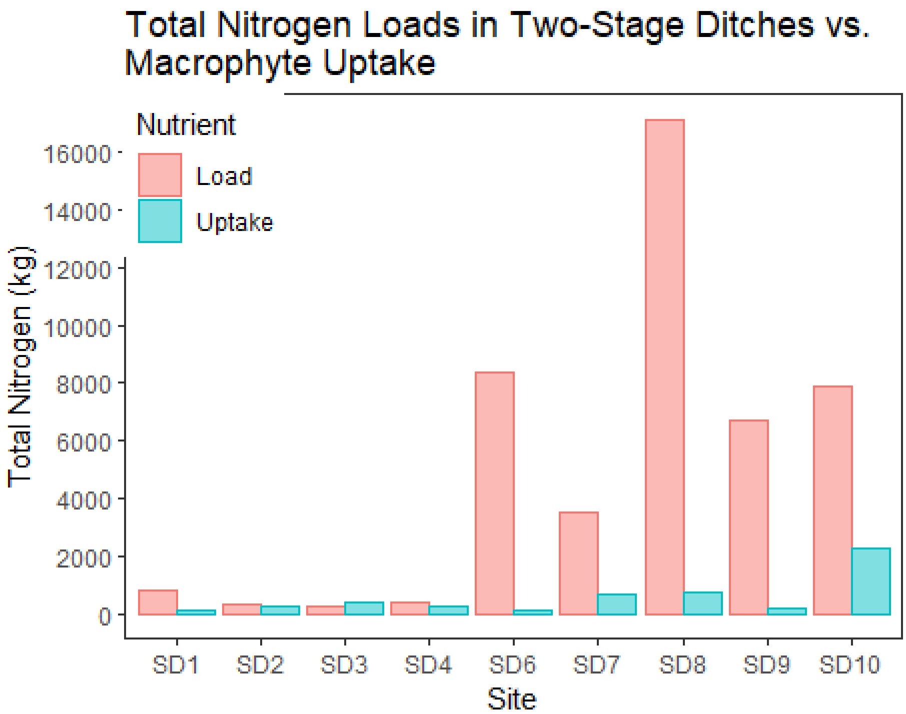

Figure 16. The total amounts of Nitrogen accumulated in plants related to the total amounts of nutrients transported through the ditches during the growth season.

TP load was generally higher than uptake by the sampled macrophytes. Shown in Figure 17, macrophyte uptake of TP was greater at sites SD3 and SD10 where *Typha, Epilobium hirsutum,* and *Phragmites austarlis* were high in abundance. Total phosphorus between plant uptake and environmental load exhibited an R-squared value of 0.2237 and a p value of 0.1985 (Appendix, Figure 42) indicating a low positive correlation that was not significant.

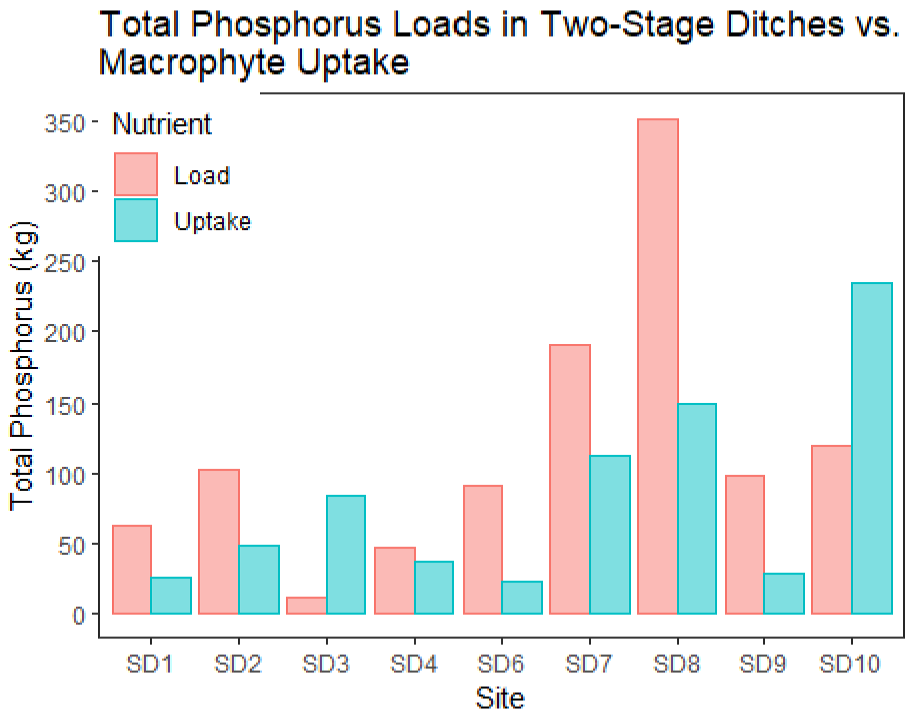

Figure 17. The total amounts of Phosphorus accumulated in plants related to the total amounts of nutrients transported through the ditches during the growth season.

5. Discussion

5.1 Objective 1 – Macrophyte Diversity and Abundance Against Environmental Factors

To log all present macrophytes and their coverage at each two-stage ditch, a survey was conducted at the beginning of June and again in August. This was done to find which environmental factors drive variation of abundance and diversity at each site; the first objective of the thesis. In answering the first research question, the most abundant species present in both June and August were the emergent species *Phragmites australis* and *Typha*. N and P availability played a large role in species diversity and the domination of emergent macrophyte species in the ditches. Some other plants saw drastic increases in abundance between the two months, namely, *Sparganium erectum* and other unidentifiable *Sparganium sp.*, whereas other macrophytes, vastly decreased in population. *Sparganium erectum* intensified the most in presence across the nine two-stage ditch sites, having only been observed once at SD9 in June to being present at seven of the nine sites in August, excluding SD4 and SD7.

An assumption could be derived that over the span of 2 months the stream environments became more optimal for *Sparganium spp.* growth, and thus it out competed previously abundant species within these plots. N and P availability as an environmental factor thus affect the preferences and tendencies for certain species to dominate in a community. As mentioned in the *Swedish Plant Geography* text, *Sparganium erectum* is a species that persists exclusively in eutrophic environments (Rydin et al. 1999). Research conducted in British rivers states that *Sparganium erectum* fully develops between early July and early September, further explaining the sudden increase in presence (Gurnell et al. 2013). This pattern seems to also occur in Denmark (personal communication). Including its vast rise population, *Sparganium erectum* increased in abundance from 1.79% across all streams against all documented species in June to 10.03% in August. Variation in macrophyte diversity is dependent on environmental factors, such as soil type, pH, and stream morphology, all altered by anthropogenic interference (Wiegleb et al. 2015). Species abundance is also dependent on stream management and disturbances. In a Danish study on the relationship between macrophyte communities and stream management, amphibious reed plants were dominant in highly disturbed streams (Baattrup-Pedersen et al. 2003). The results of that study also made mention of *Sparganium spp.* and *Typha spp.* being the most dominant amongst other obligate hydrophytes. It was also seen in this study's surveys that the same species of wetland plants populate best in disturbed environments. Like *Typha*, *Sparganium erectum* appears to be most dominant in clay soils and stream water with high amounts of dissolved oxygen and lower amounts of PO_4-P, as their relation would suggest in the August NMDS plot. However, that may challenge the prior notion that *Sparganium erectum* exclusively exists in eutrophic environments.

As research had to occur within the summer months, many time-related limitations were observed. Inexperience with the surveying process extended time spent past what was scheduled for each study site, though technique improved overtime emphasizing the need for maintained consistency. Further research into the nutrient uptake of these commonly found macrophyte species is beneficial to properly understand these patterns. Trends in macrophyte diversity in wetlands from other member states may point towards a relationship between diversity and nutrient availability. An increase in available N has been linked to macrophyte diversity loss in European streams, which in turn may be due to the slow loss of fertile soil caused by homogenous land usage affecting N availability in soil (Sardans and Peñuelas 2012). The macrophyte species found in Swedish drainage ditches appear to exist under similar conditions as found in Swedish lakes and rivers. As not as much research has been conducted in ditches and streams, these results reflecting comparable trends in Swedish lakes are insightful.

5.2 Objective 2 – The Relationship Between Macrophyte Diversity and Water Quality

Using the River LEAFPACS2 tool, EQR was determined to quantify the intensity of ecological stress at each stream. The Shannon Index, Equitability Index and EQR were all metrics used to find a correlation between macrophyte diversity with water quality; the second objective. It was found that EQR only saw negative correlation between historic nutrient loads of NO3-N in the summer and the August EQR results. A strong negative correlation between June NO3-N concentrations in 2022 and June macrophyte diversity was observed (Table 5, Appendix Figure 36), however no other response variables were correlated with nutrient concentrations or flow.

The WFD allows member states to set up their own metrics for determining ecological status. Using the UK's methodology for determining EQR, it was found that not only were most of the observed streams not compliant with WFD considerations for good ecological quality, but that EQR helps identify which streams may be heavily affected by anthropogenic disturbances and a lack of biodiversity. Due to the absence of specific algal data, some parameters in the River LEAFPACS2 were missing. As an alternative measure of EQR for SD6, SD8 and SD10 in June, and sites SD3, SD6, SD7 and SD10 in August, a theoretical final EQR was generated. By plotting the adjusted RMNI EQR (x-variable) and final EQR (y-variable) on a scatter plot, the slope-intercept form was used to solve for final EQR. Based on the estimated final EQR values the results suggest that most of the ditches were of less than moderate ecological status in June. However, unlike in the June scenario there were more ecological boundary classes classified as poor in August. After calculating the expected final EQR for SD3, SD6, SD7 and SD10, the results indicated that the sites either remained in poor state as were in June or became worse over time, despite the little improvement seen in SD6 and SD10. This may be expected as at the end of the growing period, nutrients previously trapped in macrophyte biomass leach back into the water during decay (Lu et al. 2018). It is uncertain the degree at which the calculated estimated final EQRs for SD3, SD6, SD7, and SD10 were accurately represented, but the flourish of emergent macrophyte species there would suggest that these sites were of lower than moderate ecological quality. Despite the deficiency of previously represented species in the UK taxon list for the River LEAFPACS2 tool at this study's research sites, further calibration of the tool may prove beneficial in the assessment of Swedish ditches.

In a study that compared the methodologies of nine member states (Belgium, Denmark, Estonia, Germany, Latvia, Lithuania, Netherlands, Poland, and the United Kingdom) for the ecological status assessment of lakes, class boundaries considered 'good' were highly diverse in vegetation and lakes considered 'less than good' were dominated by a minority of hydrophytes (Poikane et al. 2018). Macrophytes found at 'good' lakes spread across hydrophyte types (free-floating, submerged, emergent, etc.). Common among the good standing ecological quality were submerged macrophytes (elodeids), whereas an increase in free floating plants (such as lemnids) marked poorer ecological quality. The sites surveyed for this report's study saw a higher concentration of emergent species and free-floating species than submerged ones, aside from at SD2 in both the June and August months. The full percentage coverage of each hydrophyte type can be seen in Appendix, Table 6-23. Locations with lower EQR may expectedly be loaded with more excess nutrients than in sites with higher EQR values, as conditions where only few species thrive from higher nutrient loads harm greater diversity (van de Bund & Solimini, 2007). Lower EQR exhibits high environmental disturbances, such as excess loading of nutrients, thus the results of the expected EQR values appear to be supported by EQR trends found in the literature.

Ecological quality within streams that worsened over time could be explained by the collected macrophyte survey data. As environmental factors changed over the growing period and competition increased where *Typha* and *Sparganium* dominated, biodiversity decreased. Yet, when comparing the results of the River LEAFPACS2 with the nutrient averages of nitrate and phosphorus in the summer and winter months of 2020-2022, correlation between EQR values and nutrient trends of the past were mostly observed to be very weak. Furthermore, in the correlation tests between the 2022 environmental factors and the response variables for June and August, EQR showed no sign of statistical significance towards these factors, though macrophyte diversity did. Unfortunately, the River LEAFPACS2 tool alone could not provide a strong relationship between macrophyte diversity and water quality. However, it could help identify streams likely affected by heavy anthropogenic disturbances and a lack of biodiversity. Looking at Figure 11, the negative correlation between NO3-N concentrations over three summers and August EQR may be attributed to more functionally different macrophytes (especially of the most abundant species: *Typha,* algae, grasses, *Sparganium erectum*) taking up NO3-N more efficiently. This may also explain the significant difference in equitability means between June and August in Table 2.

5.3 Objective 3 – Most Suitable Bioremediators in Two-Stage Ditches

In finding the nutrient uptakes for the 10 most abundant species in the study, dry weight was taken, and TN and TP were analyzed per sample. From these results, nutrient uptake was calculated per sum of sampled macrophyte and by the coverage of entire stream reaches. This was to ascertain how much TN and TP could be accumulated into the biomass of the observed macrophytes at each study site for remediation; objective 3.

Answering the last research question, the species that appear to have a high remediation potential are: *Typha sp.*, *Phragmites australis*, *Epilobium hirsutum*, and *Alisma plantago-aquatica*. These are suggested for their vast coverage from July to August and capacity to uptake nutrients within their larger biomass as emergent macrophytes (Greenway, 2003). Results from both the reach-scale uptake and individual uptake suggest that *Phragmites australis* is best suited for both TN and TP uptake. Likewise, the presence of *Typha sp.* and *Epilobium hirsutum* indicate high nutrient loads of TN and TP in media. *Alisma plantago-aquatica* may not best reflect high amounts of TN in its vicinity but is a good indicator for phosphorus.

