Nutrient Release from Aquatic Plants

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Abstract

Eutrophication is among the greatest threats to global freshwater. Methods such as floating treatment wetlands (FTWs) utilize aquatic plants to remove excess nutrients directly from the water column. However, plants senesce or die off seasonally, releasing nutrients back into the water. The harvesting of plant tissues can be employed to remove nutrients from the system more permanently. The effectiveness of these strategies is dependent on the amount of nutrients a senesced or dying plant releases. This study submerged four common Missouri macrophytes in conditions that prompted senescence and examined the nutrient concentrations over time. The amount of nutrients released varied among plant species, with emergent plants offering a more permanent sequestration than either submergent or floating plants, which over time release significant amounts of phosphorous and nitrogen compounds. The findings highlight the importance of plant tissue removal in reducing the nutrient concentrations in freshwater systems using FTWs.

Introduction

Freshwater is an essential resource for many of the planet's ecosystems. Human usage of freshwater is extensive, including use for agriculture, drinking water, and recreation. One of the greatest threats to freshwater quality globally is eutrophication, the excess of nutrients in water bodies (Schindler, 2012)[1]. Excessive amounts of phosphorus- and nitrogen-based compounds, as well as other pollutants, accumulate in water from a variety of anthropogenic sources, including agricultural and urban runoff, sewage, and wastewater (Bi et al., 2019[2]; Whitfield et al, 2019[3]). These unnaturally high levels of nutrients support an increase in primary production, with detrimental effects on aquatic ecosystems. Eutrophic conditions ultimately lead to loss of water quality, ecosystem degradation, and decreased human usability. The consequences are detrimental to both the environment and humans and include loss of ecosystem services, algal blooms, fish kills, degraded aquatic habitat, and human health hazards (Bi et al., 2019[2]; Taylor et al., 2020[4]).

One strategy to mitigate eutrophication is the use of floating treatment wetlands (FTW), a type of constructed wetland designed to remove nutrients from a water body. The main structure of an FTW is a floating structure in which plants, often emergent wetland macrophytes, are secured to float at the water's surface, creating a hydroponic growth environment with the stem and leaves of the macrophyte growing above the water and the roots extending into the water. The roots form a dense system which both takes up nutrients directly from the water column and also provides a surface on which microbial biofilms grow. Both elements combine to naturally filter the water column of nutrients and sediments. (Pavlineri et al., 2016)[5]

An important component in the use of FTWs is harvesting strategies, or the removal of plant tissues, which removes nutrients from the system permanently. While nitrogen can leave a system through natural nutrient cycling processes, phosphorus cannot be directly removed from the system in this way (Taylor et al., 2020[4]). The harvesting of plant shoots is an important step to remove nutrients stored in plant tissues, although the use of this strategy alone has been noted in previous research as being insufficient to achieve maximum efficiency of FTWs (Pavlineri et al., 2016)[5]. When considering harvesting strategies, it is important to note that the concentrations of nutrients stored within different plant tissues changes temporally, with translocation to different tissues occurring at different times during the season; while shoot tissue tends to have

higher nutrient concentrations during the growing phase, these nutrients are sequestered into roots and rhizomes to be stored for use in early spring growth (Pavlineri et al., 2016[5]; Xu et al., 2017[6]).

Although macrophytes remove nutrients from the water column, the storage of nutrients in plant tissues is not permanent. At the end of a growing season, plant tissues begin to decay, and nutrients accumulated within them are released back into the system (Kumwimba et al., 2016)[7]. While stored nutrients can be retained in certain tissues, phosphorus is often released during the decomposition of plant litter, with the above-ground tissues of wetland macrophytes releasing phosphorus back into the water (Menon & Holland, 2014)[8]. The decomposition of plant litter and its resulting release of nutrients may contribute to eutrophication (Pan et al., 2017)[9]. Understanding a macrophyte's rate of nutrient release during senescence and the following decay phase is important to guide harvesting strategies for FTW maintenance.

This study conducted a months-long experiment, to explore nutrient release from aquatic plants during and after senescence, with the aim of offering insight into the potential effects of unharvested plant tissue from FTWs on freshwater quality. This microcosm-scale study was completed using four different macrophyte species along with controls to compare rates of nutrient release under conditions prompting senescence and tissue decay.

