Bachelor Research Project Report, Biology, Leiden University

The effects of planting *Stratiotes aloides* L. on the water quality of ditches

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Abstract

This study is focused on finding the effects of the planting aquatic plant *Stratiotes aloides* L. on the water quality of ditches. This is important because results can help determine whether *S. aloides* could be used for phytoremediation of polluted bodies of water and if *S. aloides* might be able to decrease the effects of nutrient pollution. Research was carried out over three months by measuring eight indicators of water quality in four ditches without *S. aloides* and four ditches where *S. aloides* was planted at the start of this study (March 2023). These indicators include pH, electrolytic conductivity, dissolved oxygen, turbidity, phosphate-levels, nitrate-levels, chlorophyll-a, and chlorophyll-b. No short-term effects of *S. aloides* on water quality were found in the studied ditches. However, evidence in one ditch where *S. aloides* has been growing since 2022 indicates that after a longer period of time, *S. aloides* might have an effect on several of the water quality indicators measured in this study. Other applications for *S. aloides* are also promising. Studies have shown *S. aloides* might be able to improve biodiversity by offering protection and nesting opportunities for many (aquatic) organisms, and the plant could potentially be used as feed for livestock, as biomass to generate bioenergy, to create nutritional supplements or could even be used for medicinal purposes. Also, if a system of planting *S. aloides* around agricultural fields was implemented, *S. aloides* could be harvested every year and used as fertilizer for the surrounding fields, creating a circular, environmentally friendly agricultural system.

Introduction

Nutrient Pollution

Natural bodies of water throughout the world are displaying a pronounced and alarming decline in water quality (Hannah et al., 2022) (Kiliç, 2021) (Manoiu et al., 2022). Decreasing quality of freshwater ecosystems due to anthropogenic pollution is an important topic that needs to be addressed (Van Rees et al., 2020). Effects of both climate change and overpopulation heighten the urgency of this issue (Gobler, 2020) (Wurtsbaugh et al., 2019). Climate change can cause extreme weather conditions, increasing pollution of water bodies due to floods and surface runoff, whilst also increasing clean water demand due to extreme droughts (Nazari-Sharabian et al., 2018) (Van Rees et al., 2020). There are various types of chemical, biological, and physical water pollution that are harmful to the environment and human health (Lin et al., 2022) (Singh et al., 2019). Pollutants include nuclear waste, pharmaceutical chemicals, pesticides, agricultural fertilizers, plastic, heavy metals, toxic chemical compounds, and many other forms of inorganic and organic waste (Lin et al., 2022) (MacLeod et al., 20210 (Schweitzer & Noblet, 2018) (Shefali et al., 2020).

Nutrient pollution is a form of water pollution that occurs when an excess of nutrients ends up into bodies of water (Wang et al., 2023). Nutrient pollution arises is mainly caused by the discharge of wastewater from industries, urban areas, and agricultural practices, as well as the combustion of fossil fuels (Withers et al., 2014). This type of pollution poses a serious threat for freshwater communities. Over-fertilisation of agricultural fields with fertilisers and animal manure are the main sources of nutrient pollution in agriculture, causing excessive amounts of nutrients to end up in surrounding ditches, rivers, and lakes (Del Rossi et al., 2023). These nutrients mostly consist of nitrogen and phosphorus. This excess in nutrients causes algal blooms, which means that algae start reproducing rapidly (Withers et al., 2014). This increase in nutrients can have several negative effects on the water quality (Dorgham, 2014) (Nazari-Sharabian et al., 2018).

Firstly, algal blooms increase the turbidity of the aquatic environment, meaning light cannot reach aquatic plants that are found in deeper parts of the water. This will limit the growth of these aquatic plant populations, which will subsequently cause the macrofauna populations that depend on these plants to decrease as well (Rabalais, 2002). Secondly, algal blooms result in an increase of dead organic material which is used by aerobic bacteria, depleting dissolved oxygen in this process. This creates an anoxic and eutrophic system where many macro-organisms cannot survive El-Sheekh et al., 2021) Lastly, another problem that can occur due to an increase in nutrients is mass growth of cyanobacteria, phytoplankton that can produce toxins that are harmful for aquatic organisms (Moustaka-Gouni & Sommer, 2020) (Wang et al., 2023). These harmful blooms are expected to increase drastically in frequency in the coming decades due to climate change (O'Neil et al., 2012) (Wang et al., 2023). This combination of water toxicity, decreasing populations of submerged macrophytes and decreases in dissolved oxygen leads to decreases in biodiversity (Glibert, 2017). A decrease in nutrient pollution, especially a decrease in pollution with high concentrations of nitrogen and phosphorus, is seen as essential for stopping these algal blooms and all related problems (Su et al., 2019).

These effects of eutrophication are not only detrimental for the local aquatic ecosystems surrounding the sources of nutrient pollution (Bailey et al., 2020). Nutrient-rich water from streams and rivers eventually flows to coastal regions, where water toxicity and a decrease in dissolved oxygen can cause dead zones, which can also lead to severe decreases in biodiversity in those areas (Nazari-Sharabian et al., 2018) (Ngatia et al., 2019) (Wurtsbaugh et al., 2019).

Legislation

The European Union Water Framework Directive states that all bodies of water in the European Union should have an acceptable level of water quality by 2027 (Van Kats, 2022). It is important that the effects of nutrient pollution are decreased to reach these levels of water quality. It seems that in the Netherlands, the chances of reaching this goal are not high, and more action should be taken to increase biotic and abiotic water quality (Van Kats, 2022). Improved legislation is essential in decreasing and mitigating the effects of nutrient pollution (Sinha et al., 2019).

Phytoremediation

Laboratory as well as field studies show the potential of aquatic phytoremediation as a solution for several types of water pollution (Ansari et al., 2020) (Fletcher et al., 2019). The idea behind aquatic phytoremediation is that aquatic plants can be used for restoration of polluted bodies of water (Li, 2022) (Singh et al., 2021). Phytoremediation uses natural processes to help eliminate organic waste like excessive nutrients as well as inorganic waste like heavy metals (Petrov et al., 2023). Recent studies even explore the potential of using phytoremediation for removing antibiotics from wastewater to decrease antibiotic resistance (McCorquodale-Bauer et al., 2023). Phytoremediation is especially interesting because it is less expensive and less invasive than technological alternatives and because the biomass produced in the process could even be used for generating biofuels or as livestock feed (Liu et al., 2021) (Singh & Pant, 2023) (Wang et al., 2020). Although combining several applications seems ideal, some combinations should always be avoided. For example, it is not recommended to use plants simultaneously for removing antibiotics and livestock feed, as this might increase the occurrence of antibiotic resistance, which is already a large problem in livestock farming (He et al., 2020).

The most common mechanisms behind phytoremediation are decreasing pollution by taking up and accumulating pollutants or by discharging chemical compounds that can mitigate the effects of pollutants (Singh & Pant, 2023) (Tripathi et al., 2020). An example is the study of Li et al. (2023), which discusses that plants could be used for phytoremediation by taking up high amounts of cadmium which is toxic to humans and many other animals. Other studies even explore possibilities for commercialisation of phytoremediation in a process called phytomining, where valuable metal ions that are taken up by plants can be extracted and used (Saxena & Bharagava, 2019).

In the case of nutrient pollution, much research has already been conducted to show how phytoremediation could be used for removal of excessive nutrients like phosphate and nitrate, therefore preventing algal blooms (Li et al., 2021) (Liu et al., 2021) (Singh et al. 2022). This approach is effective because plants use large amounts of phosphorus and nitrogen for growth and physiological processes, as both elements play a key role in photosynthesis, synthesis of DNA and RNA, structural development, and the general metabolism of plants (Dean et al., 2022). Previous research demonstrates that phytoremediation can successfully mitigate the effects of nutrient pollution by harnessing the ability of certain plant species to mimic the natural filtration function that wetlands inherently possess (Chen & Costa, 2020) (Goodson & Aziz, 2023).

Water quality indicators

After applying aquatic phytoremediation techniques, water quality indicators are used to test the effectiveness of the treatments (Nahar & Hoque, 2021). There are many different ways to measure water quality. The following eight indicators are used most often for measuring water quality, based on previous research on water quality levels and phytoremediation. These indicators are acidity (pH), electrolytic conductivity, dissolved oxygen levels, turbidity, NO_3-levels, PO_4-levels, chlorophyll-a, and chlorophyll-b (Agarin et al., 2020) (Amyati et al., 2020) (Dewi et al., 2020) (Sarkheil & Safari, 2020) (Zou et al., 2020)

The pH-value (acidity) of water is an indicator for how many hydrogen ions are present in a liquid, with a lower pH-value indicating more hydrogen ions in a substance (Omer, 2019). The pH-value is often used as an indicator for water quality and can also be an indication for the type of organisms that can be found in a body of water (Kannel et al., 2007) (Pereira et al., 2012). Most regulations state that the pH-value of water should range from 6.5 to 8.5 for drinking water and natural bodies of water (Boyacioglu, 2009) (World Health Organization, 2017). Strong alkalinity or acidity can also be lethal to many aquatic organisms (Omer, 2019).

Electrolytic or electrical conductivity (EC) is an indicator often used in research related to water quality (Puczko et al., 2018) (Sarkheil & Safari, 2020). It is a measure for the amount of dissolved ionic particles (Bhuyan et al., 2018). A variety of inorganic or organic compounds, microorganisms and toxic substances can cause electrolytic conductivity to increase (Sohail et al., 2022). A higher electrolytic conductivity indicates more dissolved particles, so if EC values are higher, the water quality is generally poorer (Bhuyan et al., 2018).

Dissolved oxygen is the concentration of oxygen that is dissolved in the water. The oxygen in the water bodies is received from aquatic plants, from phytoplankton, and from the atmosphere (Rahman et al., 2022). Although phytoplankton populations initially produce oxygen, larger algal blooms cause an excess of dead organic material, which is decomposed by aerobic microorganisms in water, resulting in low oxygen levels because those processes consume dissolved oxygen (El-Sheekh et al., 2021) (Schwarzenbach, 2010). Dissolved oxygen is an important indicator of water quality because most aquatic macrofauna are dependent on dissolved oxygen to survive (Kannel et al., 2007). According to the Universal Water Quality Index (UQWI) of Boyacioglu (2009), dissolved oxygen levels in bodies of water should always be higher than 8 mg/l.

The turbidity is a measure used for the clarity of water and is used as an indicator of potential pollution in bodies of water (Zou et al., 2020) (Stroud Water Research Center, 2022). There are many materials that can cause water to become turbid, including clay, silt, tiny inorganic and organic matter, algae and dissolved coloured organic compounds (Velthuis et al., 2023). If bodies of water become turbid due to high concentrations of pollutants, it will affect the light penetration. If light fails to penetrate the water, populations of aquatic macrophytes that grow below the surface will suffer (Stroud Water Research Center, 2022). The decline in populations of these plants will negatively affect aquatic ecosystems because of a decline in food, shelter, nesting opportunities and dissolved oxygen for macrofauna (Manca et al., 2022).