What is interesting to note about *Typha* is that the August survey saw less than 15% abundance at SD3 (14.5%), SD4 (4.90%), SD6 (6.25%), SD9 (4.48%) and

SD10 (1.82%), 5 of the 8 sites at which it was present. Yet, its biomass per area was of the highest calculated across all the sites it was sampled from (SD1, SD3, SD6, SD7, SD8). Though *Typha* did not take up a lot of space in the plots, its shoots were tall and thus had the capacity to uptake more nutrients, as echoed by Greenway (2003). *Phragmites australis* is also a sizeable plant, towering over the stream in SD10. It had the second highest biomass of the sampled areas. It too would have a broad capacity for taking up nutrients. Sampling *Sparganium erectum* would have yielded interesting results, especially given its further growth was likely influenced by the stream nutrient uptake in the late summer. In a Danish study, large canopy producing aquatic reed plants, such as *Typha angustifolia* and *Sparganium erectum* dominated in disturbed streams housing macrophyte communities (Baattrup-Pedersen et al. 2003). Interestingly, *Sparganium* and *Typha* belong to the same family, *Typhaceae*, where it may be seen as a possibility that these species in the first sampling were inaccurately classified, a potential limitation of using the PlantNet plant identification tool.

Estimating the uptake potential of each sampled species across the reach of all nine sites helped simulate the nutrient masses in above-ground biomass within these two-staged ditches. This does well to show the likely uptake of TN and TP by sampled macrophytes in the full reach as observed in Figure 16-17. Comparing those estimations with the total nutrient loads at each site further establishes the relationship between biomass, nutrient uptake, and water chemistry. Thus, using the results of the survey and sampling, species with a high capacity for remediation can be chosen based on both their coverage and nutrient uptake within the sampled two-stage ditches. From Figure 16, it can be inferred that nutrient uptake of TN does exceed TN loads transported throughout the two-stage ditches except for in SD3. Nutrient uptake of TP follows a similar trend where only SD3 and SD10 see a much higher uptake than load. These sites have a much higher abundance of *Typha, Epilobium hirsutum,* and *Phragmites austarlis*, which have the largest uptake of TN and TP of all sampled macrophytes. The data found that there was no statistical significance in the correlation between nutrient uptake and load. However, literature supports that nutrients are retained within macrophyte biomass, regardless of the extent that impact may be on the immediate environment. Species found almost exclusively in eutrophic environments benefit immensely from the high availability of nutrients (Rydin et al., 1999). *Typha, Epilobium hirsutum,* and *Phragmites austarlis* (species commonly found in eutrophic environments) follow the expectations laid out in the literature review for high nutrient uptake.

It is important to understand that these results only reflect stream macrophytes sampled above the roots. Had the roots also been sampled, it is assumed that phosphorus levels would be much higher than currently recorded, as it is more available in soil. Furthermore, the captured nutrients within the biomass of stream species can be released back into the environment when a plant dies and decays. As the abundance of some species in June decreases, availability of TN and TP may also increase, thus stimulating the growth of macrophyte that favor higher concentrations of TN and TP. It is as if water quality in two-stage ditches affect macrophyte species diversity and abundance but is in turn being affected by them. The rate at which macrophytes can regulate nutrient loads in an agricultural stream is then dependent on the management of those hydrophytes prior to the release of nutrients back into the media. However, changes to the stream's morphology and water height can adversely turn macrophytes from being a sink for nutrients to a source of it (Lu et al. 2018). Nutrients leaching back into the water from decayed macrophytes may worsen water quality, yet also provide nutrients for other macrophytes to then uptake. Though it does not appear that diversity is as important a factor in nutrient uptake, abundance seems to be.

A recent study on the capabilities of submerged macrophytes to buffer external loading of nutrients, or nutrient pulses, noted that water columns containing larger amounts of submerged macrophytes were both efficient at buffering nutrient pulses and bettering nutrient quality (Lv et al. 2023). An earlier study focused on the nutrient trapping potential of all macrophyte types for remediation purposes (Quilliam et al. 2015). It proposed that macrophyte harvesting in eutrophicated waters both removes captured excess nutrients from the waterway, and could serve additional sustainable purposes, such as: fertilizer use in agriculture, animal feed, biofuel, etc. Though the positive correlations seen in Appendix, Figures 41 and 42 between nutrient load and uptake were not statistically significant, as the above listed species persist in TN and TP rich environments as the most abundant species in their respective sites, they are suitable candidates for the remediation of eutrophicated agricultural streams in two-stage ditches. Management of these macrophytes prior to their decomposition is important for the removal of TN and TP and the upkeep of water quality in two-staged ditches.

One of the obstacles during sample collection for biomass calculations was the time between collection and weighing. Because an accurate scale could not be brought on the sampling trip, samples had to be collected for weighing upon the following week. Prolonging the time to measure fresh weight yields potential inaccuracies for fresh weight results as desiccation, or drying out, would become a problem (University of Idaho College of Natural Resources 2009). Some other challenges were in the methods for storing macrophytes. Considering limited resources (space in the rented transport vehicle and coolers for sample storage) stream samples occupied more room than initially anticipated and resulted in most of the samples resting outside of full coolers. Other issues with calculating biomass were presented during the drying of samples. Stream macrophytes required more time in the oven than was originally estimated. Though most of the samples dried after 48 hours, some required an additional 24 hours while *Typha* required more than 72 hours to reach its dry weight. Unfortunately, the exact time for *Typha* is not known as drying occurred at separate times, due to oven availability, and was dried both times for 72 hours.

6. Conclusion

In researching the remediation capabilities of macrophytes in two-stage ditches, three questions were posed. Answering the first question of what factors drive variation in macrophyte diversity and abundance in two-staged ditches, anthropogenic disturbances to stream morphology, nutrient load, soil type and pH were factors. The most evident influence in species dominance and diversity was N and P availability, especially for emergent macrophytes such as: *Phragmites australis*, *Typha*, and *Sparganium erectum*. For the second research question, can simple metrics for macrophyte diversity relate trends in water quality, correlation data for species diversity and stream nitrate concentrations support a strong negative correlation between the two, as well as for August EQR and historic trends of NO3-N loads. Lastly, identifying which of the present macrophytes are best suited for excess nutrients accumulation, the third research question, *Typha sp.*, *Phragmites australis*, *Epilobium hirsutum*, and *Alisma plantago-aquatica* had the highest remediation potential.

Of the species identified, *Typha sp.*, *Phragmites australis,* unidentified grasses and algae were of the most abundant followed closely by *Sparganium erectum* due to its population exploding in the late summer. It was found that diversity means at each site were not significantly different between the two months surveyed. By this, diversity between June and August varied slightly amongst the sites in terms of presence. However as seen in Table 2, the Shannon Equitability Index saw that the equitability means were significantly different between June and August. Thus as the dominance of functionally different species overtook, such as *Typha,* algae and *Sparganium erectum*, the concentration of NO3-N declined.

Despite some limitations with the UKTAG River LEAFPACS2 excel tool, final EQRs were estimated for both months which saw NO3-N reduction in August. It is speculated that either EQR trends are a result of historically low nitrate input, or that ecology in the late summer has a direct influence on effective nutrient uptake. Given that the River LEAFPACS2 tool could not be utilized to its utmost potential, either scenario remains speculative and would require further study.

Biomass calculations suggest that the most abundant macrophytes, *Typha* and *Phragmites australis*, may also have the largest capacity for nutrient uptake in the researched two-stage ditch sites. The literature also supports emergent macrophyte species as having the largest capacity for nutrient accumulation. Of the sampled macrophyte species, *Typha sp.*, *Phragmites australis*, *Epilobium hirsutum*, and *Alisma plantago-aquatica* are best suited for their remediation potential of eutrophic areas. A suggestion for remediating eutrophic drainage ditches, especially in two-staged ditches, would be to harvest these selected macrophytes prior to their decay at the end of the summer season and continue research into their potential for sustainable nutrient recycling.

From this data and the data collected in the August nutrient uptake samples, species abundance and their uptake capacity affect water quality (i.e., by decreasing nutrient availability in the water via uptake) and, in turn, water quality affects species abundance and biomass. Further, plans to develop this project should seek more time between the summer months and better preparation of tools and storage materials. This would increase the accuracy of biomass measurements and allow for more time at each site, increasing precision and accuracy in data collection.

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Popular Science Summary

Are Plants in Agricultural Drainage Ditches Capable of Keeping and Removing Nutrient Pollution from Streams?

Large-scale pollution cleanup of our streams, lakes, and seas tends to be costly and serves to treat the symptoms of a problem instead of the source of it. This major problem being referred to is eutrophication, excess nutrient pollution in our water from agriculture, roadways, and industrial releases to name a few. Agricultural pollution, including excess nutrients from fertilizers and pesticides, gets washed away from fields by rain and finds its way into drainage ditches that carry them through waterways harming native ecosystems. Would it not be beneficial if pollution could be predicted when it accumulates before moving on to the next location? Two-stage ditches are a great capture spot of runoff pollution, as the added terrace to the typical trapezoidal ditch design traps and collects pollution during high and low flooding periods. Even more beneficially, plants on these terraces and in the streams can absorb pollution into their bodies. This study explored the types of plants typically seen thriving in nutrient-rich environments, how data from those locations compare now to data from decades before in nutrient retention, and the capabilities of different plants to collect nutrient pollution to find the plants best suited for retaining nutrients in two-stage ditches.

Having observed two-stage ditches in 9 agricultural catchments across Central Eastern and Southern Sweden, the most commonly documented plants found were rooted plants that grow out and above the water in streams known as emergent plants. The most abundantly dominant of these emergent plants included cattail (*Typha*), common reed (*Phragmites australis*), hairy willowherb (*Epilobium hirsutum*), common water-plantain (*Alisma plantago-aquatica*), and bur-reed (*Sparganium erectum*). Other plant species found were submerged plants like waterweeds (*Elodea*), and free-floating plants like duckweed (*Lemna*) and algae.

With an understanding of the types of plants in these agricultural catchments, current levels of nutrient pollution were calculated and compared with historical data from prior decades. Although time had no direct effect on pollution in our ditches over the summer and winter months, there may be a relationship between lower nitrate pollution when plant diversity is high. It could be that having variations in plant types (emergent, submerged, and free-floating) makes absorption of different kinds of nutrients more efficient in two-stage ditches.

Examining the plants that dominated the summer season, research showed that cattails, common reeds, hairy willowherbs, and common water plantains retain nutrient pollution best from two-stage ditches. This is because, as emergent plants, they can collect both nutrients running through the stream and in the soil of the stream bed. Their large size gives them a high capacity to retain excess nutrients, making them effective for nutrient storage, like a sponge soaking up agricultural pollution in drainage ditches. So before they die and release the nutrients they collected over their growing period, it is important to remove the whole plant from the ditch. This may also benefit agriculture by providing potentially cost-effective, sustainable farming strategies. The now-dead nutrient-rich plants could be used as fertilizer on new crops, beginning a new cycle of nutrient recycling.

Acknowledgements

I would like to give special thanks to my family, for whom without their support none of my work and continued education would be possible. Special thanks to my team, Magda, Lukas, Maarten, and John for teaching me so much and providing a master's experience I could only dream of. Thank you to my friends back home for all the support y'all have given me throughout this graduate study. A special thanks to the EnvEuro programme for an insightful curriculum and for presenting me with many networking opportunities. Lastly, I would like to thank my lunch peeps: Martina, Anna, Alena, Inga, and Clara for the laughs and support on breaks, and our many fikas.

Appendix

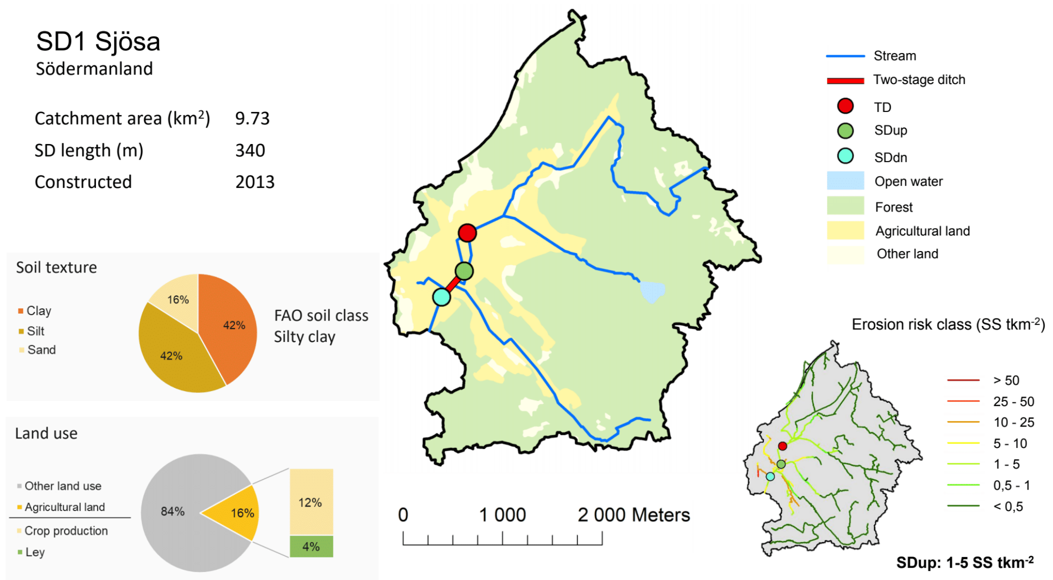

Figure 18. SD1 catchment summary and map detailing the size, soil texture, risk of erosion, and land usage.