Materials and Methods

Plant Collection

Four common Missouri plant species were utilized in a long-term microcosm experiment beginning October 6, 2021, and ending February 17, 2022: pickerelweed (*Pontederia cordata*), blue flag iris (*Iris virginica*), duckweed (genus *Lemna*), and hornwort (*Ceratophyllum demersum*). *P. cordata* and *I. virginica* are both emergent plants. *Lemna* is a genus of small, floating macrophytes. *C. demersum* is a submergent plant.

P. cordata was harvested from Ben Branch Conservation Area with the permission of Missouri Department of Conservation. *I. virginica* of a native Missouri phenotype was obtained from Millpond Plants, a nursery in Ashland, MO. *C. demersum* was harvested from Little Prairie Lake. *Lemna* was collected from Schuman Pond, a local pond close to the university. Plant specimens were brought to the lab and cleaned to remove sediment, debris, macroinvertebrates, and other material. Plants were collected in early October.

Experimental Setup and Sampling

Fifteen microcosms, three replicates of each plant species and three controls, were constructed in the lab using 17L Sterilite containers. Microcosms were filled with 10L of water collected from Schuman Pond. The water in the microcosms was aerated for the duration of the study. Specimens were assigned randomly to microcosms, and the microcosms were covered to block all light. As water evaporated from the microcosms, Milli-Q® water was used to replenish the water level to 10L. The microcosms were housed in dark conditions to prompt senescence, tissue decay, and nutrient release in the macrophytes.

Water samples were collected over a period of five months, beginning with an initial sample on October 6, 2021. Each sampling, one 50-mL filtered sample and one 50-mL unfiltered sample were collected by syringe from each microcosm and stored frozen in plastic sample bottles for later analysis. Filtered samples were filtered using GE Healthcare Whatman™

GF/C™ syringe filters. Samples were collected on a weekly basis for the first seven weeks of the experiment, then progressing to an alternating biweekly and monthly basis, for a total of twelve sample sets over nineteen total weeks.

Nutrient Testing and Data Analysis

Filtered samples were analyzed for soluble reactive phosphorus (SRP) using the ammonium molybdate blue method (Murphy & Riley, 1962)[10] and for nitrate using a Dionex LC30 ion chromatograph. Unfiltered samples were analyzed for total phosphorus (TP) using a potassium persulfate digestion followed by the ammonium molybdate blue method (APHA 1998)[11]. SRP and TP results were quantified as absorbances using a Thermo Scientific™ GENESYS™ 20 Visible Spectrophotometer. Absorbance readings for SRP and TP were used to calculate the concentration of phosphorus in µg/L using a linear regression in Microsoft Excel.

Results

Observational Data

Over the course of the five-month experimental period, visual observations were made of microcosms on each sampling date to monitor visual signs of the senescence and decay phase of each macrophyte species. At the beginning of the experiment, sediment and dead algae accumulated on the floor of control microcosms. The water within the control microcosms remained clear and colorless throughout the experiment. Each of the macrophyte microcosms initially contained whole plants. The extent of decomposition varied with each macrophyte species.

One observation noted with both emergent plant species, *I. virginica* and *P. cordata*, was the appearance of new shoot growth in spite of dark conditions aimed to prevent new growth. *I. virginica* trials also displayed new root growth later in the experiment, first observed during the fifteenth week of sampling. For both *I. virginica* and *P. cordata*, decay and decomposition appeared to occur slowly, with plant structures still distinctly visible during the nineteenth week of sampling.

In contrast, *C. demersum* and *Lemna* appeared to decompose more quickly than the emergent macrophytes. By the ninth week, much of the *C. demersum* structure had decayed, forming significant deposits of decomposed tissue at the bottom of the microcosms. *Lemna* underwent the fastest observed change of the four macrophyte species. While the *Lemna* covered much of the water's surface during the first week, by the second week, the majority of the *Lemna* had browned and settled to the floor of the microcosms, which continued throughout the experiment. For all macrophyte species, decomposing tissue from the macrophytes collected on the floor of the microcosm as browned sediment over time.