Chlorophyll-a and chlorophyll-b levels are often used in research on water quality as an indication for phytoplankton levels. Chlorophyll-a and b are both found in chloroplasts of most algae and plants (Agarin et al., 2020) (Mishra et al., 2020). Chlorophyll-levels are used as an indicator for water quality because the level of chlorophyll gives a good indication for the size of phytoplankton populations, which gives an indication for the likeliness that harmful algal blooms might occur (Zou et al., 2020). Chlorophyll-a is always more abundantly present in photosynthetic organisms and chlorophyll-b is produced in smaller amounts as a derivation from chlorophyll-a to bind and stabilize essential proteins in a later part of the photosynthetic cycle (Tanaka & Tanaka, 2011). Water bodies with high levels of nutrients from fertilisers, like agricultural ditches, normally also show high levels of chlorophyll-a and chlorophyll-b because of larger phytoplankton populations (*Indicators: Chlorophyll a | US EPA*, 2022).

Nutrient levels are also very important indicators for water quality because high nutrient levels can cause algal blooms, which eventually leads to poorer water quality (Nazari-Sharabian et al., 2018). In terms of eutrophication, nutrient levels are generally measured by measuring nitrate (NO_3) and phosphate (PO_4) levels (Zou et al., 2020). Plants use nitrogen and phosphorus for various physiological processes, so these elements are often used as essential components of fertilizers for agricultural fields (Dean et al., 2022). Through surface runoff induced by precipitation and wind, these nutrients end up polluting surrounding bodies of water (Khan & Mohammad, 2014) (Withers et al., 2014). If phytoremediation techniques are able to decrease nutrient levels in ditches of water, this indicates that the technique might be effective in preventing algal blooms and its effects.

Stratiotes aloides

Stratiotes aloides is an aquatic plant that is native to Europe and Central Asia and used to be found extensively in bodies of water in the Netherlands. It is mainly found in nutrient-rich water and is more commonly known as “water soldier” due to the serrated structure of its leaves (POWO, n.d.) (Mulderij et al., 2005). These leaves of *S. aloides* have been observed to provide protection from predators for several species, including microcrustaceans and larvae of dragonfly *Aeshna viridis* (Rantala et al., 2004) (Strzałek & Koperski, 2019). *S. aloides* is also very important for black terns (*Chlidonias niger*), as *S. aloides* provides nesting opportunities (Beintema et al., 2010).

S. aloides has the ability to take up relatively large amounts of metal ions and because the plants contain many nutrients and antioxidative compounds (Gawlik-Dziki et al., 2020). This is why the plant has been used in Poland throughout history as food, fertilizer and for its medicinal properties in treating metal-inflicted wounds (Gałczyńska et al., 2011) (Gawlik-Dziki et al., 2020). The population of *Stratiotes aloides* has been declining in the Netherlands, presumably because of a combination of several factors, including limited access to light and dissolved iron, extreme levels of sulphide and ammonium, competition, and spatial isolation (Smolders et al., 2003) (Efremov et al., 2019). Although the plant reproduces vegetatively very quickly, *S. aloides* is a native species in the Netherlands, so planting these species is not likely to have a negative ecological effect on its surrounding environment, and the plant could be harvested and removed very easily if it were to have a negative impact on its environment. Because *S. aloides* populations grow very quickly, a possible application in the future could also be using the biomass for generating biofuels (Arefin et al., 2021).

Phytoplankton

In 1979, a study conducted by E. S. Brammer showed that the water surrounding *Stratiotes aloides* always contains considerably lower levels of phytoplankton populations. This study theorised that this was due to nutrient competition and changes in ionic composition in the water. The uptake of nutrients and precipitation of phosphorus with calcium carbonate from the leaves of *S. aloides* was given as possible explanations (Brammer, 1979). Another study by Brammer and Wetzel (1984) has also shown that. *S. aloides* extracts nutrients from the water, including phosphorus, nitrogen, potassium, calcium, and sodium. However, a study by Mulderij et al. (2005) disputes this by claiming that water where *S. aloides* is found is mostly very clear and high in nutrients, which indicates that nutrient competition could not be a plausible explanation, as there appears to be an abundant supply of nutrients for phytoplankton to use as well. This study by Mulderij et al. (2005) and several other studies indicate that allelopathic inhibition is the best possible explanation for the negative effect on phytoplankton. This means that there would be a negative influence of this plant on phytoplankton through the release of chemical compounds in the surrounding water (Mulderij et al., 2006). This study by Mulderij, Smolders and van Donk (2006) revealed significantly lower phytoplankton biomass when exposed to water where S. aloides had grown. Light competition has mainly not been considered as a cause for the negative effect of *S. aloides* on phytoplankton, because both cover the water surface, and phytoplankton even occur on the surface earlier in the season than *S. aloides* (Mulderij et al., 2009). This means that *S. aloides* is not likely to have a negative influence on amount of light that phytoplankton receive. The results of all of these studies contribute to the theory that *S. aloides* could potentially help prevent algal blooms, and the effect of *S. aloides* is most likely a combination of nutrient competition and allelopathy (Mulderij et al., 2007). These studies have shown that *S. aloides* might prevent algal blooms by reducing phytoplankton populations, but this has not yet been tested on a larger scale in field studies. Moreover, there are many more factors that contribute to water quality besides phytoplankton populations, and it is not yet known whether *S. aloides* could effectively be used for phytoremediation by also improving other aspects of water quality besides phytoplankton populations.

Aim

The aim of this research is to carry out measurements on water quality indicators to find out whether a certain aquatic plant, *Stratiotes aloides*, could be used for phytoremediation, so if the water quality will improve in the area after planting species in ditches. If this research shows improvements in water quality indicators, *Stratiotes aloides* could potentially be used for phytoremediation as a solution for the problems that the Netherlands faces regarding decreasing levels of water quality and increasing occurrences of harmful algal blooms.

Experimental design

Research is conducted in ten ditches in field laboratory “Polderlab Vrouw Venne” in Oud Ade, in the Netherlands. Figure 1 (p.8) shows a schematical overview of the location of the ditches in the field laboratory. This area has the required conditions for *S. aloides* to grow, but no natural populations are present. In March 2023, *S. aloides* was planted in four of the ditches, whilst five other ditches were used as control group. In the remaining ditch, *Stratiotes aloides* had already been planted in

March 2022, one year prior to this research. As *S. aloides* has already been growing in this ditch for a year, it is used to formulate hypotheses on the effect of *S. aloides* on water quality, but not included in statistical analyses.
In each ditch, the following eight indicators of water quality are measured weekly from April till June: acidity, electrolytic conductivity, dissolved oxygen, turbidity, NO_3-levels, PO_4-levels, chlorophyll-a, and chlorophyll-b. Environmental factors including weather conditions (ambient temperature, wind strength, sunshine) and the temperature of the water in the ditch are also documented.

Hypothesis

It is expected that the water quality of ditches where *Stratiotes aloides* is growing during this study will improve in the following ways. First of all, decreasing turbidity, lower electrolytic conductivity and decreasing levels of nitrogen and phosphorus are expected because *S. aloides* seems to be able to take up many dissolved nutrients and metal ions. Second, a decrease in chlorophyll-a and chlorophyll-b because of allelopathic inhibition of phytoplankton by *S. aloides* and nutrient competition. Last, an increase in dissolved oxygen because *S. aloides* plants will produce oxygen during photosynthesis and because of lower rates of decomposition by aerobic bacteria, because decreasing populations of phytoplankton will cause decreasing amounts of dead organic material to be degraded by those bacteria. Figure 2 (p. 9) shows a schematical overview of the expected biological and chemical effects of planting *S. aloides* in ditches.
It is necessary to include that the measurements will be conducted over a relatively short period of time, which means that there is a sizeable chance that the period of adjustment necessary after planting *Stratiotes aloides* is too long to see already significant differences in water quality over this short period of time.

Figure 1. Area of "Polderlab Vrouw Venne" in Oud Ade. Green circles indicate the places where the measurements have been conducted where S. aloides *is planted (M) and the red crosses represent the places where the control group (Z) measurements have been conducted. Ditch 1M already contained* S. aloides *before this study, and has been used for the formulation of hypotheses, but is not used for statistical analysis.*

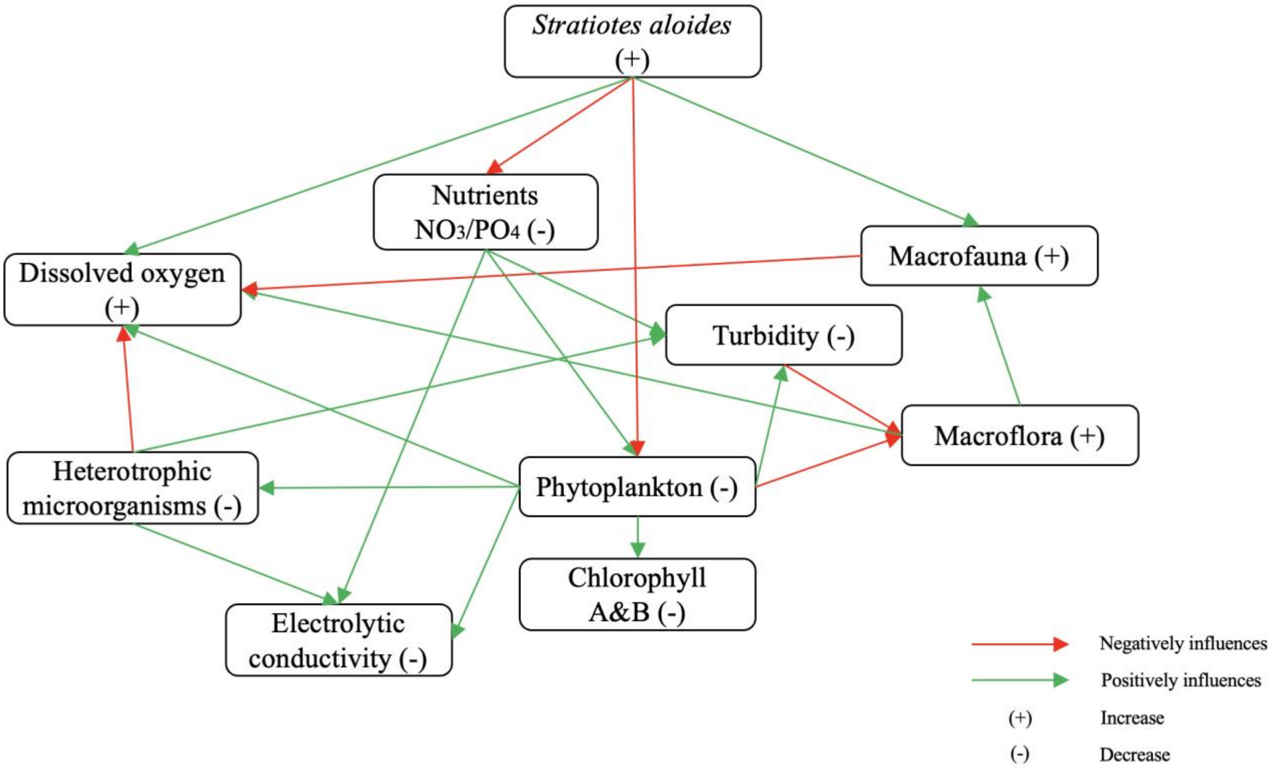

Figure 2. Schematic overview of the expected effect of S. aloides *on the surrounding aquatic environment. Red arrows indicate a negative influence and green arrows indicate a positive influence. This scenario starts with an increase in* S. aloides *in an aquatic environment.*

Materials

- Aquatic plant *Stratiotes aloides* L.
- HI98130 Combi pH, EC/TDS and temperature Tester, Hanna Instruments
- Phosphate (PO_4) Kyoritsu PackTest
- Nitrate (NO_3) Kyoritsu PackTest
- Hach Lange HQ40d Portable pH, Conductivity, Dissolved Oxygen, ORP, and ISE Multi-Parameter Meter
- AquaFluor™ Handheld Fluorometer/Turbidimeter
- Secchi Transparency Tube

Methods

Experimental design

Ten ditches in field laboratory “Polderlab Vrouw Venne” in Oud Ade, the Netherlands, are used for this research. These ditches are around 60-100 meters long and have an average breadth of 3-4 meters. The area consists of peatland, serving as a research site as well as pasture for livestock farming and horticulture (*Oud Ade – Land Van Ons*, n.d.). There are many natural populations of birds present that are typical for Dutch grasslands and farmlands, like swans, herons, geese, black-tailed godwits, lapwings, and mallards. The area of Land van Ons is used for this research because it has the required conditions for *S. aloides* to grow, but no natural populations are present. Eight indicators are measured in the ditches. These include acidity (pH), electrolytic conductivity, dissolved oxygen, turbidity, PO_4-concentrations, NO_3-concentrations, chlorophyll-a levels, and chlorophyll-b levels. Environmental factors are also considered, like weather conditions (ambient temperature, wind strength, sunshine), current, temperature of the ditch, and the presence of other plant/animal species that might affect water quality.