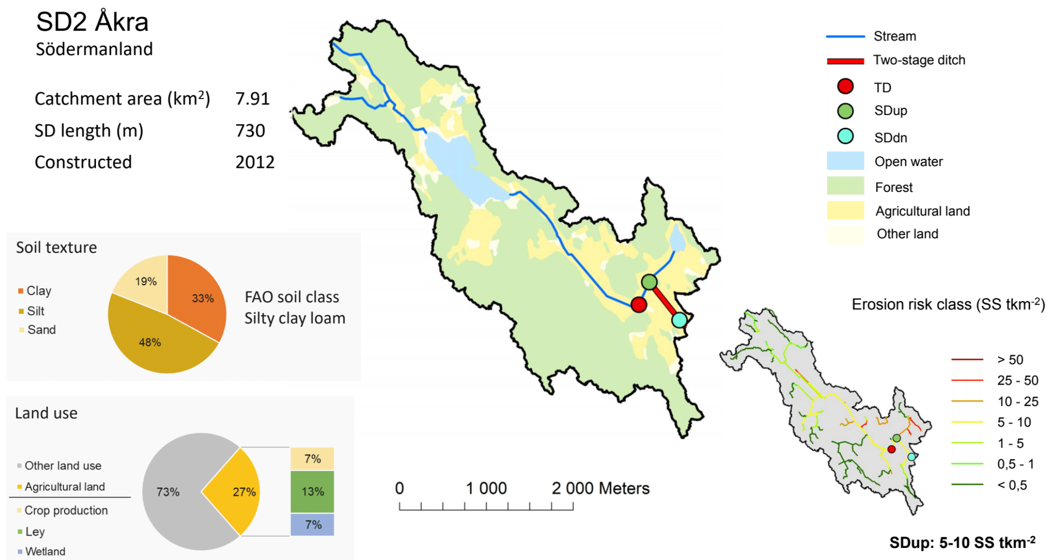

Figure 19. SD2 catchment summary and map detailing the size, soil texture, risk of erosion, and land usage.

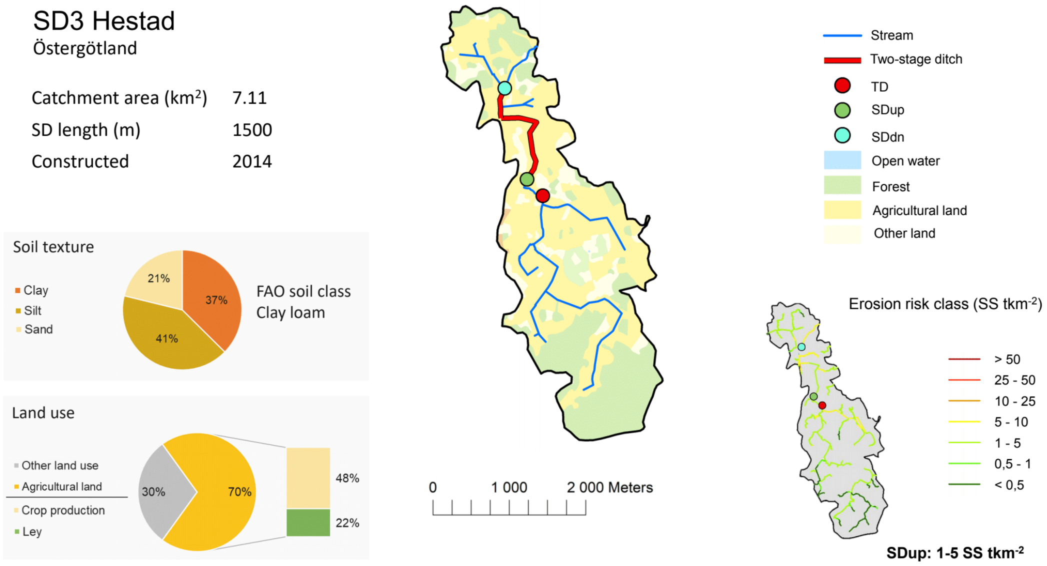

Figure 20. SD3 catchment summary and map detailing the size, soil texture, risk of erosion, and land usage.

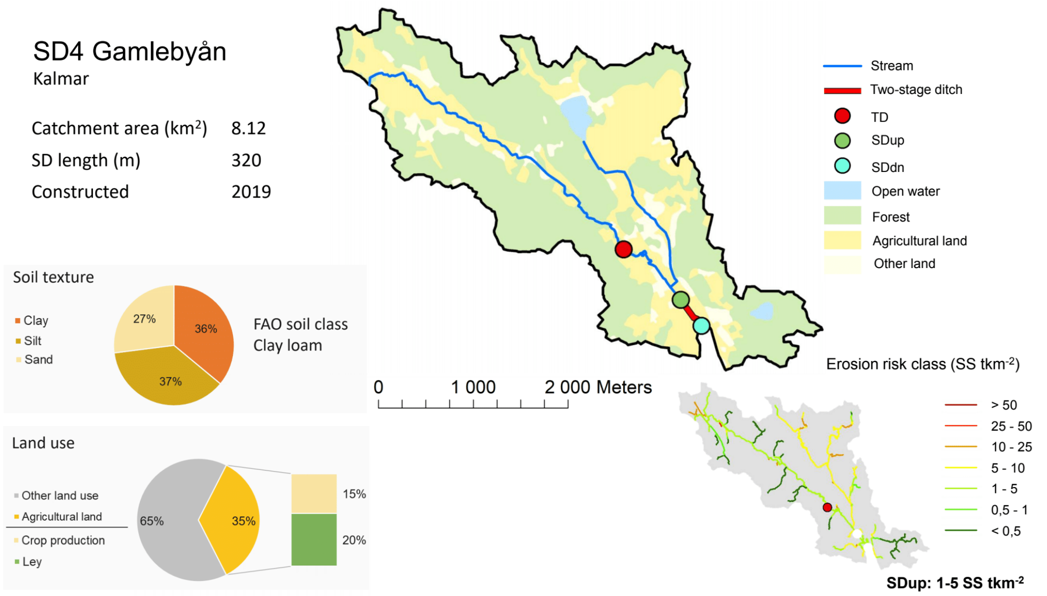

Figure 21. SD4 catchment summary and map detailing the size, soil texture, risk of erosion, and land usage.

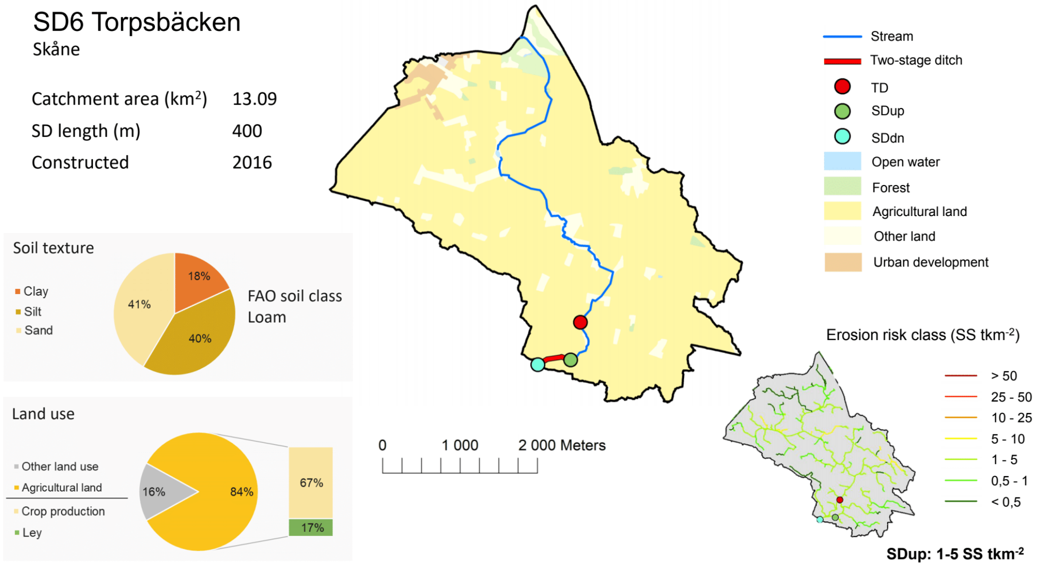

Figure 22. SD6 catchment summary and map detailing the size, soil texture, risk of erosion, and land usage.

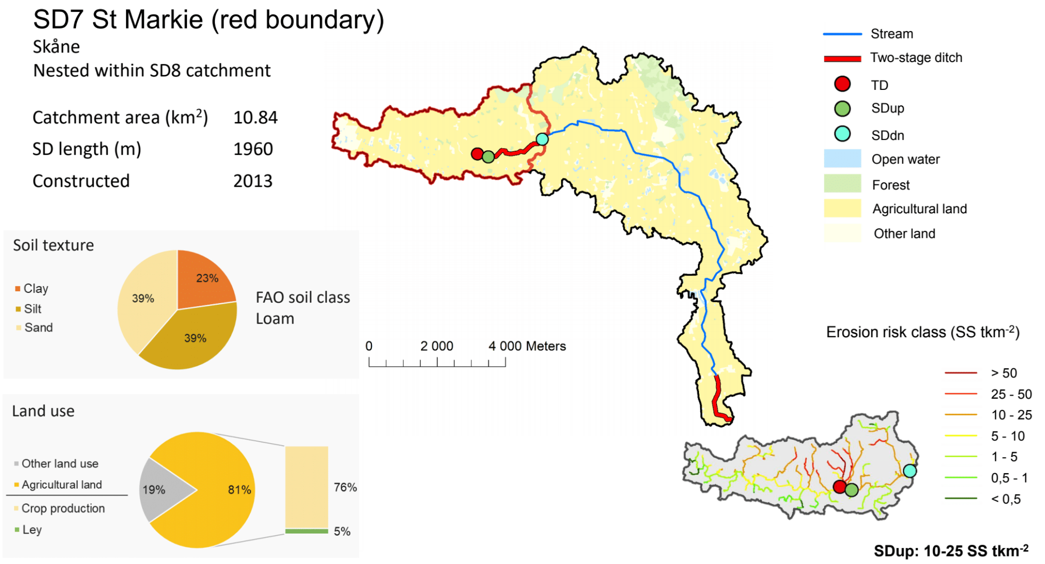

Figure 23. SD7 catchment summary and map detailing the size, soil texture, risk of erosion, and land usage.

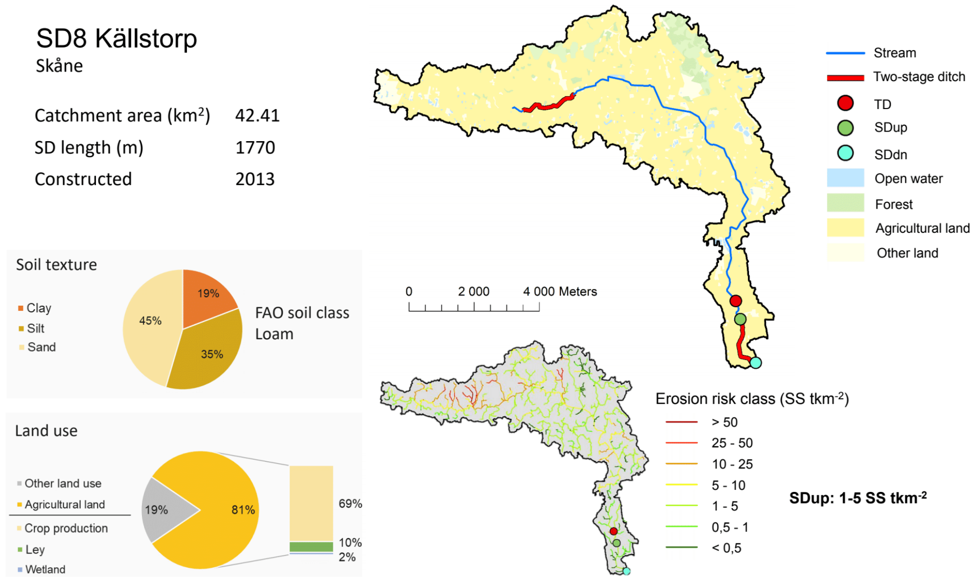

Figure 24. SD8 catchment summary and map detailing the size, soil texture, risk of erosion, and land usage.