Soluble Reactive Phosphorus (SRP) Release

The concentration of SRP in the water of each microcosm was determined for each sampling date and averaged by species to determine nutrient release rate over time, as displayed in Figure 1. The greatest concentrations of SRP appeared in the *Lemna* trials, which displayed a steep, early increase in concentration that peaked at 1669 µg/L during the final sampling week. *C. demersum* produced a more gradual increase in SRP that remained close to 0µg/L until the fourth week. A greater rate of release was displayed between the fourth and sixth weeks before

returning to a lower, more gradual rate of release. *I. virginica* released the greatest amount of SRP during the first week, but ultimately produced less total SRP than either *Lemna* or *C. demersum*, reaching a maximum concentration of 545 µg/L during the fifteenth week. *P. cordata* displayed the greatest retention of SRP, with its maximum value of only 17 µg/L during the sixth week. Its SRP concentrations consistently remained below those of even the control trials, which averaged a concentration of 27 µg/L SRP and fluctuated slightly over the course of the experiment. The low levels of SRP found in the control microcosms very likely highlight macrophyte tissues as being the source of increased SRP in plant-containing microcosms.

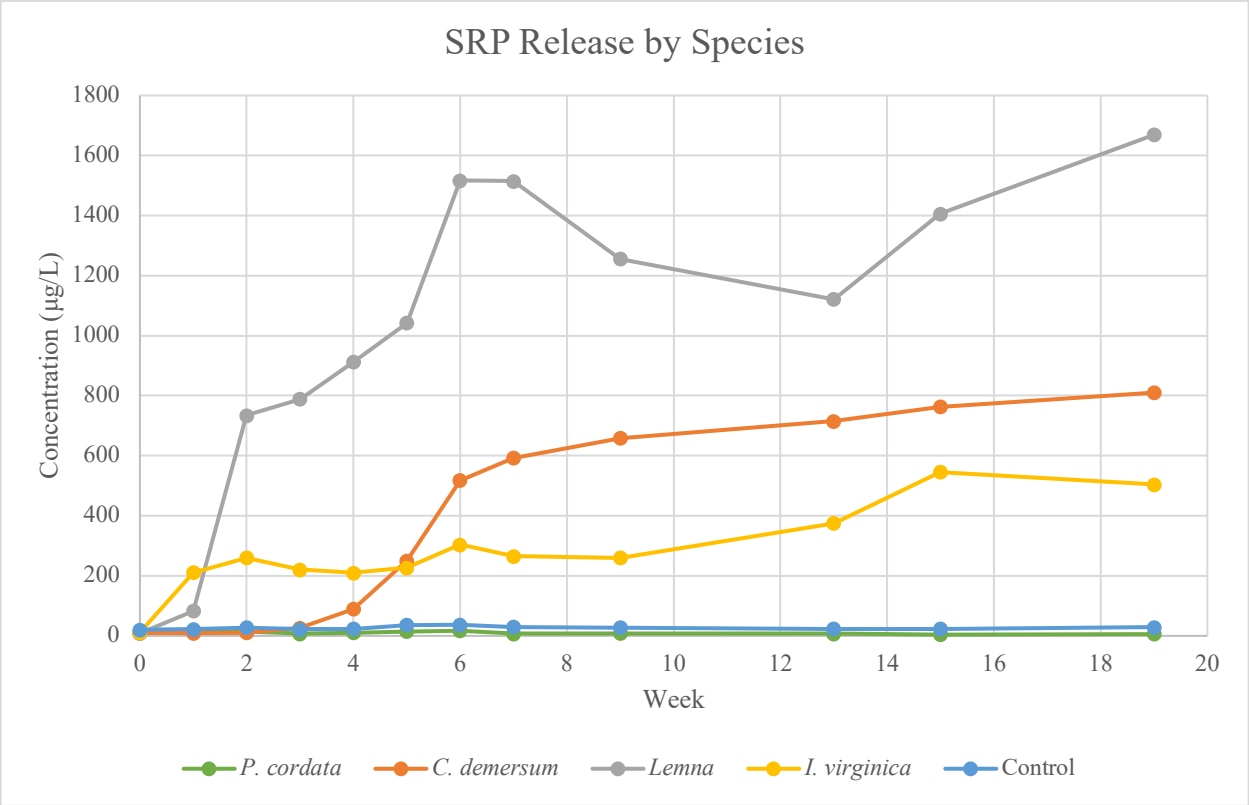

Figure 1: Changes in all macrophyte species' release of SRP over the duration of the experiment.

Total Phosphorus (TP) Release

Concentrations of TP were also monitored throughout the duration of the experiment as a means of observing the changes in the amount of non-SRP phosphorus present over time, such as organically-bound and sediment-bound phosphorus. As in the SRP trials, TP in control microcosms remained low throughout the experiment, fluctuating between a low of 22 µg/L and 39 µg/L as can be seen in Figure 2.