The ten ditches that are used are all roughly equal in size and depth. Figure 1 (p.8) indicates the location of the ten ditches on the map of the area. Five of the ditches contain *Stratiotes aloides* whilst the other five ditches are used as control group. In four of the ditches, *S. aloides* was planted in March 2023 and in one ditch *S. aloides* was planted one year prior to this research, in 2022. The remaining five ditches do not contain *S. aloides*, functioning as a control group. This research will compare the four ditches where *S. aloides* was planted in March with four ditches from the control group. The distribution of these ditches was chosen to ensure a well-balanced experimental design (Figure 1). The name of each ditch indicates which pair of ditches it belongs to (1-5) and whether *S. aloides* was planted (M) or not (Z). The ditch where *S. aloides* has been planted one year prior to this study is named 1M. This ditch will be excluded from statistical analysis and only used for formulating hypotheses on the effect of *S. aloides.*

The first three pairs of ditches are separated from each other through physical barriers. Ditch 4Z and 4M, and 5M and 5Z are technically connected, but measurements are conducted at large distances from each other and the current in the stream never flows from 4M to 4Z, or from 5M to 5Z so it is not possible for *S. aloides* plants to influence the water quality of the ditches where it is not planted.

Baseline measurements on the indicators will be conducted before planting to ensure that the chosen pairs of ditches are compatible and to see if the ditch where *S. aloides* had already been planted in previous research in 2022 differed from the other ditches before planting. Testing if ditches are all comparable for each indicator at the start of the experiment ensures that if differences are found in this study, they could more likely be attributed to the presence of *S. aloides*. Baseline measurements will also be used to formulate hypotheses on the effect of *S. aloides*, based on the ditch where *S. aloides* had been planted one year prior, in 2022.

Methods for planting *Stratiotes aloides* L.

An area of 1.5 metres left and 1.5 metres right from the marked wooden posts was covered with aquatic plants along the full breadth of the ditches (3-4 meters). *S. aloides* was extracted from ditches around the area of Meije, the Netherlands, and then transported to the field laboratory in Oud Ade. Enough of *S. aloides* was planted to cover the full bottom of the ditches of this area. The average size of *S. aloides* is around 30 by 30 cm, and it was ensured that there was enough space between the plants, resulting in around 60 plants per ditch.

However, not all plants will survive and thrive in this environment, and it is expected that around 50 plants will be able to survive. As plant populations of *S. aloides* grow very quickly, this is most likely sufficient as a starting point. The month March was chosen for planting because in March the *S. aloides* has already partly recovered from the strain that winter has exerted on the plants, so they can be removed from their original environment without inflicting too much damage. Also, in March, the plants are still submerged, but already have young shoots that can grow and thrive in the new ditches.

Methods for measuring indicators

Measurement of pH, temperature, and electrolytic conductivity

The pH, temperature, and electrolytic conductivity (EC) was measured with the HI98130 Tester. EC and pH-measurements are automatically temperature-compensated (ATC), guaranteeing accurate results (Hanna Instruments, n.d.). The instrument was held in the water until the value of the indicator was stable, after which the value was read directly from the screen. The values for all these indicators were measured twice for each ditch, and the average of those measurements was used for statistical analysis to decrease the chance of measurement errors.

Measurement of levels of NO_3 and PO_4 levels

Phosphate (PO_4) Kyoritsu PackTests and Nitrate (NO_3) Kyoritsu PackTests were used to measure the levels of NO_3 and PO_4. The undermentioned protocols according to Kyoritsu Electrical Instruments Works, Ltd. were followed (*Kyoritsu PackTest Instructions Nitrate*, n.d.) (*Kyoritsu PackTest Instructions Phosphate*, n.d.). First, a sample of water from the ditch was collected and used to fill a cuvette of 1,5 mL. After that, the green line at the top of the transparent tube with powder (for either NO_3 or PO_4) was removed to clear the opening so air could get out of the tube. The sides of the tube were pressed to expel half of the volume and then the tube was put into the sample with the sides still pressed. Then, the sides were released to fill the tube up to half of the volume (1.5 mL). The tube needed to be shaken a few times, until the powder was dissolved. After one minute for phosphate and after three minutes for nitrate, the tube was compared to colour charts to determine the value (*Kyoritsu PackTest Instructions Nitrate,* n.d.) (*Kyoritsu PackTest Instructions Phosphate*, n.d.).

Measurement of dissolved oxygen levels

A Hach Lange HQ40d Portable pH, Conductivity, Dissolved Oxygen, ORP, and ISE Multi-Parameter Meter was used to measure the dissolved oxygen levels of the water. It was held in the water until the value was stable, after which the value was read directly from the screen. The measurements were carried out twice for each ditch, using the average of those measurements for statistical analysis to decrease the chances of errors.

Measurement of turbidity

The turbidity of water was measured with a Secchi Transparency Tube. A Secchi Transparency Tube is a tube with a Secchi disk at the bottom with alternating black and white quarters. The tube was filled with sample water until the difference between the black and white quarters could not be determined anymore (*North American Lake Management Society (NALMS)*, 2019). This depth is called the Secchi depth and this method is a standardized indicator for the transparency of the water (*North American Lake Management Society (NALMS)*, 2019).

Measurement of levels of chlorophyll-a and chlorophyll-b

The AquaFluor Handheld Fluorometer/Turbidimeter was used to measure the levels of chlorophyll-a and chlorophyll-b. A 1,5 mL cuvette was filled with the sample water and placed in the sample compartment. The values for chlorophyll-a and b were read directly from the screen. Each measurement was repeated twice, using the averages for statistical analysis.

Statistical analysis

Analysis of baseline measurements

Two types of t-tests were carried out for each indicator at the start of this study. The first type of t-tests was carried out for each indicator to test if ditches 1Z, 2Z, 2M, 3Z, 3M, 4Z, 4M, 5Z and 5M were all comparable before starting the experiment. The second type of t-test that was carried out was used to compare the values of the water quality indicators in ditch 1M, where *S. aloides* had already been planted one year ago in 2022, to the values of the indicators of all other ditches.

Analysis of differences between treatment ditches and control ditches

Linear mixed models were used to compare four control ditches (1Z, 2Z, 3Z, and 4Z without *S. aloides*) and three treatment ditches (2M, 3M and 4M with *S. aloides* planted in March 2023) with each other. Linear mixed models are often used in studies related to phytoremediation, as they are able to take into account that discovered differences might have been due to seasonal differences, differences in water temperature or inherent differences between measuring sites (Balázs et al., 2018) (Gervais-Bergeron et al., 2021) (Wang et al., 2021).

Ditch 1M is not used in these linear mixed models because *S. aloides* has already been growing there for one year. During the research, as is further explained in the discussion, ditch 5Z and 5M have been deemed unfit for measurements due to the smaller size and shallow depth of the ditches. *S. aloides* was unable to grow in this aquatic environment, therefore the results of those ditches have been excluded from the statistical analysis.

Analysis of differences between ditch 1M and control ditches

Linear mixed models were also created for comparing ditch 1M to four control ditches. However, as one data point is not enough to ensure reliability, instead of using these linear models, visualisations were created to illustrate differences between ditch 1M, three treatment ditches and four control ditches.

Results

Statistical analysis

Baseline measurements

Baseline measurements showed that the studied ditches did not differ significantly from each other, except for the ditch 1M where *S. aloides* had been planted one year prior to this study. When analysing the baseline measurements, this ditch showed significant differences with regards to electrolytic conductivity, dissolved oxygen, turbidity, PO_4-levels, chlorophyll-a, and chlorophyll-b. Visualisations and p-values for all t-tests are found in Appendix A and B. The results for NO_3-levels were inconclusive because NO_3-concentrations were too small to be measured with the material used in this study.

Comparison of treatment ditches and control ditches

There were no significant differences found for any of the indicators, so no significant differences for dissolved oxygen, phosphate, pH, electrolytic conductivity, turbidity, chlorophyll-a, or chlorophyll-b. The results for NO_3-levels were inconclusive. Visualisations below show the values for the indicators in control group and treatment group (ditches where *S. aloides* was planted in March 2023) in the weeks that measurements were conducted. The results of the linear mixed models used to determine the effect that *S. aloides* has had since planting in March are also indicated below. All linear mixed models do not include ditch 1M.

Comparison of ditch 1M and control ditches

Visualisations below show the differences between ditch 1M, three treatment ditches and four control ditches during the weeks in which measurements were conducted (April till June).

Acidity (pH)

The results of the linear mixed model that compares the control group (no *S. aloides*) to the treatment group (containing ditches where *S. aloides* was planted March 2023) revealed a p-value of 0.977 for the short-term influence of *S. aloides* on pH.