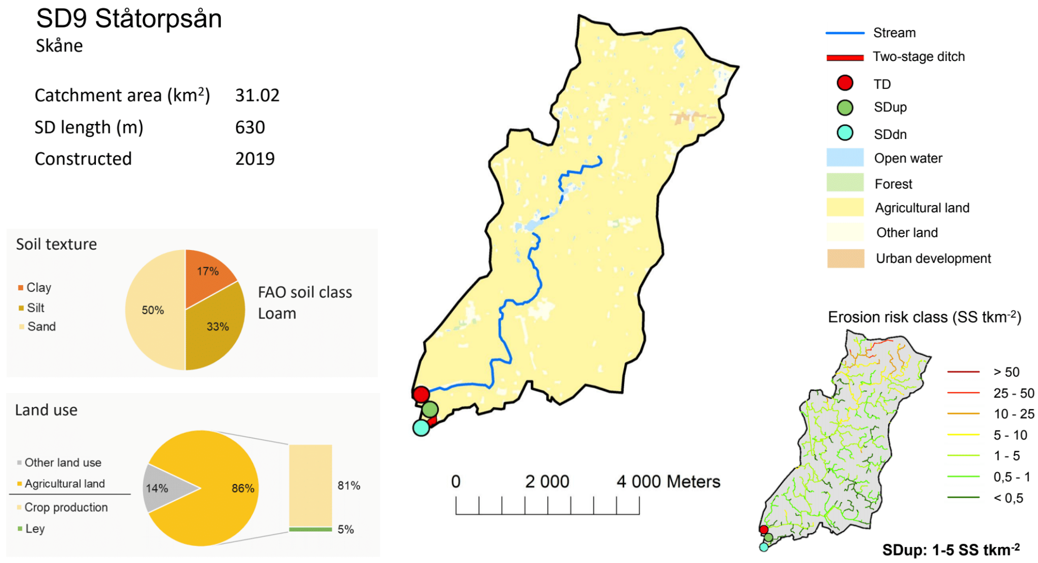

Figure 25. SD9 catchment summary and map detailing the size, soil texture, risk of erosion, and land usage.

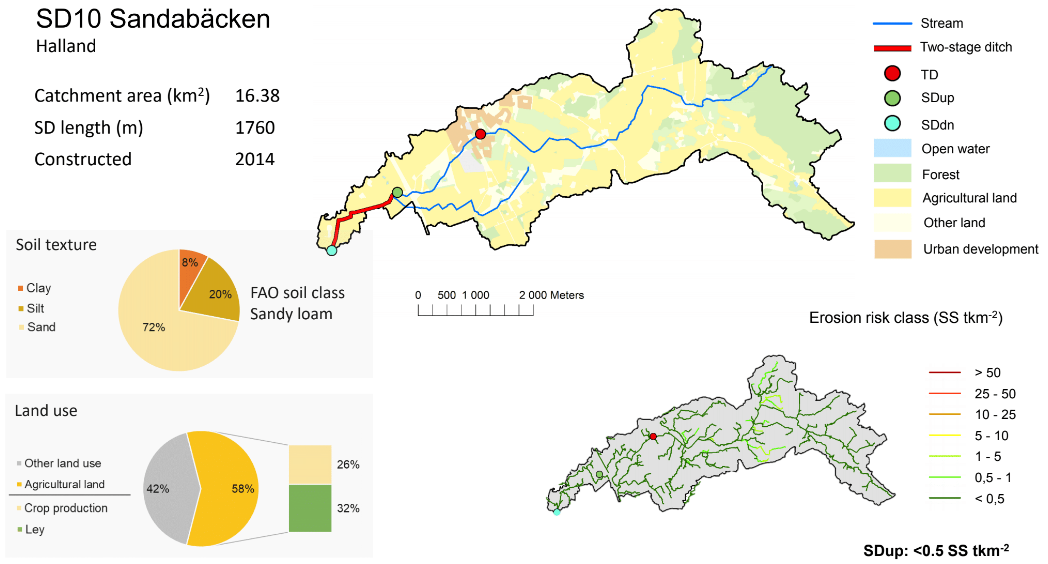

Figure 26. SD10 catchment summary and map detailing the size, soil texture, risk of erosion, and land usage.

	A	B	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
1		LEAFPACS 2 RIVER RECORD SHEET														
2		*100 m survey*														
3																
4																
5							river name					river name				
6							Site 1	SD1	SD2	SD3	SD4	SD6	SD7	SD8	SD9	SD10
7			Taxon RMNI	Aquatic TAXA	Functional group	Filamentous alga	Example SCV	SCV	SCV	SCV	SCV	SCV	SCV	SCV	SCV	SCV
8		Acorus calamus	9.49													
9		Alisma lanceolatum	8.47													
10		Alisma plantago-aquatica	7.82					5	3							
11		Anthelia julacea	2.70													
12		Apium inundatum	4.34	1	8											
13		Apium nodiflorum	8.64	1	8		3									
14		Azolla filiculoides	9.71	1	1											
15		Baldellia ranunculoides	4.34	1	4											
16		**Batrachospermum sp(p)**	5.46	1	19											
17		Berula erecta	8.24	1	8									7	7	
18		Bidens cernua	8.13													
19		Bidens tripartita	8.39													
20		Blindia acuta	1.09	1	22											
21		Blue-green algal scum/pelts	5.10	1	3											
22		Bolboschoenus maritimus	7.65													
23		Brachythecium plumosum	2.92	1	21											
24		Brachythecium rivulare	3.56	1	21											
25		Bryum alpinum	3.83													
26		Bryum dixonii	5.22													
27		Bryum pseudotriquetrum	2.71													
28		Butomus umbellatus	8.89	1	13											
29		**Calliergon cuspidatum**	3.49													
30		Callitriche brutia var hamulata	4.51	1	6											
31		Callitriche hermaphroditica	5.75	1	5											
32		Callitriche obtusangula	8.04	1	6		1									
33		Callitriche platycarpa	7.56	1	6											

Figure 27. Using the numeric scale designated for this tool, a number represents the coverage seen by the species of macrophytes labelled in column B. Column I is an example column for the tool.

	A	B	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
1		LEAFPACS 2 RIVER RECORD SHEET														
2		*100 m survey*														
3																
4																
5							river name					river name				
6							Site 1	SD1	SD2	SD3	SD4	SD6	SD7	SD8	SD9	SD10
7			Taxon RMNI	Aquatic TAXA	Functional group	Filamentous alga	Example SCV	SCV	SCV	SCV	SCV	SCV	SCV	SCV	SCV	SCV
449																
451	Metrics	**RMNI**			RMNI		7.52	8.07	8.31	7.41	7.42	8.21	7.70	8.64	8.30	7.75
452		**NTAXA**			NTAXA		8.00	2.00	3.00	0.00	2.00	0.00	0.00	1.00	3.00	0.00
453		**NFG**			NFG		7.00	2.00	2.00	0.00	2.00	0.00	0.00	1.00	3.00	0.00
454		**ALG**			ALG		3.80	0.00	0.00	0.00	0.00	0.00	0.00	0.00	17.50	0.00
455					FG1		2	5	12	0	0	0	0	0	4	0
456					FG2		0	0	0	0	0	0	0	0	0	0
457		7.522391755			FG3		0	0	0	0	0	0	0	0	0	0
458		8			FG4		0	0	0	0	0	0	0	0	0	0
459		7			FG5		0	0	8	0	0	0	0	0	0	0
460		3.8			FG6		1	0	0	0	0	0	0	0	0	0
461					FG7		0	0	0	0	0	0	0	0	0	0
462					FG8		4	0	0	0	0	0	0	7	7	0
463					FG9		0	0	0	0	0	0	0	0	0	0
464					FG10		0	0	0	0	0	0	0	0	0	0
465					FG11		0	0	0	0	0	0	0	0	0	0
466					FG12		0	0	0	0	7	0	0	0	0	0
467					FG13		0	4	0	0	7	0	0	0	0	0
468					FG14		0	0	0	0	0	0	0	0	0	0
469					FG15		0	0	0	0	0	0	0	0	0	0
470					FG16		0	0	0	0	0	0	0	0	0	0
471					FG17		0	0	0	0	0	0	0	0	0	0
472					FG18		8	0	0	0	0	0	0	0	0	0
473					FG19		4	0	0	0	0	0	0	0	6	0

Figure 28. The results of the survey are calculated in the blue-colored cells to represent the values for RMNI, NTAXA, NRG and ALG. Column I is an example column for the tool.

Table 6. June survey data for SD1 stream and terrace/floodplain macrophytes. Columns separated species into categories and each row per section equates to 100%.

JUNE	Stream					Floodplains						
Site SD1	Emergent macrophytes	Floating macrophytes	Submerged macrophytes	Filamentous algae	Bare sediments	Reed species	Lower grasses	Herbs	Bryophytes	Organic litter	Bare soil	Woody Vegetation
UP 1	5	0	0	30	65	0	20	40	0	0	40	0
UP 2	40	0	0	35	25	0	25	55	0	5	15	0
UP 3	35	0	0	40	25	0	47.61904762	28.57142857	0	19.04761905	4.761904762	0
MD 1	44.55445545	0.99009901	0	44.55445545	9.900990099	0	30	20	0	25	25	0
MD 2	14.85148515	0.99009901	0	64.35643564	19.8019802	0	25	25	0	40	10	0
MD 3	49.5049505	0.99009901	0	39.6039604	9.900990099	0	25.51020408	3.06122449	0	61.2244898	10.20408163	0
DN 1	19.8019802	0.99009901	0	64.35643564	14.85148515	0	20.40816327	3.06122449	0	56.12244898	20.40816327	0
DN 2	10	10	5	45	30	0	20	5	0	70	5	0
DN 3	45	5	0	40	10	0	36.08247423	20.6185567	2.06185567	20.6185567	20.6185567	0

Table 7. June survey data for SD2 stream and terrace/floodplain macrophytes. Columns separated species into categories and each row per section equates to 100%.

JUNE	Stream					Floodplains						
Site SD2	Emergent macrophytes	Floating macrophytes	Submerged macrophytes	Filamentous algae	Bare sediments	Reed species	Lower grasses	Herbs	Bryophytes	Organic litter	Bare soil	Woody Vegetation
UP 1	0	1.960784314	19.60784314	0	78.43137255	0	50	35	0	10	5	0
UP 2	8.928571429	1.785714286	62.5	8.928571429	17.85714286	0	39.6039604	0.99009901	0	54.45544554	4.95049505	0
UP 3	4.901960784	0.980392157	2.941176471	2.941176471	88.23529412	0	39.6039604	0.99009901	0	29.7029703	29.7029703	0
MD 1	2.923976608	0.584795322	46.78362573	46.78362573	2.923976608	0	15	5	0	75	5	0
MD 2	4.854368932	2.912621359	58.25242718	4.854368932	29.12621359	0	55	15	0	25	5	0
MD 3	1.960784314	0	49.01960784	0	49.01960784	0	12.5	50	16.66666667	16.66666667	4.166666667	0
DN 1	14.85148515	0.99009901	4.95049505	14.85148515	64.35643564	0	49.01960784	1.960784314	9.803921569	39.21568627	0	0
DN 2	24	4	8	56	8	0	50	9.090909091	4.545454545	27.27272727	9.090909091	0
DN 3	35	20	5	0	40	0	60	5	0	15	20	0

Table 8. June survey data for SD3 stream and terrace/floodplain macrophytes. Columns separated species into categories and each row per section equates to 100%.

JUNE	Stream					Floodplains						
Site SD3	Emergent macrophytes	Floating macrophytes	Submerged macrophytes	Filamentous algae	Bare sediments	Reed species	Lower grasses	Herbs	Bryophytes	Organic litter	Bare soil	Woody Vegetation
UP 1	0	0	2	0	98	0	55	40	0	5	0	0
UP 2	50	0	0	0	50	0	90	3	0	7	0	0
UP 3	40	0	0	0	60	0	59.60264901	0.662251656	0	0	0	39.73509934
MD 1	75	0	0	0	25	0	45	35	0	20	0	0
MD 2	10	0	0	0	90	0	50	15	0	35	0	0
MD 3	50	0	0	0	50	0	85	5	0	10	0	0
DN 1	45	20	0	0	35	0	75	5	0	20	0	0
DN 2	49.5049505	0.99009901	0	0	49.5049505	0	40.81632653	3.06122449	0	10.20408163	0	45.91836735
DN 3						0	99.00990099	0.99009901	0	0	0	0

Table 9. June survey data for SD4 stream and terrace/floodplain macrophytes. Columns separated species into categories and each row per section equates to 100%.

JUNE	Stream					Floodplains						
Site SD4	Emergent macrophytes	Floating macrophytes	Submerged macrophytes	Filamentous algae	Bare sediments	Reed species	Lower grasses	Herbs	Bryophytes	Organic litter	Bare soil	Woody Vegetation
UP 1	50	0	0	0	50	0	38.88888889	33.33333333	0	27.77777778	0	0
UP 2	75	0	0	0	25	0	48	24	0	20	8	0
UP 3	65	0	0	0	35	0	43.75	31.25	0	12.5	12.5	0
MD 1	70	0	0	0	30	0	68.42105263	5.263157895	0	21.05263158	5.263157895	0
MD 2	95	0	0	0	5	0	12.5	66.66666667	0	4.166666667	16.66666667	0
MD 3	80	0	0	0	20	0	9.523809524	76.19047619	0	4.761904762	9.523809524	0
DN 1	50	0	0	0	50	0	4.310344828	86.20689655	0	8.620689655	0.862068966	0
DN 2	60	0	0	0	40	0	5.263157895	84.21052632	0	5.263157895	5.263157895	0
DN 3	75	0	0	0	25	0	4.166666667	75	0	16.66666667	4.166666667	0

Table 10. June survey data for SD6 stream and terrace/floodplain macrophytes. Columns separated species into categories and each row per section equates to 100%.