Trends in TP concentrations appear to resemble those of SRP to some extent within the different trials. As with SRP concentrations, *P. cordata* presented the lowest concentrations of TP (Figure 3). *I. virginica* (Figure 4) and *C. demersum* (Figure 5) offered intermediate amounts of TP similar to their SRP concentrations. *Lemna* produced the highest TP concentrations, as it did with SRP, with a maximum concentration of 2124 µg/L during the sixth week of sampling (Figure 6).

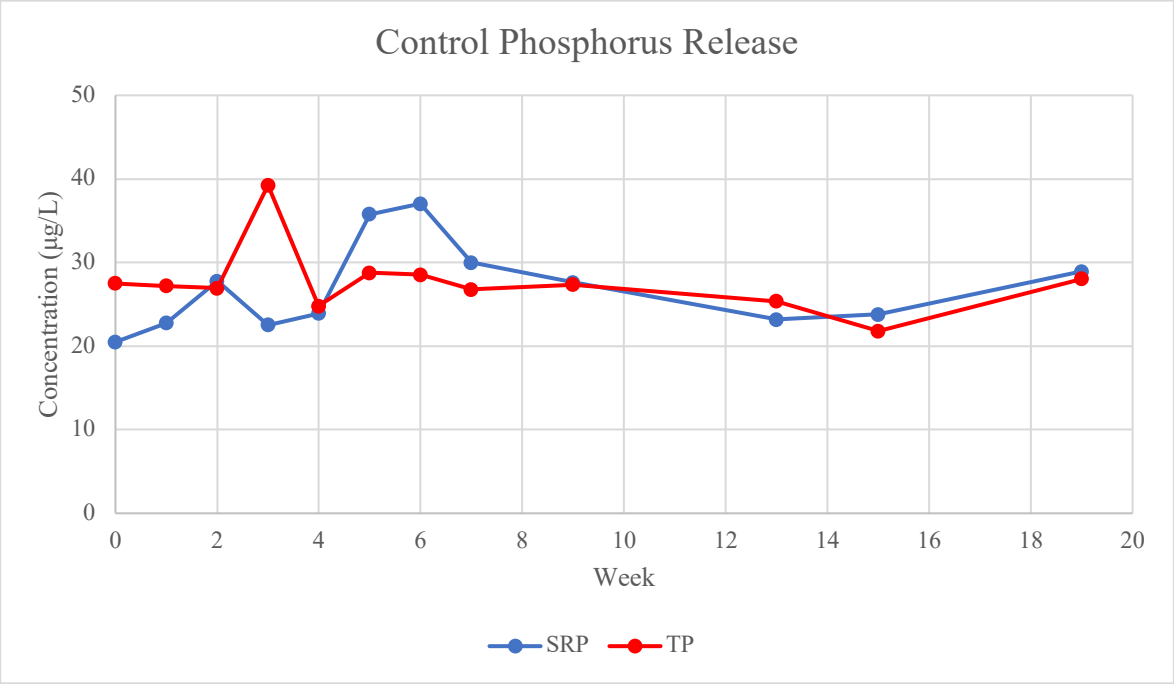

Figure 2: Changes in SRP and TP concentrations of control trials over the duration of the experiment.