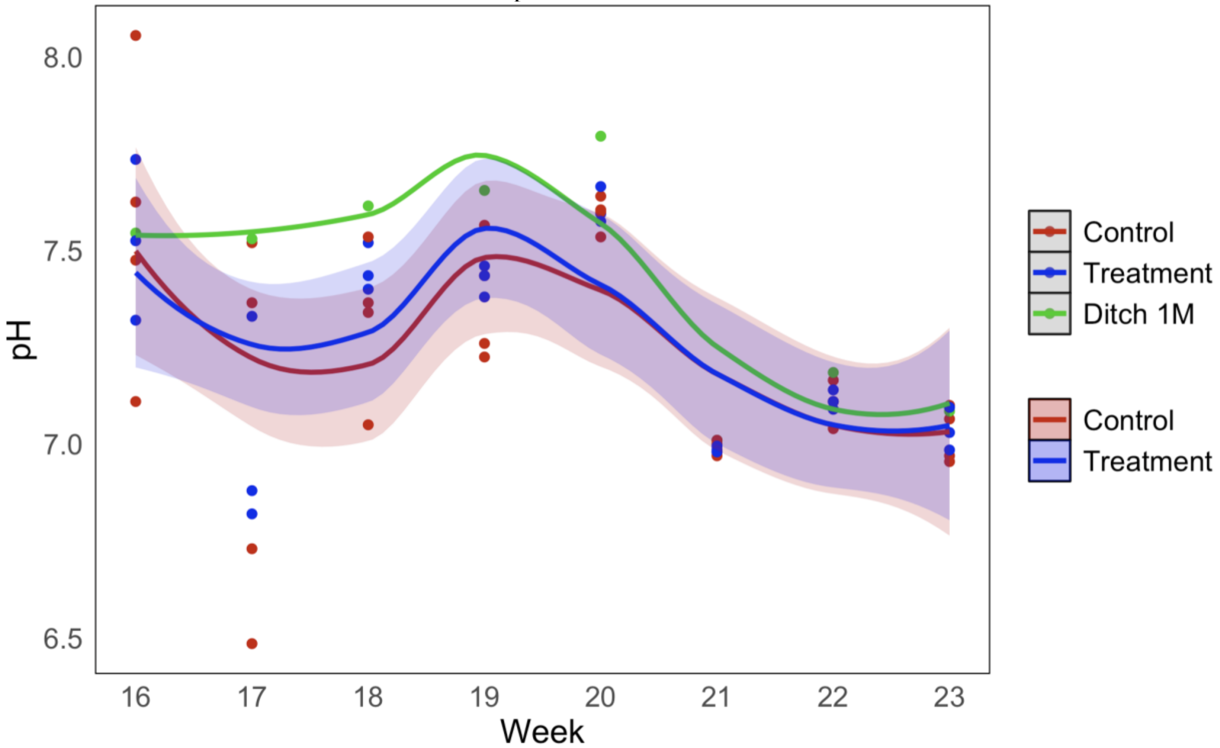

Figure 3. This graph shows the fluctuations in pH-values for the control group without S. aloides *(red), the treatment group in which* S. aloides *has been planted in March 2023 (blue), and Ditch 1M where* S. aloides *was planted one year prior to the start of this study (green). Measurements were conducted from April (week 16) till June (week 23).*

Electrolytic conductivity

The results of the linear mixed model demonstrated a p-value of 0.845 for the short-term influence of *S. aloides* on electrolytic conductivity after planting in March 2023.

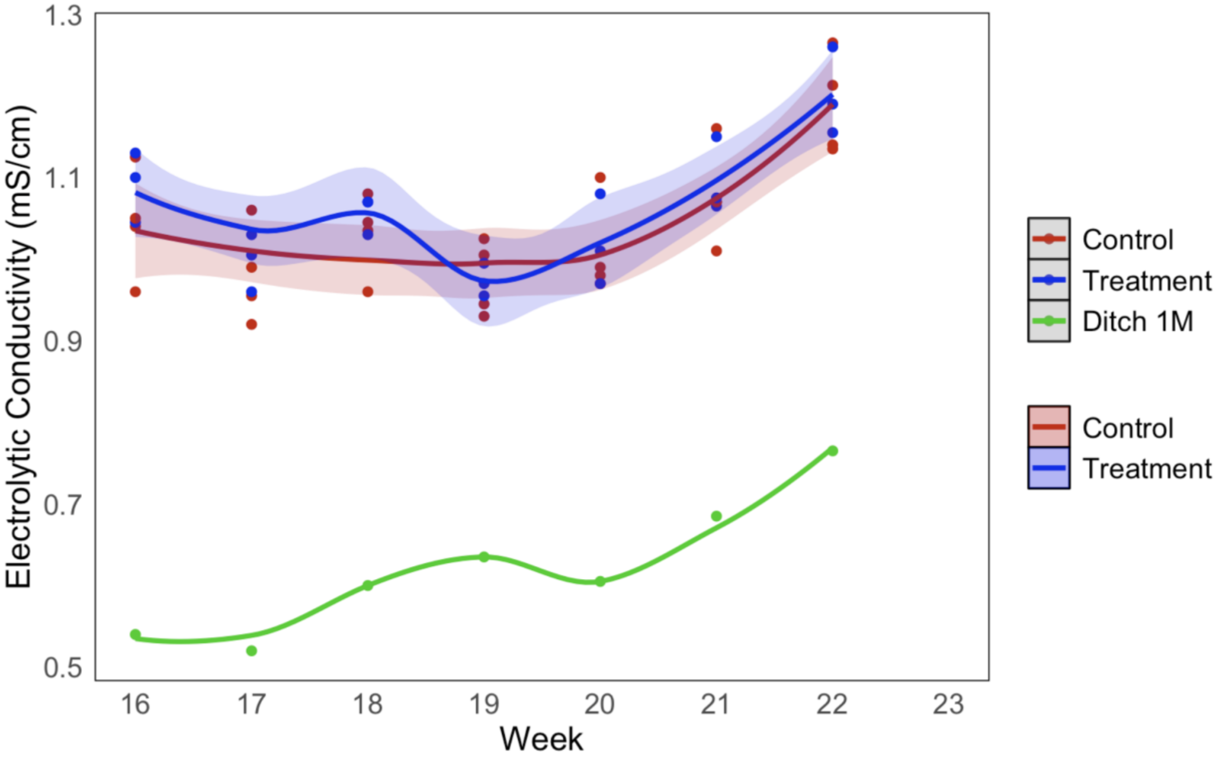

Figure 4. This graph shows the weekly changes in electrolytic conductivity for the control group, the treatment group in which S. aloides *has been planted in March 2023, and Ditch 1M where* S. aloides *was planted one year ago.*

Dissolved oxygen

The results of the linear mixed model showed a p-value of 0.226 for the short-term influence of *S. aloides* on dissolved oxygen levels.

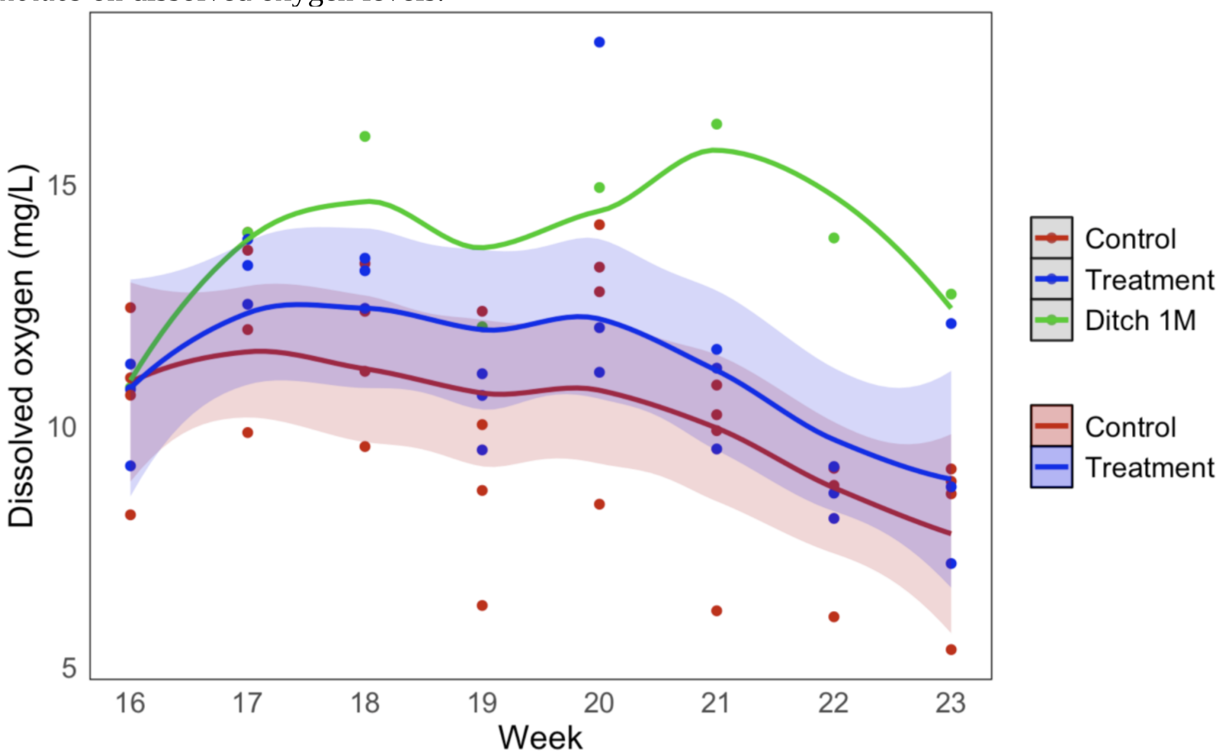

Figure 5. This graph shows the levels of dissolved oxygen for the control group, the treatment group and Ditch 1M where S. aloides *was planted one year ago.*

Turbidity
The results of the linear mixed model revealed a p-value of 0.164 for the short-term influence of *S. aloides* on turbidity.

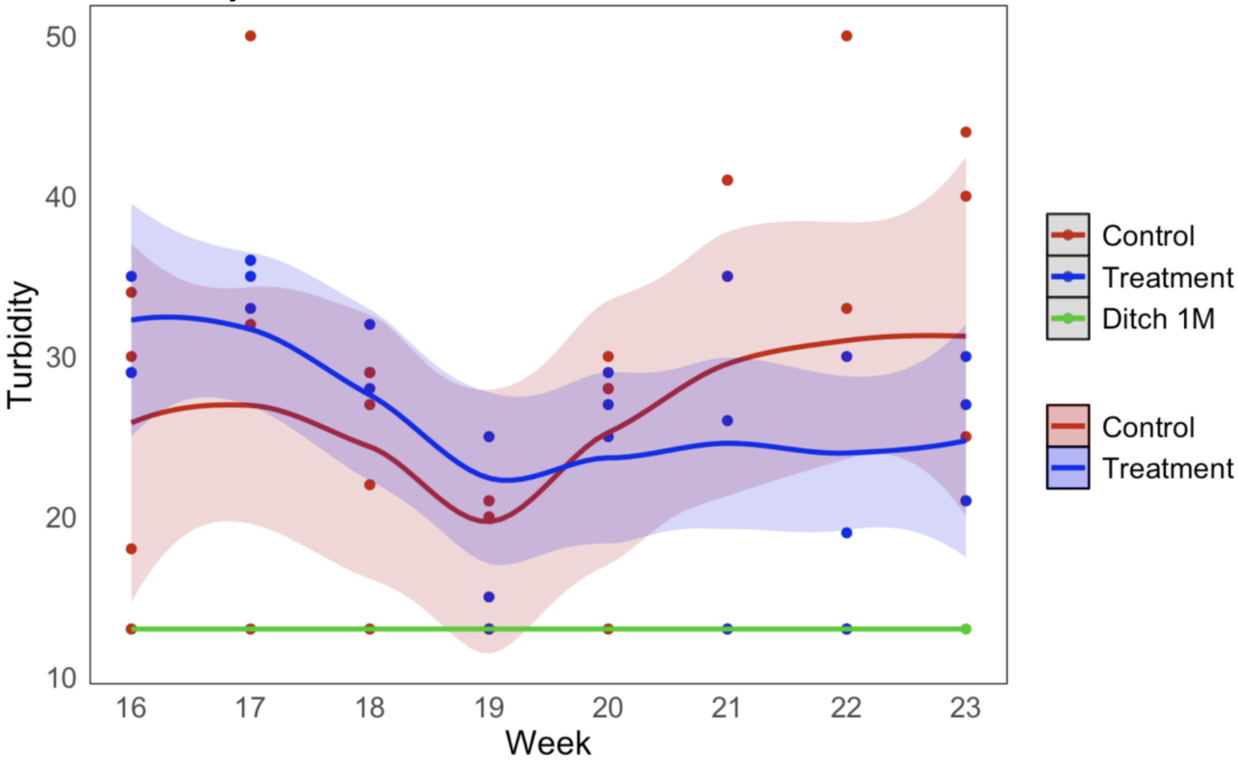

Figure 6. This graph shows the levels of turbidity for the control group, the treatment group and Ditch 1M for each week that measurements were conducted.