JUNE	Stream					Floodplains						
Site SD6	Emergent macrophytes	Floating macrophytes	Submerged macrophytes	Filamentous algae	Bare sediments	Reed species	Lower grasses	Herbs	Bryophytes	Organic litter	Bare soil	Woody Vegetation
UP 1	50	0	0	0	50	0	57.14285714	3.571428571	0	0	10.71428571	28.57142857
UP 2	80	0	0	0	20	0	68.96551724	6.896551724	0	0	0	24.13793103
UP 3	40	0	0	0	60	0	47.05882353	52.94117647	0	0	0	0
MD 1	50	0	0	0	50	0	30.76923077	65.38461538	0	3.846153846	0	0
MD 2	90	0	0	0	10	0	25	20	0	5	0	50
MD 3	100	0	0	0	0	0	47.05882353	52.94117647	0	0	0	0
DN 1	100	0	0	0	0	0	60	40	0	0	0	0
DN 2	75	0	0	0	25	0	24	40	0	4	32	0
DN 3	75	0	0	0	25	0	48	48	0	4	0	0

Table 11. June survey data for SD7 stream and terrace/floodplain macrophytes. Columns separated species into categories and each row per section equates to 100%. Due to the length and collapse of this catchment's terrace along the stretch, the upstream has two plots, the midstream has three, and the downstream has four.

JUNE	Stream					Floodplains						
Site SD7	Emergent macrophytes	Floating macrophytes	Submerged macrophytes	Filamentous algae	Bare sediments	Reed species	Lower grasses	Herbs	Bryophytes	Organic litter	Bare soil	Woody Vegetation
UP 1	25	0	0	0	75	0	80	0	0	20	0	0
UP 2	80	0	0	0	20	0	83.33333333	0	0	16.66666667	0	0
MD 1	40	2	0	0	58	0	60	0	0	40	0	0
MD 2	14.70588235	1.960784314	0	0	83.33333333	0	19.60784314	73.52941176	0	1.960784314	4.901960784	0
MD 3	39.21568627	1.960784314	0	0	58.82352941	0	100	0	0	0	0	0
DN 1	20	0	0	0	80	0	72	8	0	20	0	0
DN 2	10	0	0	0	90	0	54.54545455	36.36363636	0	4.545454545	4.545454545	0
DN 3	30	0	0	0	70	0	61.8556701	30.92783505	0	5.154639175	2.06185567	0
DN 4	25.92592593	0	0	29.62962963	44.44444444	0	57.14285714	38.0952381	0	4.761904762	0	0

Table 12. June survey data for SD8 stream and terrace/floodplain macrophytes. Columns separated species into categories and each row per section equates to 100%.

JUNE	Stream					Floodplains						
Site SD8	Emergent macrophytes	Floating macrophytes	Submerged macrophytes	Filamentous algae	Bare sediments	Reed species	Lower grasses	Herbs	Bryophytes	Organic litter	Bare soil	Woody Vegetation
UP 1	40	0	0	0	60	0	85.71428571	9.523809524	0	4.761904762	0	0
UP 2	50	0	0	0	50	0	80	0	0	20	0	0
UP 3	80	0	0	0	20	0	24.75247525	49.5049505	0	24.75247525	0.99009901	0
MD 1	80	0	0	0	20	0	45.87155963	4.587155963	0	2.752293578	0.917431193	45.87155963
MD 2	40	0	0	0	60	0	90	5	0	0	0	5
MD 3	40	0	0	0	60	0	60	10	0	0	25	5
DN 1	25	0	0	0	75	0	70	10	0	0	0	20
DN 2	80	0	0	0	20	0	66.66666667	19.04761905	0	4.761904762	4.761904762	4.761904762
DN 3	50	0	0	0	50	0	20	65	0	10	5	0

Table 13. June survey data for SD9 stream and terrace/floodplain macrophytes. Columns separated species into categories and each row per section equates to 100%.

JUNE	Stream					Floodplains						
Site SD9	Emergent macrophytes	Floating macrophytes	Submerged macrophytes	Filamentous algae	Bare sediments	Reed species	Lower grasses	Herbs	Bryophytes	Organic litter	Bare soil	Woody Vegetation
UP 1	20	0	0	80	0	0	63.7254902	34.31372549	0	1.960784314	0	0
UP 2	39.39393939	0	0	45.45454545	15.15151515	0	40	60	0	0	0	0
UP 3	0	0	5	90	5	0	75	25	0	0	0	0
MD 1	33.33333333	0	0	63.33333333	3.333333333	0	90	10	0	0	0	0
MD 2	30	0	0	10	60	0	70	30	0	0	0	0
MD 3	40	0	0	0	60	0	70	20	0	10	0	0
DN 1	40	0	0	40	20	0	63.15789474	36.84210526	0	0	0	0
DN 2	26.47058824	0	0	73.52941176	0	0	73.52941176	26.47058824	0	0	0	0
DN 3	6.542056075	0	0	93.45794393	0	0	74.25742574	25.74257426	0	0	0	0

Table 14. June survey data for SD10 stream and terrace/floodplain macrophytes. Columns separated species into categories and each row per section equates to 100%.

JUNE	Stream					Floodplains						
Site SD10	Emergent macrophytes	Floating macrophytes	Submerged macrophytes	Filamentous algae	Bare sediments	Reed species	Lower grasses	Herbs	Bryophytes	Organic litter	Bare soil	Woody Vegetation
UP 1	0	0	0	0	100	0	0	37.03703704	0	11.11111111	51.85185185	0
UP 2	2	0	0	0	98	0	63.63636364	27.27272727	0	9.090909091	0	0
UP 3	5	0	0	0	95	0	49.01960784	14.70588235	1.960784314	4.901960784	29.41176471	0
MD 1	10	0	0	0	90	0	9.615384615	28.84615385	1.923076923	1.923076923	57.69230769	0
MD 2	10	0	0	0	90	0	40	10	0	0	50	0
MD 3	50	0	0	0	50							
DN 1	50	0	0	0	50							
DN 2	20	0	0	0	80							
DN 3	50	0	0	0	50							

Table 15. August survey data for SD1 stream and terrace/floodplain macrophytes. Columns separated species into categories and each row per section equates to 100%.

AUGUST	Stream					Floodplains						
Site SD1	Emergent macrophytes	Floating macrophytes	Submerged macrophytes	Filamentous algae	Bare sediments	Reed species	Lower grasses	Herbs	Bryophytes	Organic litter	Bare soil	Woody Vegetation
UP 1	52.63157895	0	0	0	47.36842105	0	30	35	0	20	15	0
UP 2	80	5	0	0	15	0	9.900990099	60.3960396	0	19.8019802	9.900990099	0
UP 3	80	10	0	0	10	0	40	10	0	20	30	0
MD 1	80	2	0	0	18	0	30	45	0	15	10	0
MD 2	80	5	0	0	15	0	9.900990099	35.64356436	0	39.6039604	14.85148515	0
MD 3	80	5	0	0	15	0	40	30	0	10	20	0
DN 1	40	2	0	0	58	0	10	45	0	15	30	0
DN 2	55	5	0	0	40	0	35	0	0	20	45	0
DN 3	35	5	10	0	50	0	45	0	0	35	20	0

Table 16. August survey data for SD2 stream and terrace/floodplain macrophytes. Columns separated species into categories and each row per section equates to 100%.

AUGUST	Stream					Floodplains						
Site SD2	Emergent macrophytes	Floating macrophytes	Submerged macrophytes	Filamentous algae	Bare sediments	Reed species	Lower grasses	Herbs	Bryophytes	Organic litter	Bare soil	Woody Vegetation
UP 1	0	5	95	0	0	0	45	50	0	0	5	0
UP 2	20	10	70	0	0	0	49.01960784	1.960784314	0	24.50980392	24.50980392	0
UP 3	18.18181818	40.90909091	40.90909091	0	0	0	30	10	0	10	50	0
MD 1	0	10	90	0	0	0	80	0	0	20	0	0
MD 2	0	13.26530612	86.73469388	0	0	0	90	0	0	10	0	0
MD 3	0	13.26530612	86.73469388	0	0	0	40	40	0	20	0	0
DN 1	0	60	40	0	0	0	60	15	0	25	0	0
DN 2	14.89361702	56.73758865	28.36879433	0	0	0	58.55855856	27.92792793	0	9.009009009	4.504504505	0
DN 3	10	45	0	5	40	0	25.51020408	43.87755102	0	10.20408163	20.40816327	0

Table 17. August survey data for SD3 stream and terrace/floodplain macrophytes. Columns separated species into categories and each row per section equates to 100%.

AUGUST	Stream					Floodplains						
Site SD3	Emergent macrophytes	Floating macrophytes	Submerged macrophytes	Filamentous algae	Bare sediments	Reed species	Lower grasses	Herbs	Bryophytes	Organic litter	Bare soil	Woody Vegetation
UP 1	50	0	0	0	50	0	60	20	0	15	0	5
UP 2	75	0	0	0	25	0	80	10	0	10	0	0
UP 3	20	0	0	0	80	0	20	5	0	40	0	35
MD 1	40	0	0	0	60	0	60	30	0	10	0	0
MD 2	25	0	0	0	75	0	45	10	0	45	0	0
MD 3	30	0	0	0	70	0	45	45	0	10	0	0
DN 1	20	0	0	0	80	0	78	12	0	10	0	0
DN 2	75	0	0	0	25	0	35	10	0	20	0	35
DN 3	10	0	0	0	90	0	40	40	0	20	0	0

Table 18. August survey data for SD4 stream and terrace/floodplain macrophytes. Columns separated species into categories and each row per section equates to 100%.

AUGUST	Stream					Floodplains						
Site SD4	Emergent macrophytes	Floating macrophytes	Submerged macrophytes	Filamentous algae	Bare sediments	Reed species	Lower grasses	Herbs	Bryophytes	Organic litter	Bare soil	Woody Vegetation
UP 1	35	0	0	0	65	0	0	80	0	20	0	0
UP 2	40	0	0	0	60	0	30	45	0	20	5	0
UP 3	45	0	0	0	55	0	0	75	0	15	10	0
MD 1	90.0990099	0	0	0	9.900990099	0	45	25	0	5	25	0
MD 2	90.0990099	0	0	0	9.900990099	0	30	35	0	25	10	0
MD 3	90	0	0	0	10	0	25	25	0	20	30	0
DN 1	60	0	0	0	40	0	25	40	0	30	5	0
DN 2	65	0	0	0	35	0	20	25	0	15	40	0
DN 3	95	0	0	0	5	0	30	20	0	40	10	0

Table 19. August survey data for SD6 stream and terrace/floodplain macrophytes. Columns separated species into categories and each row per section equates to 100%.

AUGUST	Stream					Floodplains						
Site SD6	Emergent macrophytes	Floating macrophytes	Submerged macrophytes	Filamentous algae	Bare sediments	Reed species	Lower grasses	Herbs	Bryophytes	Organic litter	Bare soil	Woody Vegetation
UP 1	30	0	0	0	70	0	40	35	0	0	0	25
UP 2	30	0	0	0	70	0	45	30	0	0	0	25
UP 3	30	0	0	0	70	0	25	75	0	0	0	0
MD 1	25	0	0	0	75	0	40	60	0	0	0	0
MD 2	25	0	0	0	75	0	19.8019802	0.99009901	0	0	0	79.20792079
MD 3	0	0	0	0	100	0	50	35	0	15	0	0
DN 1	0	0	0	0	100	0	85	10	0	5	0	0
DN 2	0	0	0	0	100	0	60	25	0	10	5	0
DN 3	20	0	0	0	80	0	75	20	0	0	5	0

Table 20. August survey data for SD7 stream and terrace/floodplain macrophytes. Columns separated species into categories and each row per section equates to 100%.

AUGUST	Stream					Floodplains						
Site SD7	Emergent macrophytes	Floating macrophytes	Submerged macrophytes	Filamentous algae	Bare sediments	Reed species	Lower grasses	Herbs	Bryophytes	Organic litter	Bare soil	Woody Vegetation
UP 1	60	0	0	0	40	0	90	0	0	10	0	0
UP 2	70	0	0	0	30	0	75	20	0	5	0	0
MD 1	25	5	0	0	70	0	80	10	0	10	0	0
MD 2	20	0	0	0	80	0	80	15	0	5	0	0
MD 3	40	0	0	0	60	0	50	45	0	5	0	0
DN 1	20	0	0	0	80	0	85	0	0	15	0	0
DN 2	55.44554455	0	0	0	44.55445545	0	65	20	0	15	0	0
DN 3	55	0	0	0	45	0	80	15	0	5	0	0
DN 4	20	0	0	0	80	0	75	10	0	15	0	0

Table 21. August survey data for SD8 stream and terrace/floodplain macrophytes. Columns separated species into categories and each row per section equates to 100%.

AUGUST	Stream					Floodplains						
Site SD8	Emergent macrophytes	Floating macrophytes	Submerged macrophytes	Filamentous algae	Bare sediments	Reed species	Lower grasses	Herbs	Bryophytes	Organic litter	Bare soil	Woody Vegetation
UP 1	50	0	0	0	50	0	25	60	0	15	0	0
UP 2	30	0	0	0	70	0	75	20	0	5	0	0
UP 3	5	0	0	0	95	0	50	45	0	0	0	5
MD 1	75	0	0	0	25	0	90	10	0	0	0	0
MD 2	50	0	0	0	50	0	45	40	0	10	0	5
MD 3	70	0	0	0	30	0	73	1	10	1	5	10
DN 1	65	0	0	0	35	0	90	5	0	5	0	0
DN 2	90	0	0	0	10	0	75	20	0	5	0	0
DN 3	90	0	0	0	10	0	99	1	0	0	0	0

Table 22. August survey data for SD9 stream and terrace/floodplain macrophytes. Columns separated species into categories and each row per section equates to 100%.