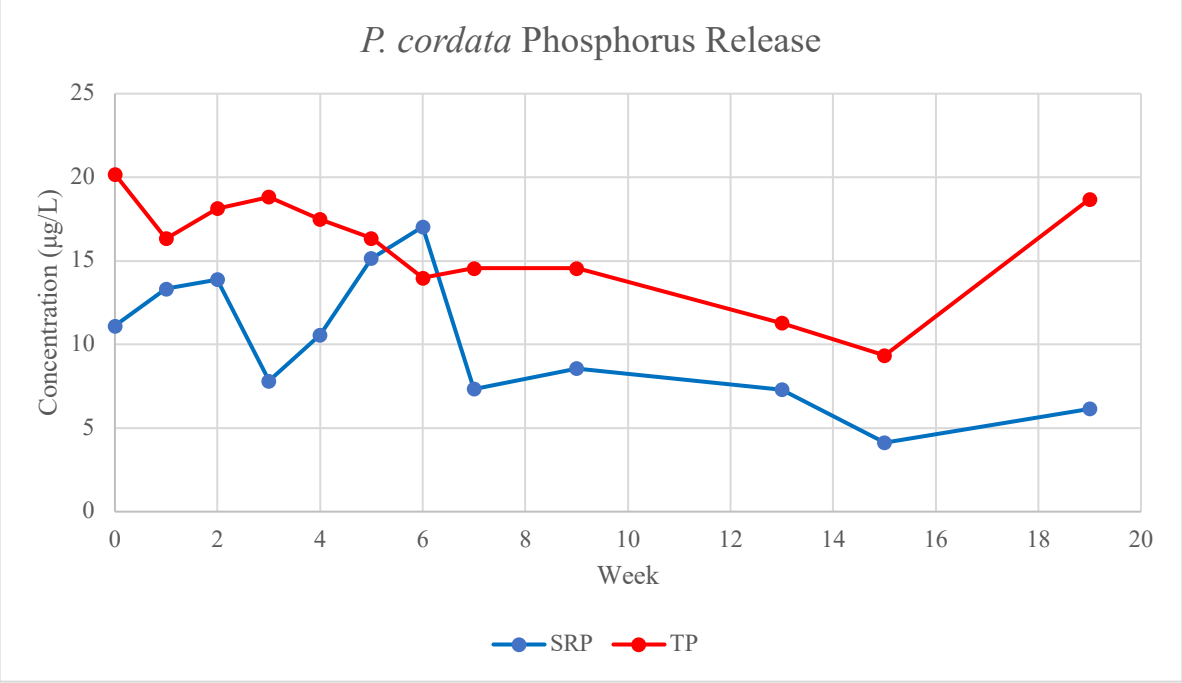

Figure 3: Changes in SRP and TP concentrations of P. cordata *(pickerelweed) over the duration of the experiment.*

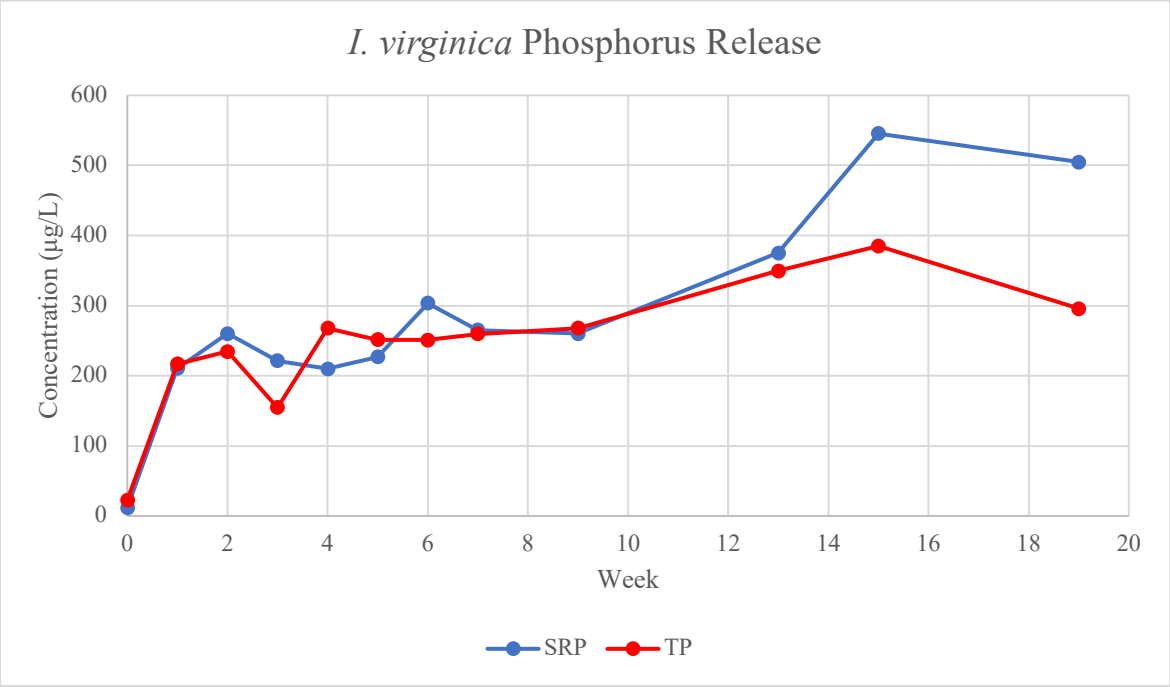

Figure 4: Changes in SRP and TP concentrations of I. virginica *(blue flag iris) over the duration of the experiment.*