Phosphate
The results of the linear mixed model demonstrated a p-value of 0.521 for the short-term influence of *S. aloides* on phosphate levels.

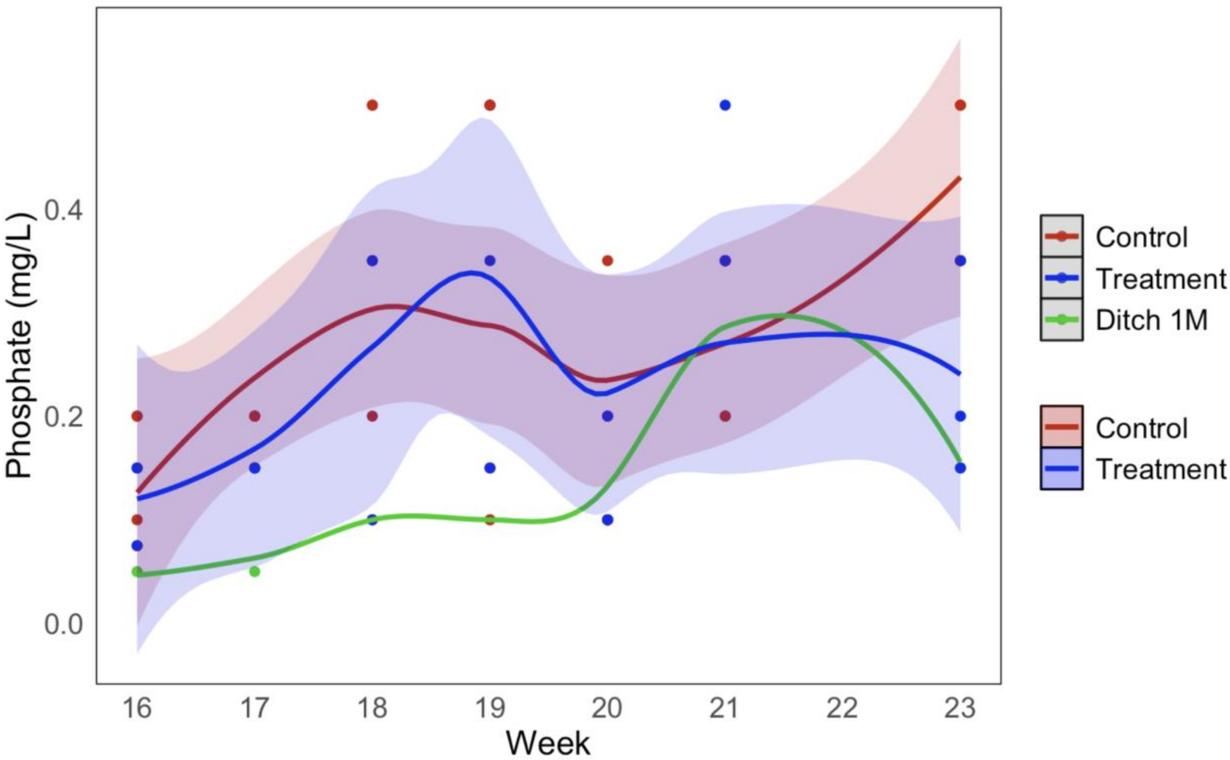

Figure 7. This graph shows the levels of phosphate for the control group, the treatment group and Ditch 1M.

Chlorophyll-a

The results of the linear mixed model showed a p-value of 0.804 for the short-term influence of *S. aloides* on chlorophyll-a.

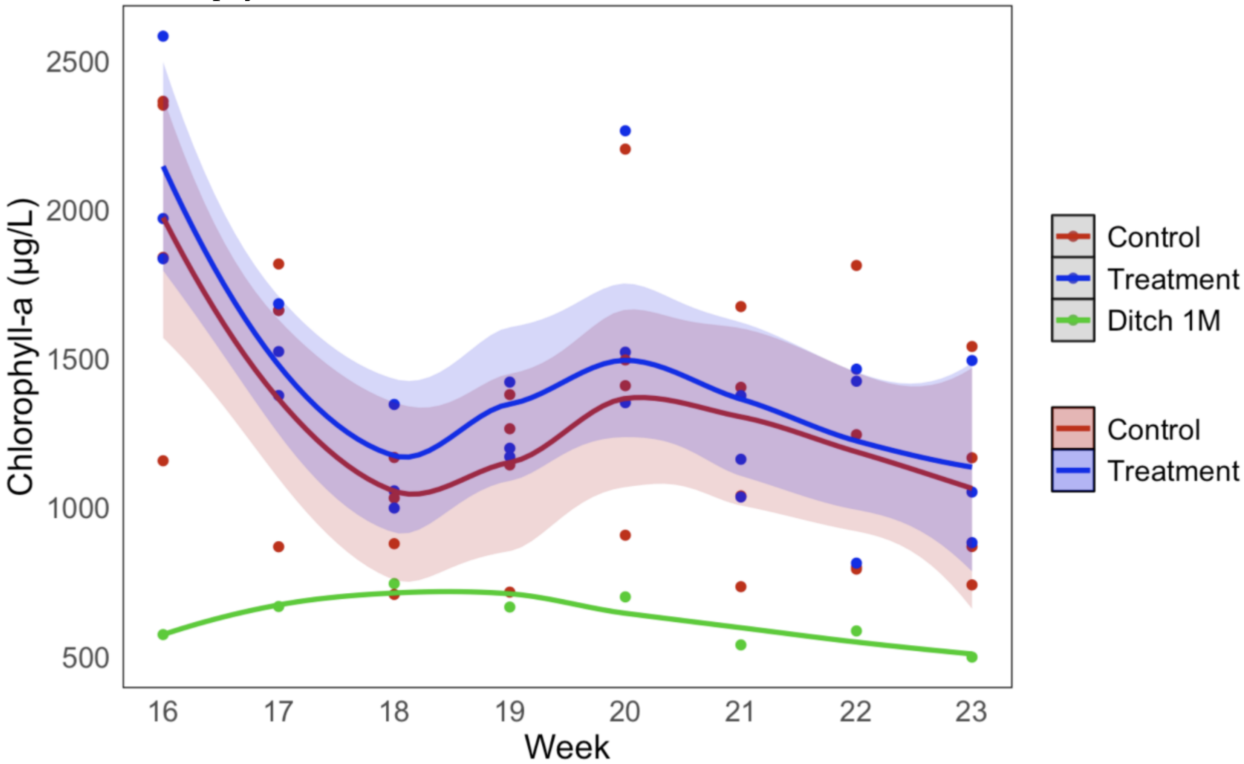

Figure 8. This graph shows the levels of chlorophyll-a for the treatment group, the control group and Ditch 1M.

Chlorophyll-b

The results of the linear mixed model demonstrated a p-value of 0.241 for the short-term influence of *S. aloides* on chlorophyll-b.

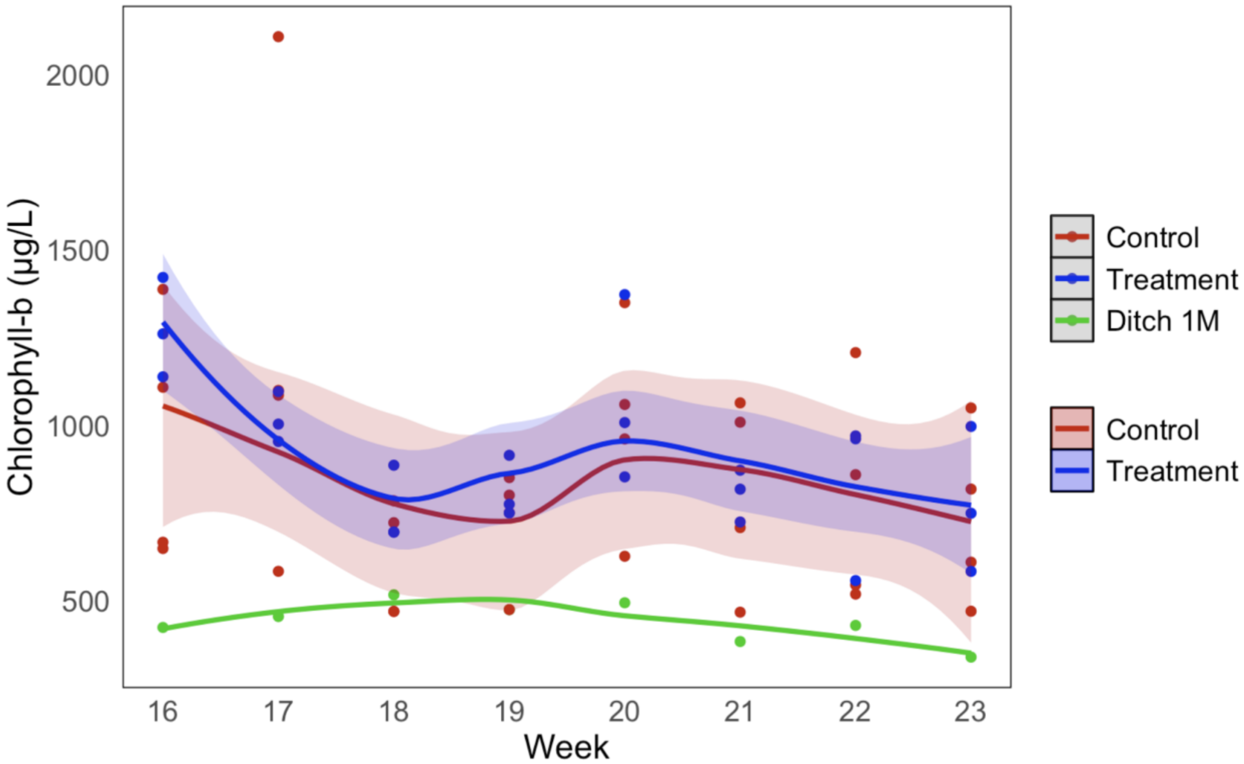

Figure 9. This graph shows levels of chlorophyll-b for the treatment group, the control group and Ditch 1M.

Discussion

Key findings indicators

The results have shown that there are no significant differences yet for any of the indicators between the ditches where *S. aloides* was planted in March 2023 and the control ditches. Results for NO_3-levels were inconclusive. Visualisations show visible differences between ditch 1M (where *S. aloides* has been growing for over one year) and the control ditches in terms of electrolytic conductivity, dissolved oxygen, turbidity, phosphate-levels, chlorophyll-a and chlorophyll-b. As there is just one ditch where *S. aloides* has been growing for one year, creating linear mixed models for comparing ditch 1M to other ditches were not reliable. Therefore, visualisations are used to interpret the possible effects of *S. aloides* after one year.