AUGUST	Stream					Floodplains						
Site SD9	Emergent macrophytes	Floating macrophytes	Submerged macrophytes	Filamentous algae	Bare sediments	Reed species	Lower grasses	Herbs	Bryophytes	Organic litter	Bare soil	Woody Vegetation
UP 1	25	0	0	0	75	0	20	50	0	10	20	0
UP 2	80	0	0	0	20	0	30	70	0	0	0	0
UP 3	5	0	0	0	95	0	40	55	0	5	0	0
MD 1	80	0	0	0	20	0	55	40	0	5	0	0
MD 2	10	0	0	0	90	0	75	25	0	0	0	0
MD 3	50	0	0	0	50	0	0	0	0	100	0	0
DN 1	10	0	0	60	30	0	0	0	0	100	0	0
DN 2	0	15	0	0	85	0	65	30	0	5	0	0
DN 3	0	0	0	0	100	0	0	67	0	33	0	0

Table 23. August survey data for SD10 stream and terrace/floodplain macrophytes. Columns separated species into categories and each row per section equates to 100%.

AUGUST	Stream					Floodplains						
Site SD10	Emergent macrophytes	Floating macrophytes	Submerged macrophytes	Filamentous algae	Bare sediments	Reed species	Lower grasses	Herbs	Bryophytes	Organic litter	Bare soil	Woody Vegetation
UP 1	0	0	0	0	100	0	0	40	0	20	40	0
UP 2	72.72727273	0	0	0	27.27272727	0	80	20	0	0	0	0
UP 3	5	0	0	0	95	0	55	40	0	0	5	0
MD 1	10	0	0	0	90	0	0	55	0	5	30	10
MD 2	20	0	0	0	80	0	0	1	0	20	69	10
MD 3	40	0	0	0	60							
DN 1	50	0	0	0	50							
DN 2	30	0	0	0	70							
DN 3	40	0	0	0	60							

Table 24. Stream vegetation coverage of the macrophyte species found in the streams of the nine surveyed SD sites in June. Highlighted are the most abundant macrophyte species. The counts present in the 'Sum' are a summation of the individual percentages for each two-stage ditch site that make up 900 total counts (100 per SD site). The 'Normalized %' column reflects the percentage of each documented species found at all study sites totaling 100%.

	June										Normalized
	SD1	SD2	SD3	SD4	SD6	SD7	SD8	SD9	SD10	SUM	%
alder sp.							1.00			1.00	0.11
alisma plantago-aquatica		3.18								3.18	0.35
alisma sp.	6.05									6.05	0.67
alopecurus pratensis						10.7		0.55		11.3	1.25
berula erecta								3.28		3.28	0.36
brown algea								20.8		20.8	2.31
butomus umbellatus								0.11		0.11	0.01
callitriche sp.		0.24				1.61				1.85	0.21
carex rostrata		2.39								2.39	0.27
cicuta virosa							11.0			11.0	1.22
eleocharis palastris		1.59								1.59	0.18
elodea sp.		48.2								48.2	5.36
epilobium hirsutum			8.75		7.69	8.04	18.0	4.92		47.4	5.27
equisetum fluviatile			5.10	4.69						9.79	1.09
equisetum sp.	0.43	5.57	3.64	14.1		5.36				29.1	3.23
galium palustre		0.80								0.8	0.09
green algae						10.7		26.8		37.5	4.17
hydrocharis morsus-ranae						1.07				1.07	0.12
leersia oryzoides			40.1		12.3					52.4	5.82
lemna sp.	1.59	2.95	6.71							11.3	1.25
lolium perenne							2.00			2.00	0.22
lysimachia thrysiflora				1.56						1.56	0.17
myosotis sp.		0.32								0.32	0.04
nuphar lutea				31.3						31.3	3.48
phalaris arundinacea								3.39		3.39	0.38
phragmites australis				3.91	61.5	13.4	2.00	3.83	95.8	**180.4**	**20.05**
poa pratensis					3.85					3.85	0.43
ranunculus sceleratus	0.43									0.43	0.05
ranunculus tricophyllus	4.03									4.03	0.45
salix sp.			1.46						2.99	4.45	0.49
schoenoplectus lacustris	2.16									2.16	0.24
schoenoplectus sp.				25.0						25.0	2.78
scirpus sylvaticus		1.59				9.65				11.2	1.25
solanum dulcamara				6.25		8.04				14.3	1.59
sparganium erectum								16.1		16.1	1.79
stachys palustris							1.00	0.55		1.55	0.17
tarragon sp.							0.20			0.20	0.02
typha sp.	22.3		29.2	13.3	12.3	31.4	52.9			**161.4**	**17.93**
unidentified filamentous algae	58.4	29.1	5.1					19.1		**111.7**	**12.41**
unidentified grass	3.89	1.59			2.31		12.0			19.8	2.20
unidentified herb	0.72							0.55	1.20	2.47	0.27
veronica scutelleta		2.39								2.39	0.27

Table 25. Stream vegetation coverage of the macrophyte species found in the streams of the nine surveyed SD sites in August. Highlighted are the most abundant macrophyte species.

	August										Normalized
	SD1	SD2	SD3	SD4	SD6	SD7	SD8	SD9	SD10	SUM	%
alisma plantago-aquatica	6.46	2.22								8.68	0.96
berula erecta							26.7	29.9		56.60	6.29
carex spp.		1.11					11.4			12.51	1.39
elodea nuttalli		61.0								61.00	6.78
epilobium hirsutum			17.4			2.70	21.9	10.4		52.40	5.82
equisetum arvense			4.35							4.35	0.48
equisetum fluviatile		1.22								1.22	0.14
equisetum palustre				18.3						18.30	2.03
equisetum spp.	1.62									1.62	0.18
filipendula ulmaria			4.35			5.39	2.86			12.60	1.40
hydrocharis morsus-ranae		11.2								11.20	1.24
lemna spp.	6.30	20.0						4.48		30.78	3.42
lythrum salicaria			4.35							4.35	0.48
nuphar lutea				31.9						31.90	3.54
phalaris arundinacea						4.04				4.04	0.45
phragmites australis				3.27	68.8	5.39		1.49	65.5	144.40	**16.04**
salix cinera			2.90	1.63					1.82	6.35	0.71
schoenoplectus lacustris	3.23			27.0						30.23	3.36
schoenoplectus spp.	0.81									0.81	0.09
scirpus sylvaticus		1.11				10.8			7.27	19.18	2.13
solanum dulcamara				8.17						8.17	0.91
sparganium erectum		2.22			12.5		3.81	11.9	20.0	50.43	5.60
sparganium sp.	28.3		11.6							39.90	4.43
stachys palustris				4.90				2.99		7.89	0.88
typha spp.	48.5		14.5	4.90	6.25	29.9	33.3	4.48	1.82	143.65	**15.96**
unidentified bryophyte						1.35				1.35	0.15
unidentified filamentous algae								17.9		17.90	1.99
unidentified grass	4.85		40.6		12.5	40.4			3.64	101.99	**11.3**
unidentified herb								16.4		16.40	1.82

Table 26. Vegetation coverage of the most abundant species of their respective stream sites found in June and August for June.

	June								
	SD1	SD2	SD3	SD4	SD6	SD7	SD8	SD9	SD10
Alisma plantago-aquatica		3.18							
Alisma sp.	6.05								
Alopecuris pratensis						10.7		0.55	
Berula erecta								3.28	
Brown algae								20.8	
Elodea nutalli									
Elodea sp.		48.2							
Epilobium hirsutum			8.75		7.69	8.04	18	4.92	
Equisetum palustre									
Equisetum spp.	0.43	5.57	8.74	18.8		5.36			
Green algae						10.7		26.8	
Hydrocharis morsus-ranae						1.07			
Leersia orzyoides			40.1		12.3				
Lemna sp.	1.59	2.95	6.71						
Nuphar lutea				31.3					
Phragmites australis				3.91	61.5	13.4	2	3.83	95.8
Salix sp.			1.46						2.99
Schoenoplectus lacustris	2.16								
Schoenoplectus sp.				25					
Scirpus sylvaticus		1.59				9.65			
Solanum dulcamara				6.25		8.04			
Sparganium erectum								16.1	
Sparganium sp.									
Typha sp.	22.3		29.2	13.3	12.3	31.4	52.9		
Unidentified Filamentous Algae	58.4	29.1						19.1	
Unidentified Grass	3.9	1.59	5.1		2.31		12		
Unidentified Herb	0.72							0.55	1.2

Table 27. Vegetation coverage of the most abundant species of their respective stream sites found in June and August for August.

	August								
	SD1	SD2	SD3	SD4	SD6	SD7	SD8	SD9	SD10
Alisma plantago-aquatica	6.46	2.22							
Alisma sp.									
Alopecuris pratensis									
Berula erecta							26.7	29.9	
Brown algae									
Elodea nutalli		61							
Elodea sp.									
Epilobium hirsutum			17.4			2.7	21.9	10.4	
Equisetum palustre				18.3					
Equisetum spp.	1.62	1.22	4.35						
Green algae									
Hydrocharis morsus-ranae		11.2							
Leersia orzyoides									
Lemna sp.	6.3	20						4.48	
Nuphar lutea				31.9					
Phragmites australis				3.27	68.75	5.39		1.49	65.5
Salix sp.			2.9	1.63					1.82
Schoenoplectus lacustris	3.23			27					
Schoenoplectus sp.	0.81								
Scirpus sylvaticus		1.11				10.8			7.27
Solanum dulcamara				8.17					
Sparganium erectum		2.22			12.5		3.81	11.9	20
Sparganium sp.	28.3		11.6						
Typha sp.	48.5		14.5	4.9	6.25	29.9	33.3	4.48	1.82
Unidentified Filamentous Algae								17.9	
Unidentified Grass	4.85		40.6		12.5	40.4			3.64
Unidentified Herb								16.4	

Table 28. Vegetation coverage of the most abundant species of their respective stream sites found in June and August.

	June									August									
	SD1	SD2	SD3	SD4	SD6	SD7	SD8	SD9	SD10	SD1	SD2	SD3	SD4	SD6	SD7	SD8	SD9	SD10
Alisma plantago-aquatica		3.18								6.46	2.22							
Alisma sp.	6.05																	
Alopecuris pratensis						10.7		0.55										
Berula erecta								3.28								26.7	29.9	
Brown algae								20.8										
Elodea nutalli											61							
Elodea sp.		48.2																
Epilobium hirsutum			8.75		7.69	8.04	18	4.92				17.4			2.7	21.9	10.4	
Equisetum palustre													18.3					
Equisetum spp.	0.43	5.57	8.74	18.8		5.36				1.62	1.22	4.35						
Green algae						10.7		26.8										
Hydrocharis morsus-ranae						1.07					11.2							
Leersia orzyoides			40.1		12.3													
Lemna sp.	1.59	2.95	6.71							6.3	20						4.48	
Nuphar lutea				31.3									31.9					
Phragmites australis				3.91	61.5	13.4	2	3.83	95.8				3.27	68.75	5.39		1.49	65.5
Salix sp.			1.46						2.99			2.9	1.63					1.82
Schoenoplectus lacustris	2.16									3.23			27					
Schoenoplectus sp.				25						0.81								
Scirpus sylvaticus		1.59				9.65					1.11				10.8			7.27
Solanum dulcamara				6.25		8.04							8.17					
Sparganium erectum								16.1			2.22			12.5		3.81	11.9	20
Sparganium sp.										28.3		11.6						
Typha sp.	22.3		29.2	13.3	12.3	31.4	52.9			48.5		14.5	4.9	6.25	29.9	33.3	4.48	1.82
Unidentified Filamentous Algae	58.4	29.1						19.1									17.9	
Unidentified Grass	3.9	1.59	5.1		2.31		12			4.85		40.6		12.5	40.4			3.64
Unidentified Herb	0.72							0.55	1.2								16.4	

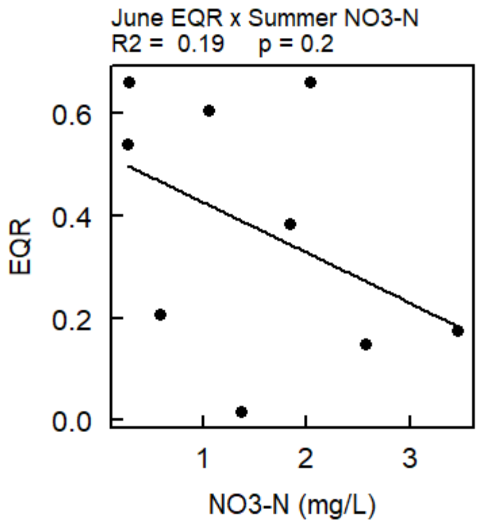

Figure 29. A scatterplot depicting the relationship between the June final EQR results and the average NO3-N concentrations from the summers of 2020-2022 at all two-stage ditch sites.