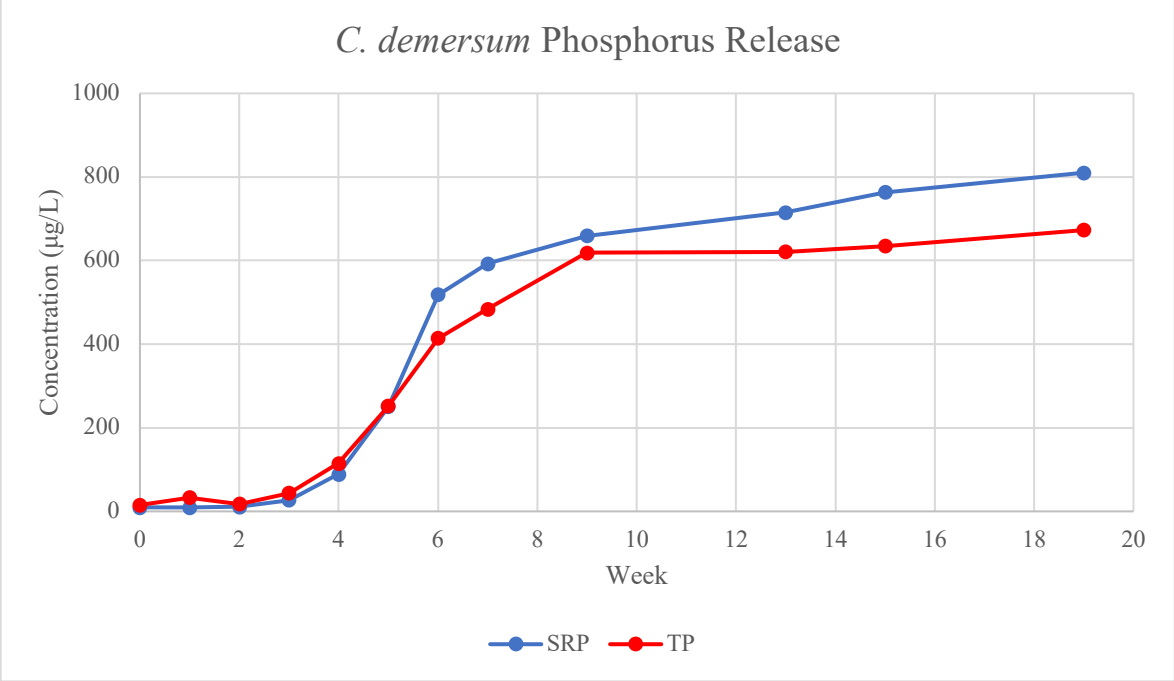

Figure 5: Changes in SRP and TP concentrations of C. demersum *(hornwort) over the duration of the experiment.*