Acidity (pH)

During the first weeks, pH-values of ditch 1M with *S. aloides* for one year seemed to be consistently higher than all other ditches, before levels decreased around week 21. Unfortunately, the measuring instrument that was used, was calibrated incorrectly after week 20, and measurements from week 21 till 23 should be considered untrustworthy. If only the measurements from week 16-20 are considered, it seems that the pH of ditch 1M is slightly higher than other ditches. Regardless of whether or not the results of week 21-23 are reliable, *S. aloides* has generally not had a large influence on the pH of the ditches. Some studies have also shown that *S. aloides* is mostly indifferent to pH and it is therefore logical that planting *S. aloides* does not have a large influence on the pH of water (Efremov et al., 2019). However, there are two ways in which *S. aloides* might still slightly influence the acidity of the ditches. The first way is very simple. In all bodies of water, there is the following chemical equilibrium: $HCO_3^- + H^+ \rightarrow H_2CO_3 \rightarrow CO_2 + H_2O$. When CO_2 is used by aquatic macrophytes like *S. aloides* for photosynthesis, this equilibrium shifts to the right, which means more H^+-ions will form bonds with HCO_3^-, which will increase the pH-value to make the water more alkaline (Ansari et al., 2020) (Thind & Rowell, 1999). Another way is through the uptake of nitrogen. Although results for NO_3-levels in this research were inconclusive, if *S. aloides* might have a small effect on NO_3-levels, studies have shown that an excess of nitrogen fertilizers can cause more acidity. So, if *S. aloides* does cause an increased uptake of nitrogen in water, *S. aloides* might also be able to increase the pH of bodies of water (Venkatesan et al., 2004).

Electrolytic conductivity

The results have shown that electrolytic conductivity is consistently and considerably lower in ditch 1M compared to all other ditches. Electrolytic conductivity is an indicator of how many dissolved ionized particles are present in water that is studied (Bhuyan et al., 2018) (Saalidong et al., 2022). It is influenced by many different factors, like the presence of inorganic or organic compounds and microorganisms, but generally, lower electrolytic conductivity (fewer dissolved ionized particles) indicates better water quality (Sohail et al., 2022). The results of this study show variations in electrolytic conductivity between all ditches, but the levels of electrolytic conductivity in ditch 1M seem to be relatively low enough to assume that *S. aloides* has had a substantial effect. The presumed mechanism behind this phenomenon is that *S. aloides* takes up pollutants and (in)organic compounds like dissolved metal ions or excessive nutrients. Removing these chemicals causes a decrease in dissolved ions, which causes the electrolytic conductivity to decrease (Li et al., 2021) (Li et al., 2023) (Singh & Pant, 2023).

Dissolved oxygen

During the baseline measurements, it seemed like the ditch where *S. aloides* had grown a year showed lower levels of dissolved oxygen. This is a different result from all later measurements that were carried out on dissolved oxygen and seems out of the ordinary. *S. aloides*, like all plants, take up CO_2 and produce O_2, therefore more aquatic plants should contribute to more dissolved oxygen. Although phytoplankton populations also produce oxygen, excessive dead organic material produced in algal blooms is decomposed by aerobic bacteria, depleting oxygen in this process (Nazari-Sharabian et al., 2018). Other studies have shown that aquatic plants like *S. aloides* generally have a positive effect on the level of oxygen in aquatic ecosystems (Ansari et al., 2020). Therefore, two possible explanations might be that the dissolved oxygen levels were just temporarily lower due to temporary higher activity of micro- and macrofauna in this ditch or that a measuring error has occurred. The results indicate that generally, ditch 1M as well as the ditches where *S. aloides* was planted in March had slightly higher levels of dissolved oxygen, although differences are small. Next year, in spring 2024, research should be carried out on all ditches again to see if the ditches with *S. aloides* show significantly higher levels of dissolved oxygen.

Turbidity

The levels of turbidity differed quite noticeably within all ditches. On average the turbidity of the ditches of the control group and treatment group was higher than the turbidity of ditch 1M, although it is clearly visible that the turbidity varied a lot within each group. For example, ditch 2Z always seemed much clearer than most ditches (except for 1M) throughout the season and ditch 5M was generally less clear. The linear mixed models that were used in the statistical analysis take these standard differences linked to the individual characteristics of each ditch into account. It is difficult to determine what caused specific differences between ditches as turbidity is influenced by many factors, including current, activity of organisms, inorganic and organic matter, algae, and particles from sediments (Stroud Water Research Center, 2022) (Velthuis et al., 2023). It must therefore be noted that using turbidity as an indicator is not always optimal, because if *S. aloides* were to attract more biodiversity of macro-organisms, the presence of organisms like dragonflies, snails or even swans might also cause water to become more turbid, through their physical activity and respiration. However, it is still important to take turbidity into account, as it is important that water is clear enough for light to penetrate below the surface to submerged aquatic vegetation that supports the aquatic ecosystems (Manca et al., 2022) (Stroud Water Research Center, 2022).

It is also essential to highlight the shortcomings of the way that turbidity was measured in this research. The Secchi tube that was used gives an indication on a scale of 13-90, with 13 representing the clearest and 90 the most turbid water. As this scale is limited, there might have been a difference between the clearest ditches that could not be measured with the material that was used in this research.

Nitrate (NO_3)

In this study, the nitrate levels were too low to detect with the instruments that were used. A possible explanation for low nitrate-levels found in this study is that the relative N:P ratio must be low, meaning there is relatively more phosphorus than nitrogen available for phytoplankton and plants. This would cause nitrogen from fertilizers that ended up in aquatic ecosystems to be quickly used by phytoplankton and plants, resulting in lower levels of dissolved nitrate (Howarth et al., 2021). Another explanation is the difference in chemical bonds between nitrogen and phosphorus in detritus (Vitousek & Howarth, 1991). In dead organic material, nitrogen is usually bonded with carbon, and phosphorus is bonded within esters, meaning the chemical bonds of phosphorus are weaker than those of nitrogen (Vitousek & Howarth, 1991). When decomposition is slow, like is usual in systems rich in peat, nitrogen is released more slowly than phosphorus. This causes lower nitrogen levels as all nitrogen that is released is quickly taken up (Vitousek & Howarth, 1991).

The instruments used in this study could not detect these low levels of nitrate in the ditches, so further research could use equipment that can detect lower levels or equipment that measures both nitrate and nitrite. Although this was not possible for this study, other researchers might have access to more resources or equipment to measure nitrite levels as well. If the same research were carried out next year with the same materials, the Kyoritsu PackTests that were used for nitrate could also be used to detect nitrite if the sample is first boiled for two minutes (*Kyoritsu PackTest Instructions Nitrate, n.d.)*.

Phosphate (PO_4)

Results have shown that the levels of phosphate vary substantially within the groups and there is no difference visible yet between the control and treatment group. Phosphate levels of ditch 1M, however, seem to be generally lower than in the other ditches. Further research is necessary to show if this trend of ditch 1M is also visible in the other ditches next year. If this were the case, this would contribute to the theory that *S. aloides* could be used for phytoremediation to increase water quality by taking up nutrients and therefore decreasing nutrient pollution.

Chlorophyll-a and chlorophyll-b

Chlorophyll-levels are used in this research to represent the sizes of phytoplankton populations in ditches. It is assumed that when chlorophyll-levels are lower, the phytoplankton populations are smaller, so the likeliness that algal blooms will occur is smaller (Tsybekmitova & Fedorov, 2023). Like the other indicators, there were no significant differences between the treatment group and the control group. Chlorophyll-levels fluctuated throughout the season, but both chlorophyll-a and chlorophyll-b levels seemed to be consistently lower in ditch 1M than the ditches in the treatment group or control group. In the graphs, it is clearly visible that the levels of chlorophyll-a and chlorophyll-b fluctuate with striking similarity to each other for each different group. The levels of chlorophyll-a and b in ditch 1M where *S. aloides* has been growing a year also seem to be far more stable than the levels in the other ditches. Lower chlorophyll-levels in this ditch contributes to the theory that *S. aloides* could potentially be used for phytoremediation by inhibiting phytoplankton populations by nutrient competition and allelopathic inhibition (Mulderij et al., 2007). If further research demonstrates the same effect, *S. aloides* could potentially be used to prevent phytoplankton populations from becoming too large, therefore preventing algal blooms.

Limitations of study design

The most likely explanation as to why the ditches where *S. aloides* has been planted in March have not yet shown many significant improvements is that there is a longer period of adjustment necessary. However, other factors could also have contributed to why this study has not yet found many significant effects of *S. aloides* on the water quality of ditches. First of all, the period of March till May has exhibited exceptionally low temperatures and high precipitation. This could have inhibited the growth of the new plants and therefore inhibited the effects on the ditches. Second, predation could have slightly inhibited plant growth as well. Although the tough structure and spines of the leaves prevent most organisms from eating *S. aloides*, American crayfish have been theorized to consume roots, and waterfowl has been observed eating the younger parts of the plant (Smolders et al., 1995). Another reason why this study has found fewer effects than expected of *S. aloides* is that measurements were conducted once every week, and conducting more measurements during this short period of time might have given a better representation of the changes in the values of the indicator, whilst simultaneously enhancing the power of the statistical analysis to detect possible effects. Another explanation could be that the plant population needs to be larger to show more significant effects. In ditch 1M, there are also more individual plants present because they have had the opportunity to reproduce vegetatively for a longer period of time. So, if this research were to be repeated in one year with other ditches, it would be even better to plant more individual *S. aloides* plants per ditch, because if *S. aloides* has more effects on water quality, planting more individuals will increase the likelihood that these effects are detected. However, because *S. aloides* reproduces very quickly, it is still believed that in one year, like the ditch with the one-year-old plants, the four ditches where *S. aloides* was planted in March will be fully covered with these plants as well. Lastly, from a statistical point of view, the small sample size of this study is an important limitation. In general, the statistical power of models, so the probability of a model to detect an effect if there truly is one, is dependent on several factors, including the sample size and size of the effect. If the sample size in a study is low, the studied effect must be very large for models to detect this effect. As the effect of *S. aloides* in this study has likely been small due to the small time the water of the ditches has had to adjust and the inhibited growth of *S. aloides* populations due to weather conditions and fewer individuals at the start, it is logical that no significant effects were found after 3 months.

Limitations of statistical analysis

Ditch 5Z and 5M were excluded from the statistical analysis. In ditch 5M, *S. aloides* did not grow as well as in the other ditches. The shallow depth of this particular ditch is most likely to blame for why very few individuals have survived, as research has shown that depth is an important factor for the survival of *S. aloides* (Toma, 2019). Because ditch 5Z also has a very shallow depth, it was decided to exclude both ditches 5M and 5Z from the statistical analysis in this research.

Results show much variation between the ditches, meaning some ditches seem to have certain range of values for indicators as a standard characteristic that they possess. For example, ditch 2Z always seemed to have lower levels of electrolytic conductivity and turbidity than all other ditches except ditch 1M where *S. aloides* had grown 1 year. The linear mixed models used in this study can take these standard characteristics of ditches into consideration. However, it is still important to consider that when there is much variation in measured values, there is always a lower likelihood that an effect is detected through any type of statistical analysis.

The linear mixed models created in this study were used to compare the three ditches where *S. aloides* was planted in March where growth of *S. aloides* was successful (2M, 3M and 4M) to the four control ditches (1Z, 2Z, 3Z and 4Z). Creating linear mixed models to compare ditch 1M (with one-year-olde plants) to the four control ditches was not trustworthy because there was only one ditch where *S. aloides* has been growing for over a year, and it is nearly impossible to determine whether differences that are found can be attributed to *S. aloides*, or if they can simply be explained by the standard characteristics of that particular ditch. This is why models on ditch 1M were not used for drawing conclusions in this study. Instead of using models, the visualisations of the changes of values for each water quality indicator were used to show the potential effect of *S. aloides*.