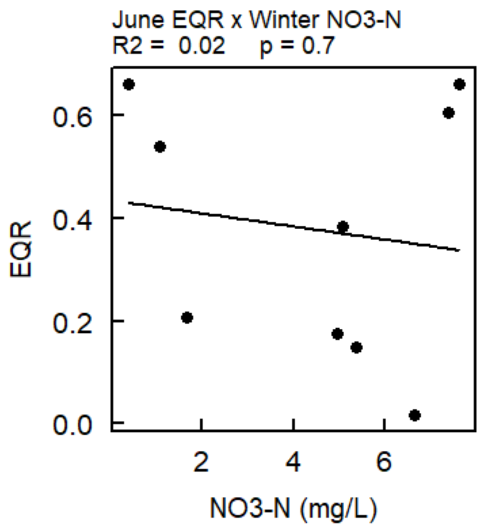

Figure 30. A scatterplot depicting the relationship between the June final EQR results and the average NO3-N concentrations from the winters of 2020-2022 aat all two-stage ditch sites.

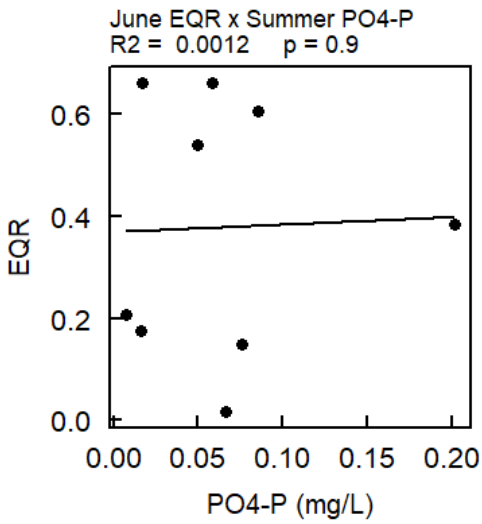

Figure 31. A scatterplot depicting the relationship between the June final EQR results and the average PO4-P concentrations from the summers of 2020-2022 at all two-stage ditch sites.

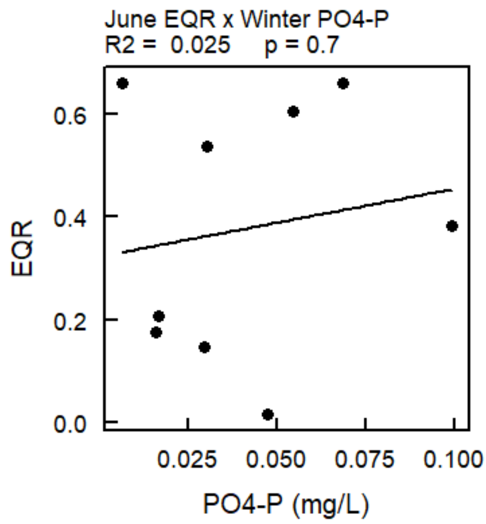

Figure 32. A scatterplot depicting the relationship between the June final EQR results and the average PO4-P concentrations from the winters of 2020-2022 at all two-stage ditch sites.

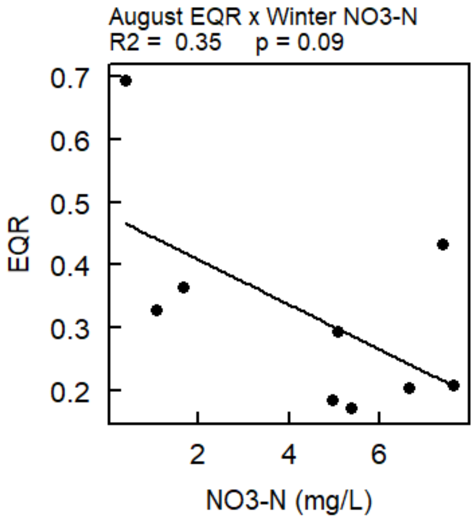

Figure 33. A scatterplot depicting the relationship between the August final EQR results and the average NO3-N concentrations from the winters of 2020-2022 at all two-stage ditch sites.

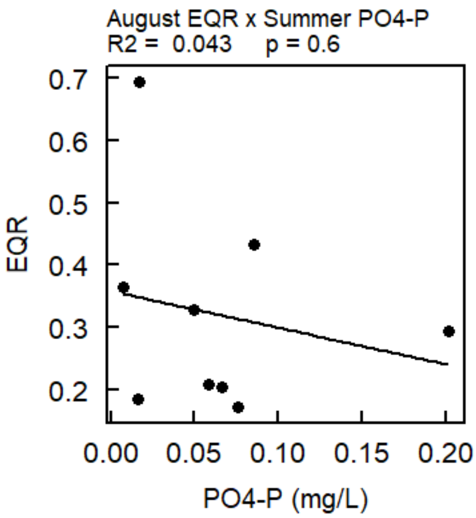

Figure 34. A scatterplot depicting the relationship between the August final EQR results and the average PO4-P concentrations from the summers of 2020-2022 at all two-stage ditch sites.

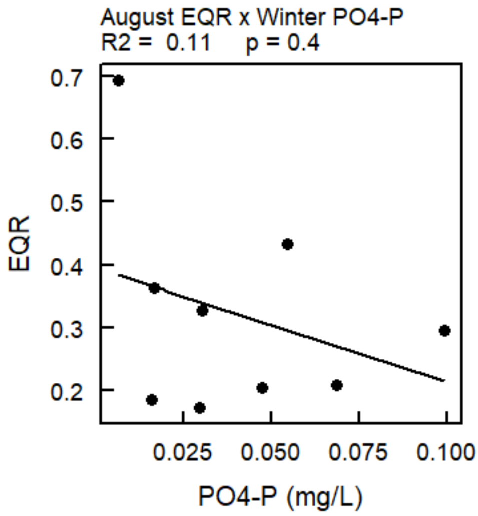

Figure 35. A scatterplot depicting the relationship between the August final EQR results and the average PO4-P concentrations from the winters of 2020-2022 at all two-stage ditch sites.

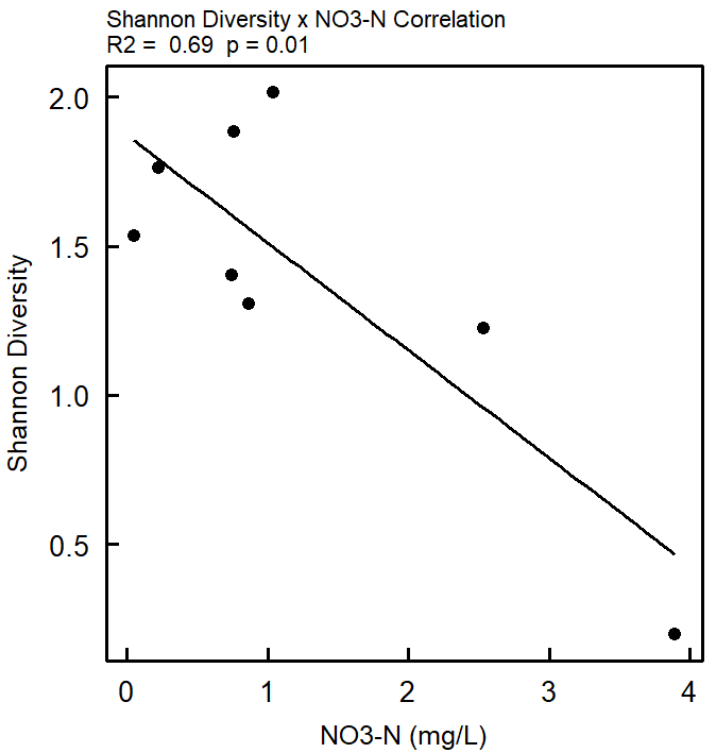

Figure 36. Correlation plot showing the strong negative correlation of Shannon Diversity and NO3-N concentration from June 2022.

Table 29. Correlated test between environmental factors (August Q50, and August 2022 NO3-N and PO4-P concentrations) and response variables (August Shannon Diversity, Shannon Equitability, and EQR). The test looked at stream values available for each SD site, excluding SD7 and SD10.

	Response variable											
	Shannon Diversity				Shannon Equitability				EQR			
Factors	Multiple R	R Square	p	df	Multiple R	R Square	p	df	Multiple R	R Square	p	df
Q50	0.20	0.04	0.67	5	0.61	0.38	0.14	5	0.32	0.10	0.48	5
NO3-N	0.26	0.07	0.57	5	0.11	0.01	0.81	5	0.18	0.03	0.69	5
PO4-P	0.17	0.03	0.71	5	0.54	0.29	0.21	5	0.50	0.25	0.25	5

Table 30. Fresh and dry weights for August samples from the channel's upstream (CU) and downstream (CD) in 0.04 m2 plots.

Site	Location	Sample ID	Species Name	Fresh Weight (g 0.04 m^2)	Dry Weight (g 0.04 m^2)	Dry Weight (kg m^2)
SD1	CU	1	Typha sp.	225.7	99.91	2.49775
SD1	CU	2	Alisma sp.	26.97	16.06	0.4015
SD1	CD	3	Typha sp.	355.6	128.9	3.22175
SD1	CD	4	Alisma sp.	74.62	32.72	0.818
SD2	CU	5	Elodea sp.	45.52	4.72	0.118
SD2	CU	6	Alisma plantago-aquatica	259.6	128.5	3.2125
SD2	CD	7	Elodea sp.	57.86	7.38	0.1845
SD2	CD	8	Alisma plantago-aquatica	90.41	27.18	0.6795
SD3	CU	9	Typha sp.	738.9	162.3	4.0575
SD3	CU	10	Epilobium hirsutum	469.3	136.0	3.40075
SD3	CD	11	Typha sp.	701.1	188.7	4.7175
SD4	CU	12	Nuphar lutea	228.5	35.91	0.89775
SD4	CU	13	Schoenoplectus sp.	129.0	39.80	0.995
SD4	CU	14	Equisetum sp.	30.68	9.79	0.24475
SD4	CD	15	Nuphar lutea	90.93	16.41	0.41025
SD4	CD	16	Schoenoplectus sp.	129.4	35.36	0.884
SD4	CD	17	Equisetum sp.	74.88	22.69	0.56725
SD6	CU	18	Phragmites australis	236.9	132.1	3.3035
SD6	CU	19	Typha sp.	817.5	184.6	4.615
SD6	CD	20	Phragmites australis	43.28	25.12	0.628
SD7	CU	21	Typha sp.	167.6	28.00	0.7
SD7	CU	22	Phragmites australis	151.2	70.16	1.754
SD7	CD	23	Typha sp.	899.0	198.1	4.9525
SD7	CD	24	Phragmites australis	287.5	143.8	3.5955
SD8	CU	25	Typha sp.	758.2	153.9	3.8475
SD8	CU	26	Epilobium hirsutum	188.6	40.01	1.00025
SD8	CD	27	Typha sp.	327.3	87.90	2.1975
SD8	CD	28	Epilobium hirsutum	311.7	80.66	2.0165
SD9	CU	29	Sparangium erectum	137.2	29.50	0.7375
SD9	CU	30	Epilobium hirsutum	275.0	91.55	2.28875
SD9	CD	31	Unidentified filamentous algea	115.7	45.94	1.1485
SD9	CD	32	Sparangium erectum	68.26	14.73	0.36825
SD9	CD	33	Epilobium hirsutum	463.3	162.9	4.0715
SD10	CU	34	Phragmites australis	415.4	187.2	4.68025
SD10	CD	35	Phragmites australis	348.0	168.0	4.1995

Table 31. Total nutrient uptake in g/sq. m for the sites SD1-SD10 normalized.

Site	Species	Sum of Tot-N (g)	Sum of Tot-P (g)
SD1	Alisma	4.7540236	0.816205147
SD1	Typha	12.18905132	1.877864157
SD2	Alisma	11.6752579	2.144812639
SD2	Elodea	1.4555009	0.238307007
SD3	Epilobium	7.18714505	1.823548095
SD3	Typha	18.7859565	3.590868064
SD4	Equisetum	2.62124125	0.411260643
SD4	Nuphar	7.52621475	0.815766311
SD4	Schoenoplectus	5.0086428	0.808582435
SD6	Phragmites	12.4163345	1.278982631
SD6	Typha	7.8554684	1.644577207
SD7	Phragmites	11.5539592	1.142658928
SD7	Typha	12.406429	2.659423945
SD8	Epilobium	10.64857875	1.925882806
SD8	Typha	9.10622265	1.936194122
SD9	Epilobium	12.67275591	1.850132847
SD9	Sparangium	3.8776437	0.448683278
SD9	Algea	2.3351302	0.403651298
SD10	Phragmites	20.77351315	2.06226253

Table 32. Total nutrient mass in tonnes for the entire reach of sites SD1-SD10.