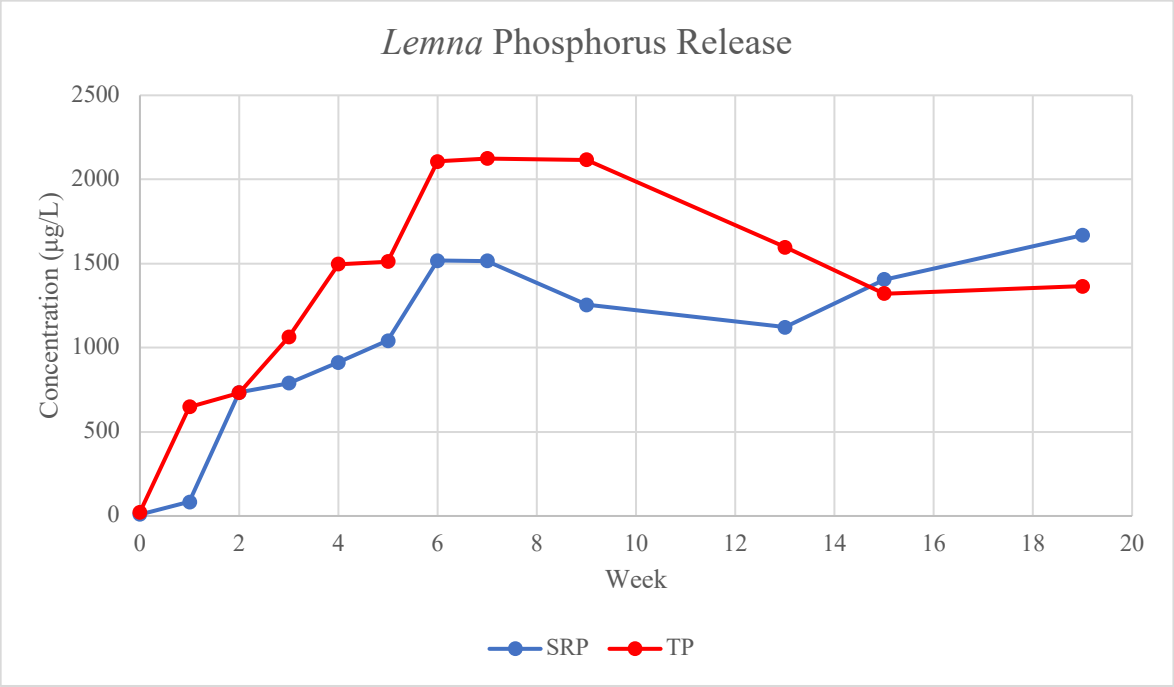

Figure 6: Changes in SRP and TP concentrations of Lemna *(duckweed) over the duration of the experiment.*

Nitrate Release

Changes in water concentrations of nitrate were recorded with each sampling to explore nitrogen release within the microcosms as plants decayed. The control microcosms fluctuated between an initial concentration of 0 µg/L nitrate and a maximum of 385 µg/L nitrate. In comparison to phosphorus release, nitrate was observed to have a delayed release for all macrophytes, with the first increase in nitrate levels observed during the third week of sampling.

The results indicate that as with phosphorus release, *Lemna* released the largest amount of nitrate, with a maximum concentration of 16466 µg/L nitrate recorded during the thirteenth week of the experiment. Similarly to *Lemna*, *C. demersum* also released higher amounts of nitrate, with its maximum of 6337 µg/L also recorded during the thirteenth week. In contrast to the phosphorus results, *I. virginica* appeared to retain nitrate much better than it did phosphorus, with a 0 µg/L concentration recorded for several dates and a maximum concentration of 176 µg/L. Similarly, *P. cordata* also retained nitrate well throughout the experiment, with a maximum concentration of 127 µg/L.