It must also be noted that the assumptions for the linear mixed models of dissolved oxygen and pH did not meet fully meet the assumptions for linear mixed models, so these models could be used as an indication, but more research is necessary to confirm these findings.

Further research

As already briefly touched upon in a previous part of the discussion, it is recommended that this study is continued in the coming years to see whether ditches where *S. aloides* has been growing for one year show the same effect of *S. aloides* as is seen in ditch 1M. During this next research, equipment should be used that is able to detect low levels of nitrate or detect nitrite as well, to draw conclusions on the influence of *S. aloides* on nitrogen levels. If in the future, other researchers would be inclined to repeat this study in an alternative location, it is recommended to carry out this research over a longer period of time than three months, place as many individual *S. aloides* plants in each ditch as possible, preferably measure more than once a week, and use a larger sample size (more ditches) for their research.

There are many other interesting, related studies that should be conducted on *Stratiotes aloides* to determine the value of using this plant for phytoremediation. Two aspects that are particularly interesting to study include determining if *S. aloides* could potentially be used for carbon capture and if it might be able to reduce methane emissions from ditches.

Carbon capture using aquatic macrophytes like *S. aloides* is very interesting because it does not cause direct competition with agricultural land use and studies show that aquatic macrophytes are able to take up far more CO_2 than terrestrial vegetation (Khalid et al., 2022). If *S. aloides* is able to capture many carbon emissions, it might be more likely that commercialization and application of *S. aloides* as a phytoremediation technique would be used on a large scale in the future.

Reducing methane emissions from aquatic ecosystems is also a very interesting potential application of *S. aloides* that requires further research. Approximately half of the global atmospheric methane emissions are produced by aquatic ecosystems (Rosentreter et al., 2021). Methane possesses a warming potential that is 25 times greater than CO_2 and natural methane emissions will continue to increase in the coming years (Tiwari et al., 2019). Decreasing methane emissions from ditches is seen as essential for battling climate change, and further research should be conducted to find out whether *Stratiotes aloides* might be able to decrease these emissions (Malerba et al., 2022).

Conclusion

The results of this study have shown no short-term effects of *Stratiotes aloides* on the water quality in ditches. However, this study has also found evidence in one ditch that one year after planting, *S. aloides* might have an effect for several of the indicators measured in this study. These should be further examined in the coming years, but seem to include positive effects on electrolytic conductivity, turbidity, dissolved oxygen, phosphate, chlorophyll-a, and chlorophyll-b. Other applications for *S. aloides* besides potentially improving water quality seem very promising as well. Studies have shown the potential role of *S. aloides* in improving biodiversity by offering protection and nesting opportunities for many organisms, and there are also many other interesting applications of planting *S. aloides*. The plant could potentially be used as feed for livestock, as biomass to generate bioenergy, to create nutritional supplements or could even be used for medicinal purposes in treating metal-inflicted wounds. If a system of planting *S. aloides* around agricultural fields is implemented, *S. aloides* could be harvested every year and used as fertilizer for the surrounding fields. Further research should be conducted on the long-term effects of planting *S. aloides* on water quality and on other potential applications, including carbon capture and decreasing methane emissions.

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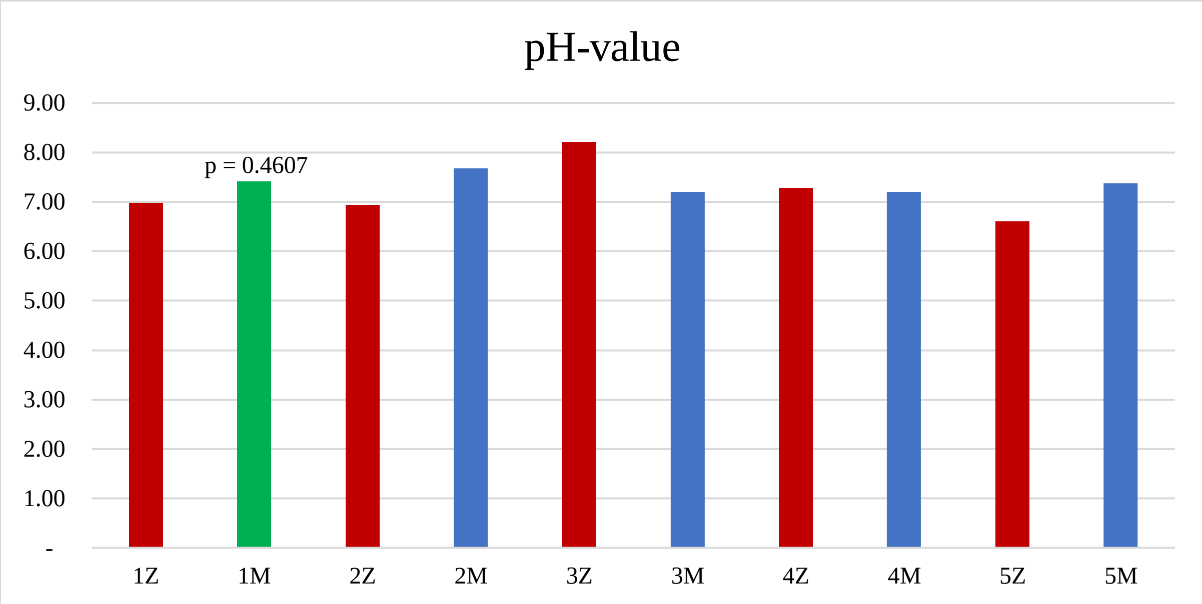
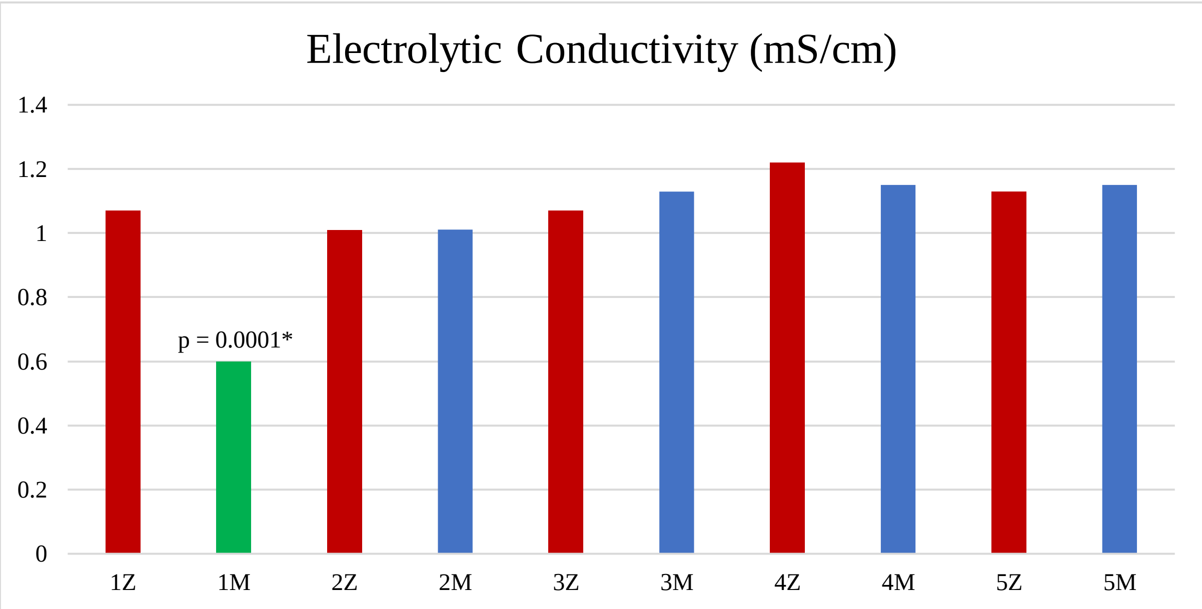
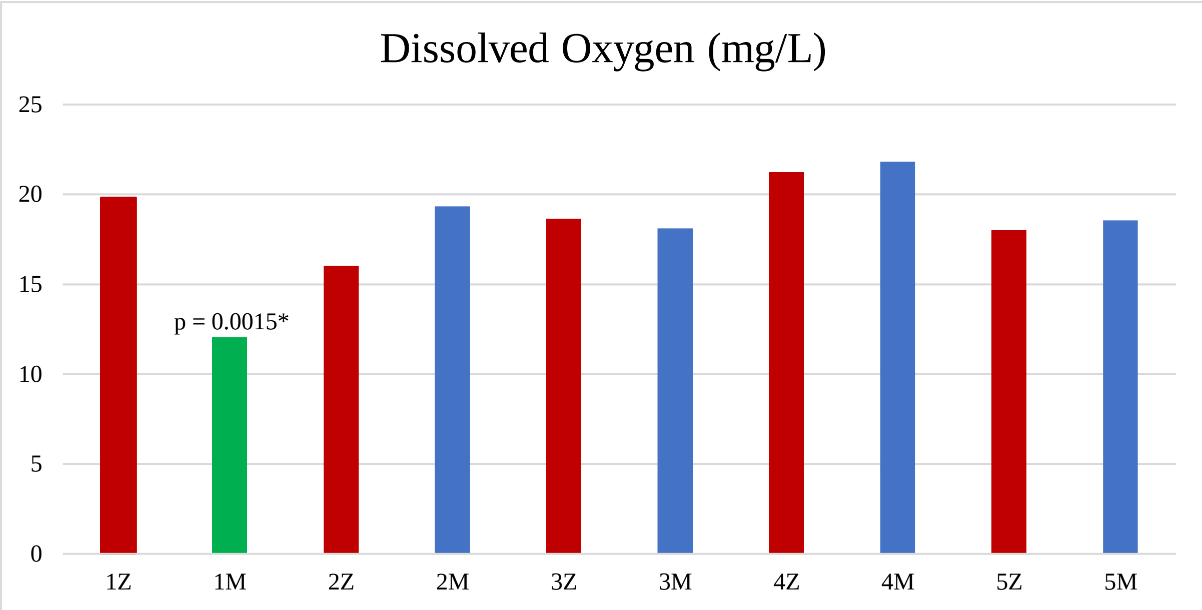
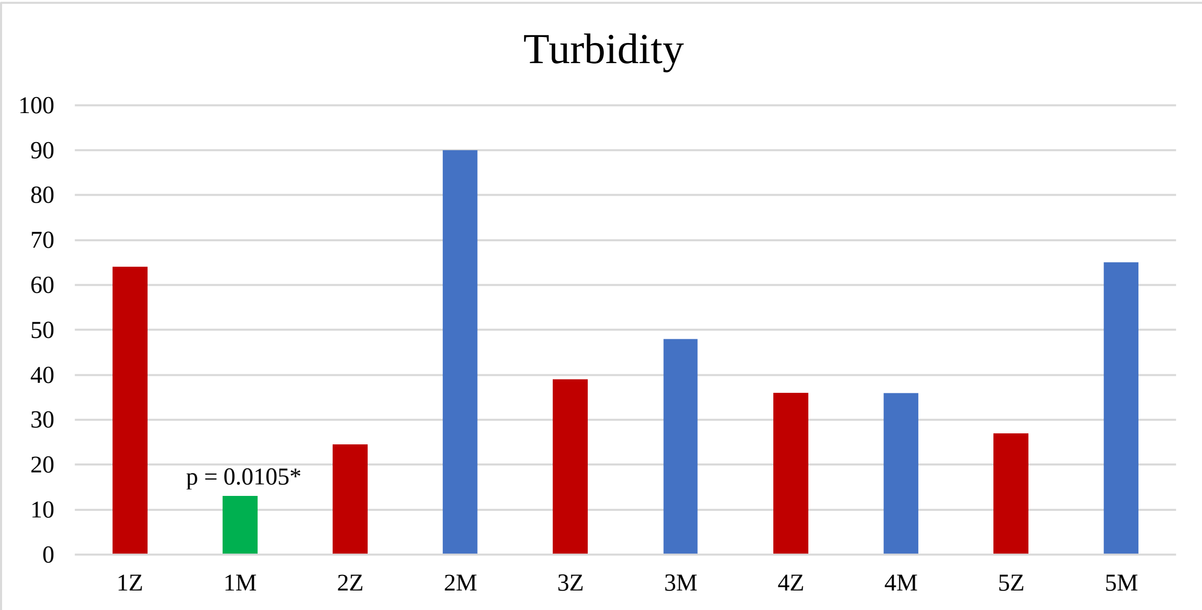
Appendix A: baseline measurements