SITE	Species	Sum of reach-scale TN uptake (tonne)	Sum of reach-scale TP uptake (tonne)
SD1	Alisma	0.044639104	0.007698831
SD1	Typha	0.114605135	0.017876205
SD2	Alisma	0.240570587	0.044150936
SD2	Elodea	0.029529586	0.004837559
SD3	Epilobium	0.100116931	0.025402025
SD3	Typha	0.304504366	0.057870472
SD4	Equisetum	0.045936337	0.007237323
SD4	Nuphar	0.140063801	0.01534535
SD4	Schoenoplectus	0.091342771	0.014479338
SD6	Phragmites	0.095482385	0.009803194
SD6	Typha	0.061743982	0.012926377
SD7	Phragmites	0.3396864	0.033594172
SD7	Typha	0.364749013	0.078187064
SD8	Epilobium	0.41578028	0.075626326
SD8	Typha	0.347480348	0.073749713
SD9	Epilobium	0.136278697	0.019641424
SD9	Sparganium	0.042898941	0.004920146
SD9	Algae	0.023514761	0.004064769
SD10	Phragmites	2.297376342	0.234842998

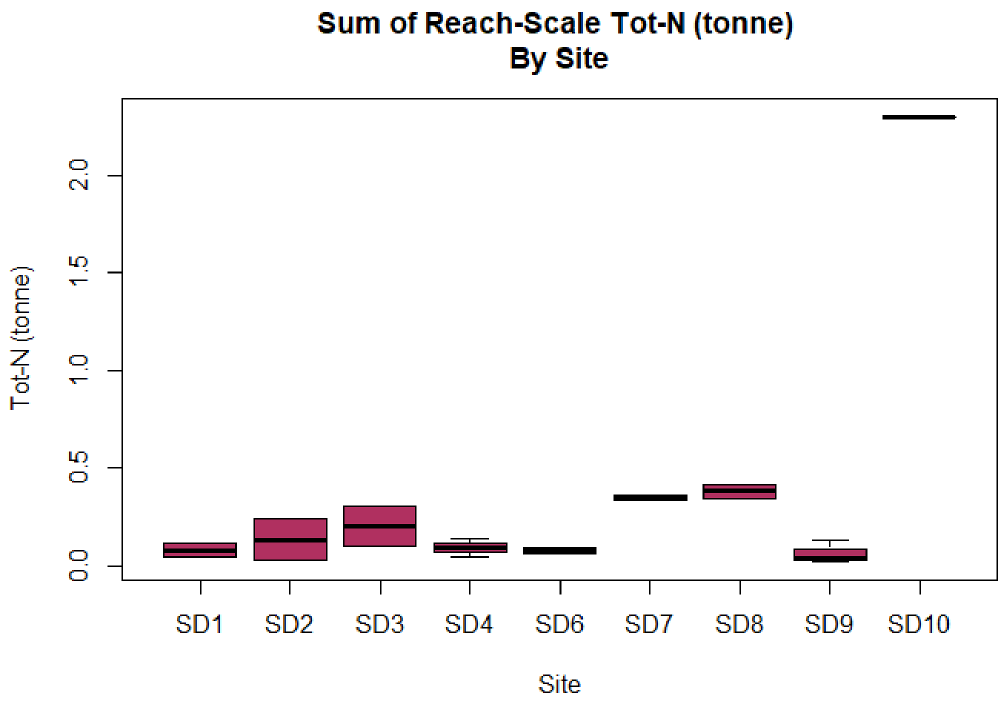

Figure 37. Total nitrogen uptake in tonnes for the entire reach of sites SD1-SD10. This is for all recorded macrophyte samples per site.

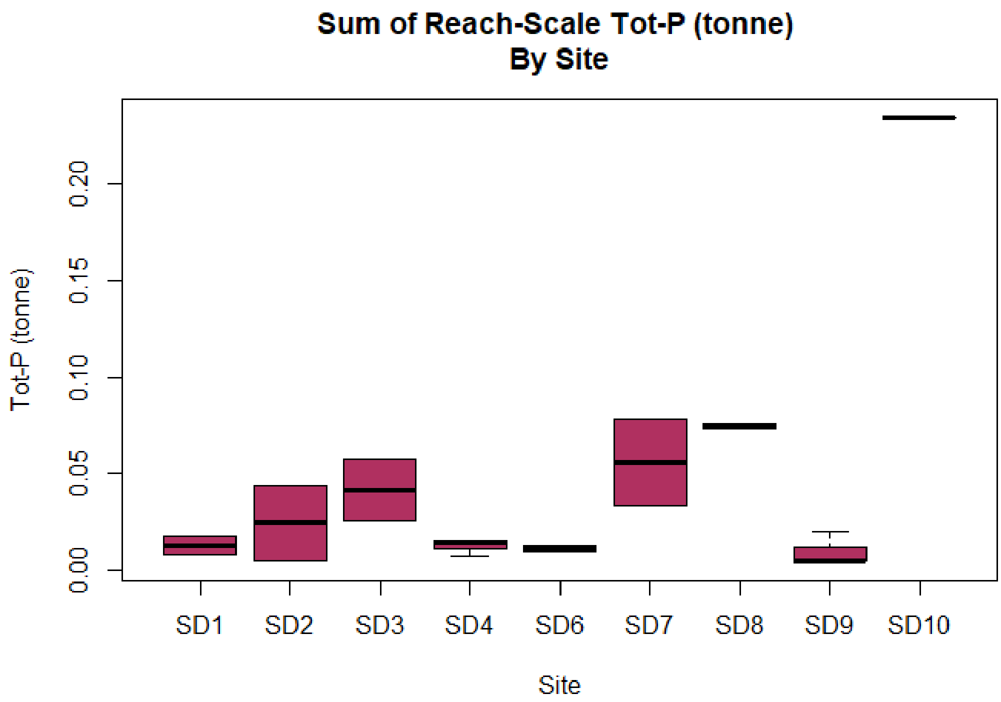

Figure 38. Total phosphorus uptake in tonnes for the entire reach of sites SD1-SD10. This is for all recorded macrophyte samples per site.

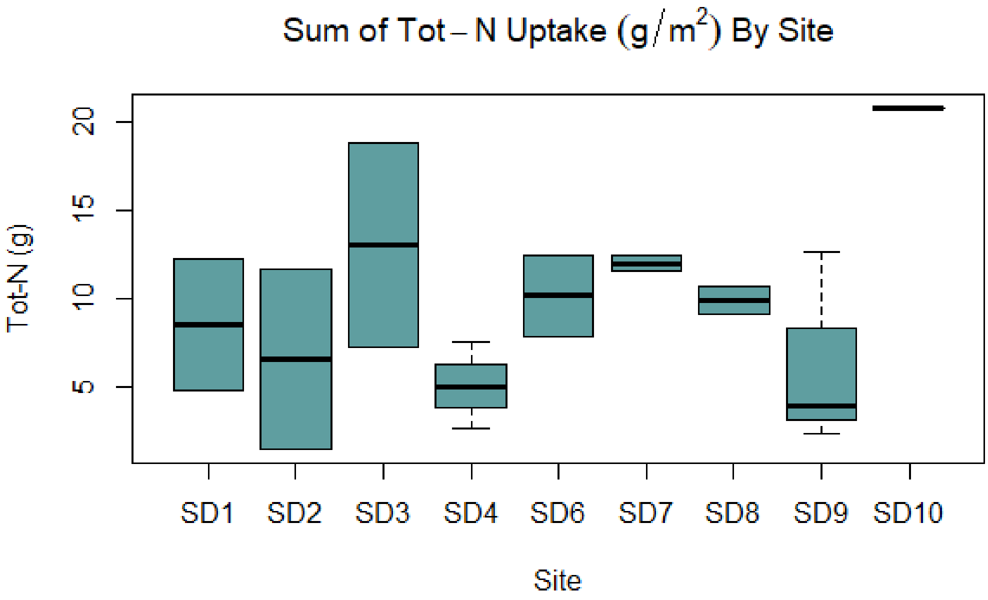

Figure 39. Total nitrogen uptake in g/m2 for the sites SD1-SD10 normalized. This is for all recorded macrophyte samples per site.

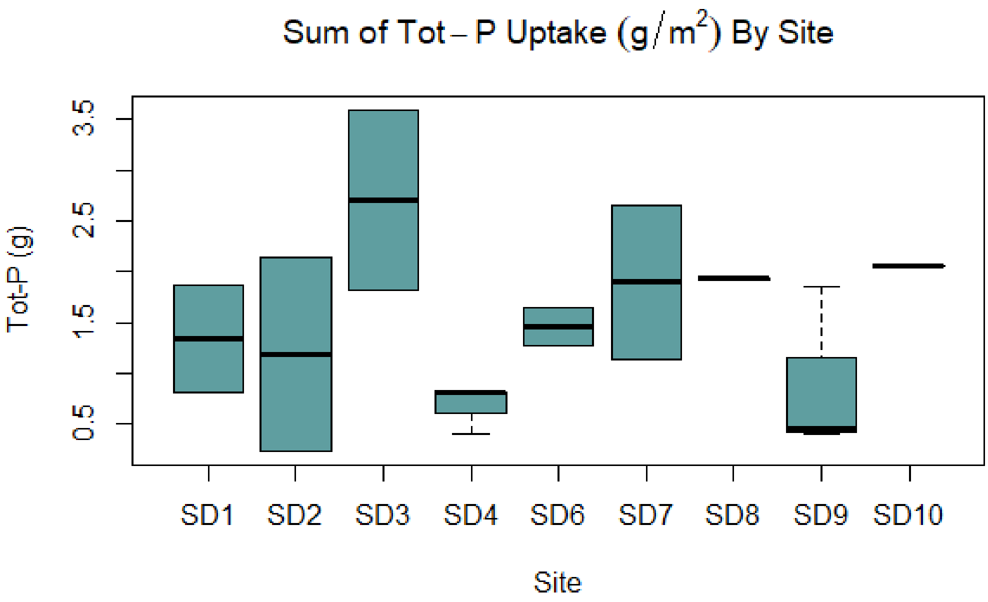

Figure 40. Total phosphorus uptake normalized in g/m2 for the entire reach of sites SD1-SD10. This is for all recorded macrophyte samples per site.

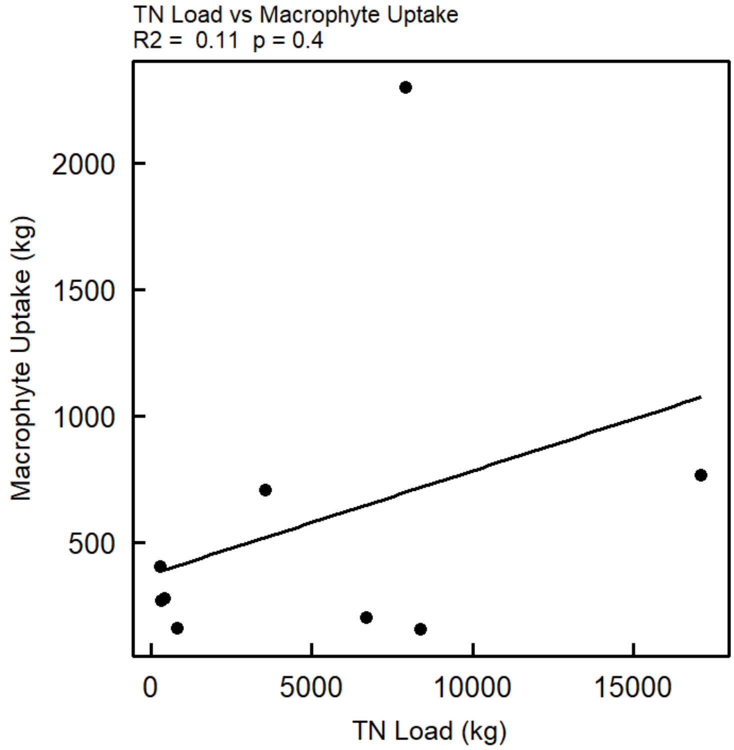

Figure 41. A scatterplot correlating the relationship between the influx of total nitrogen in the two-stage ditches and the uptake by the sampled macrophytes at those sites.

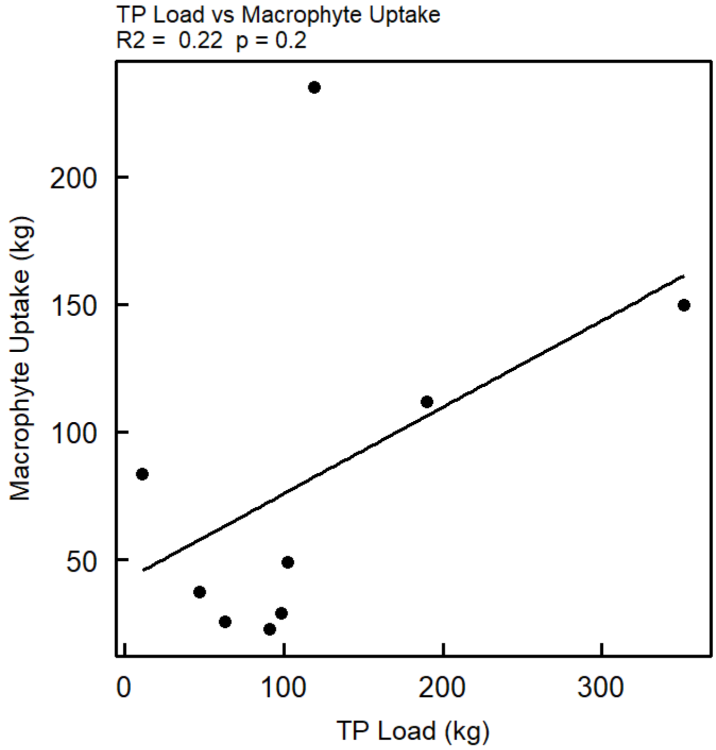

Figure 42. A scatterplot correlating the relationship between the influx of total phosphorus in the two-stage ditches and the uptake by the sampled macrophytes at those sites.

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