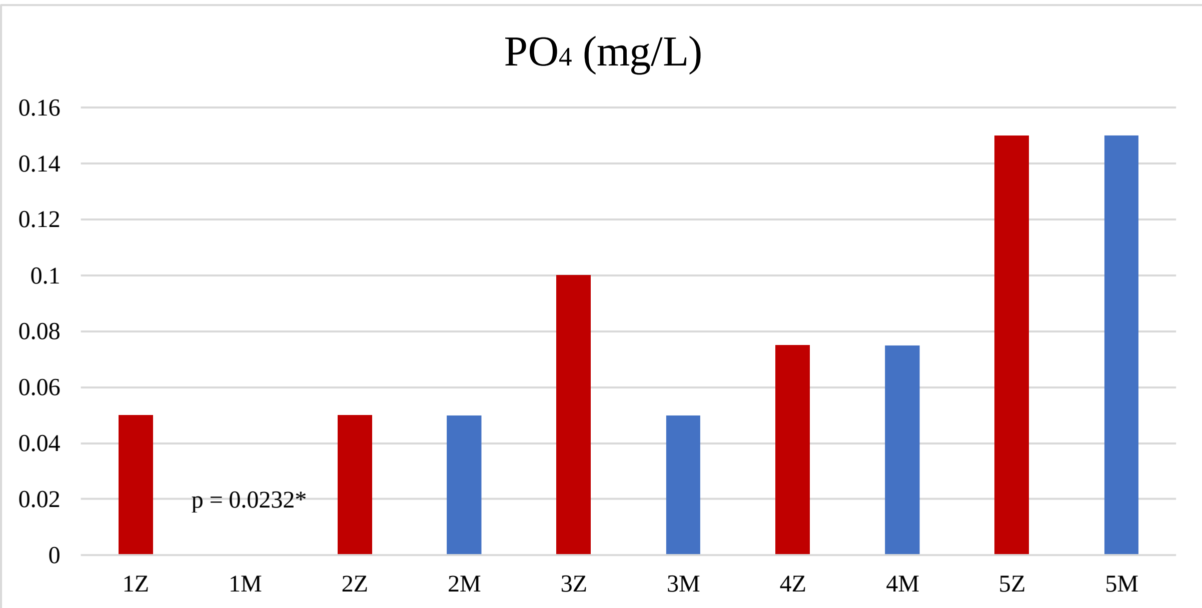
Below are the visualisations of the baseline measurements. Two t-tests were carried out for each water quality indicator. First, t-tests were carried out for each indicator to confirm if ditches 1Z, 2Z, 2M, 3Z, 3M, 4Z, 4M, 5Z and 5M were all comparable before starting the experiment. The results confirmed that those ditches did not differ significantly from each other.
The second type of t-test that was carried out compared the values of the water quality indicators in ditch 1M, where *S. aloides* had already been planted one year ago, to the values of all other ditches. The results showed that *S. aloides* differed significantly from the other ditches with regards to electrolytic conductivity, turbidity, dissolved oxygen, PO_4-levels, chlorophyll-a and chlorophyll-b, but did not differ significantly in terms of acidity (pH). The p-values of the t-tests carried out to determine whether ditch 1M differed significantly from the other ditches are indicated in each graph.

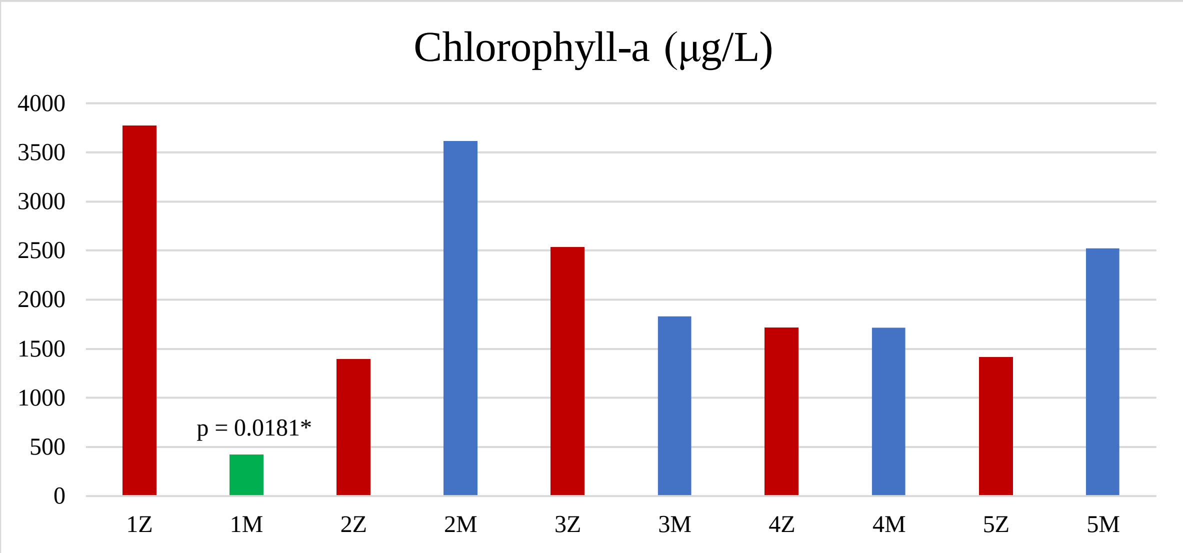


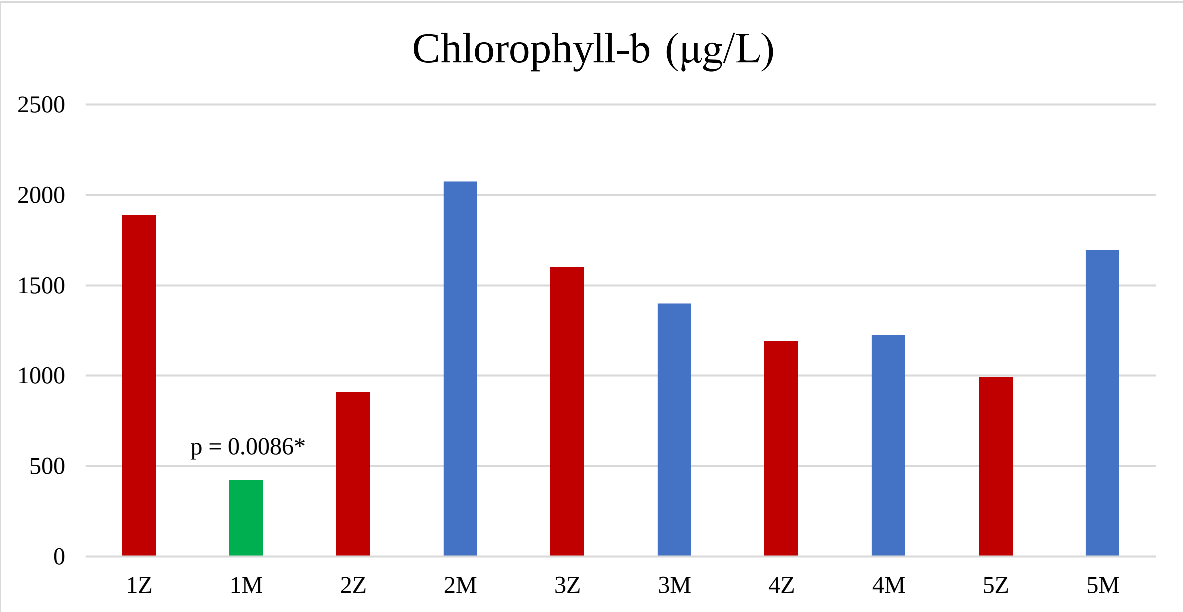



Dissolved Oxygen (mg/L)
25
20
15
10
5
0
p = 0.0015*
1Z
1M
2Z
2M
3Z
3M
4Z
4M
5Z
5M


Turbidity
100
90
80
70
60
50
40
30
20
10
0
p = 0.0105*
1Z
1M
2Z
2M
3Z
3M
4Z
4M
5Z
5M


PO4 (mg/L)
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
p = 0.0232*
1Z
1M
2Z
2M
3Z
3M
4Z
4M
5Z
5M


Chlorophyll-a (µg/L)
4000
3500
3000
2500
2000
1500
1000
500
0
p = 0.0181*
1Z
1M
2Z
2M
3Z
3M
4Z
4M
5Z
5M


Chlorophyll-b (µg/L)
2500
2000
1500
1000
500
0
p = 0.0086*
1Z
1M
2Z
2M
3Z
3M
4Z
4M
5Z
5M

Appendix B: t-tests baseline measurements

Comparison of ditches 2, 3, 4 and 5

Two-sample, paired t-tests for paired ditches 2, 3, 4 and 5 show non-significant results, so the ditches do not differ significantly from each other.

- Temperature ($p = 0.9126$)
- pH-value ($p = 0.7508$)
- Turbidity ($p = 0.1548$)
- Electrolytic conductivity ($p = 0.9326$)
- Dissolved oxygen ($p = 0.316$)
- Chlorophyll-a ($p = 0.3822$)
- Chlorophyll-b ($p = 0.2687$)
- NO_3-concentration (p = NA)
- PO_4-concentration ($p = 0.391$)

Comparison of ditch 1M to the other ditches

One-sample, non-paired t-tests carried out to compare 1M to the other ditches show significant differences in values between the 1M ditch and other ditches for electrical conductivity, dissolved oxygen concentration, turbidity, PO_4, chlorophyll-a and chlorophyll-b. There was no significant difference in pH-value and temperature, and the results for NO_3 were too low to compare. Significance is marked with the sign "*".

- Temperature ($p = 0.1429$)
- pH-value ($p = 0.4607$)
- Turbidity ($p = 0.01047$*)
- Electrolytic conductivity ($p = 0.0001474$*)
- Dissolved oxygen ($p = 0.001556$*)
- Chlorophyll-a ($p = 0.01805$*)
- Chlorophyll-b ($p = 0.008628$*)
- NO_3-concentration (p = NA)
- PO_4-concentration ($p = 0.02321$*)