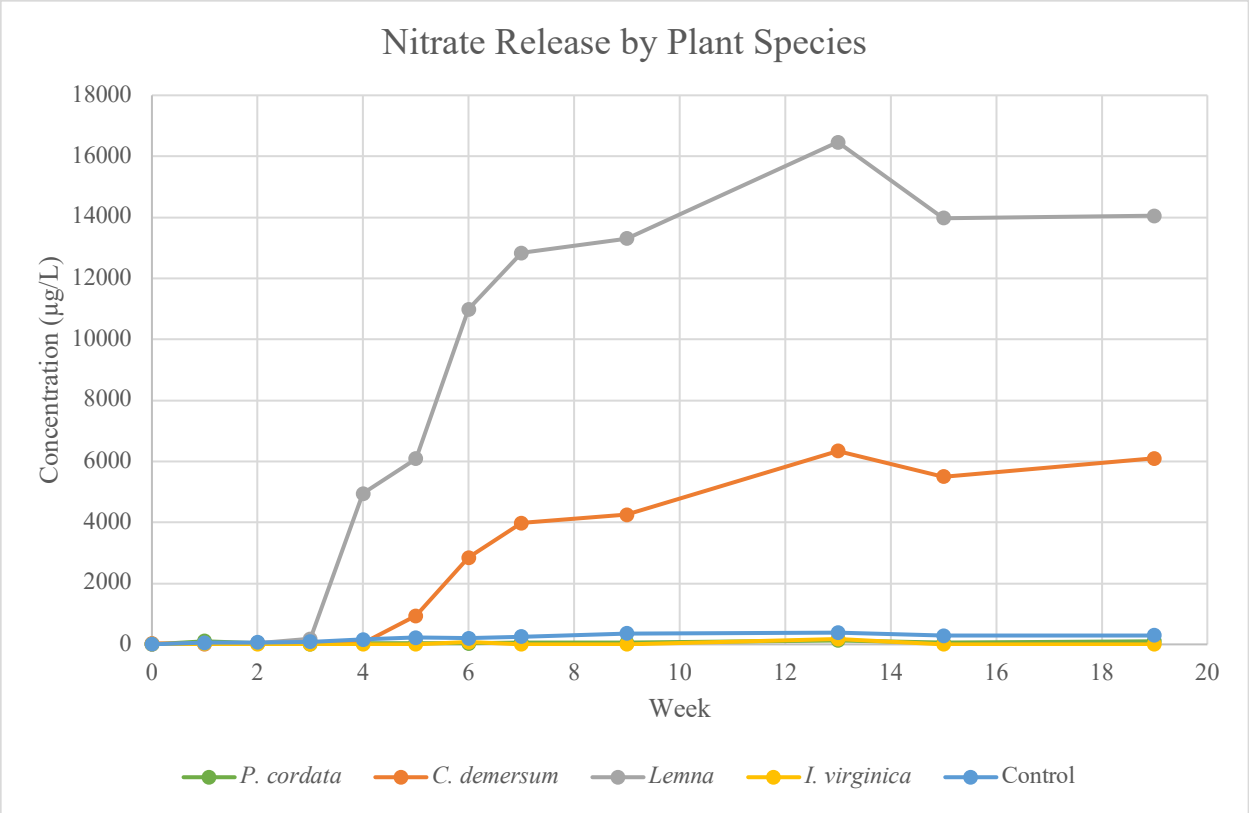

Figure 7: Changes in all macrophyte species' release of nitrate over the duration of the experiment.

Discussion

Each of the four macrophyte species displayed different responses and rates of decay. *Lemna*, the only floating macrophyte tested, quickly entered the decay phase and released large amounts of both phosphorus and nitrate over the course of the experiment. *C. demersum*, the only submergent plant tested, displayed a more gradual, but still significant, release of nutrients and a slower rate of decay. Both *P. cordata* and *I. virginica*, the two emergent macrophyte species, demonstrated much slower decay and high nitrate retention, but differed in the retention of phosphorus, with *P. cordata* releasing very low amounts of phosphorus and *I. virginica* releasing a higher concentration.

It is important to note that the results for TP and SRP may have been affected by test interferences, resulting in the discrepancies between the values shown in Figures 2-6, and that all nutrient tests may have been affected by sampling error, testing error, or dilution error. With this in mind, concentrations of SRP that appear to exceed those of TP likely indicate that a very high percentage of TP is made up of SRP for those samples.

Whitfield et al. (2019)[3] described the decomposition of macrophytes as occurring in several stages, beginning with rapid release of nutrients early in the decay phase, a slower release of nutrients following colonization by microbes, and a final stage of slow release as structural tissues decompose. As seen in the results of this study, nutrient release varied greatly between the macrophytes used. However, increased release of phosphorus was observed in the first weeks of the experiment for *I. virginica* and *Lemna*, with both appearing to have slowing rates during

the later weeks of the experiment. Similarly, once *C. demersum* began to release nutrients, the data appeared to change from a higher rate of release to a slower rate of release over time.

As described by Pan et al. (2017)[9], a species' growth form may significantly impact the rates of nutrient release from plants. This 2017 study found that the highest concentrations of nutrients in their trials were found in mesocosms with floating plant tissue, potentially due to floating plant tissue being softer and more easily decomposable. *Lemna*, the only floating plant utilized in this study, similarly had poor nutrient retention. This may also explain the higher release of nutrients from *C. demersum*, which also has a softer structure. Given the high amounts of nutrient release from floating plants, their removal from an ecosystem before plants begin decomposition has been recommended in previous research to prevent eutrophication (Yi et al., 2021)[12].

In contrast, the emergent plant species used were both found to release lower amounts of nutrients. Specimens used of *I. virginica* and *P. cordata* had already begun their seasonal senescence when they were first placed into the microcosms, which could explain this difference. Xu et al. (2017)[6] notes that emergent wetland plants undergo an important seasonal process of nutrient translocation, during which nutrients important to growth are moved from tissues bound for decay, such as shoots, to more permanent structures like roots and rhizomes. This would contribute to better retention of nutrients.

Given the extremely low levels of SRP and TP observed in the *P. cordata* microcosms, it is also possible that the plants were still able to uptake nutrients from the water. Senescence typically occurs during colder seasonal conditions, which can interrupt nutrient cycling and transport (Whitfield et al., 2019)[3]. However, I was unable to replicate colder temperatures in my study, and biological processes such as nutrient uptake that would be reduced by cold temperature conditions would not have been impacted. Further research would be necessary to confirm this hypothesis.

Previous research has emphasized the impact of phosphorus as the main contributor to harmful algal blooms, while nitrogen is less influential (Schindler, 2012)[1]. Changes in the N:P ratios of waterbodies also affect phytoplankton growth and diversity within systems (Lu et al., 2018)[13]. In the context of this study, this would suggest that macrophyte species with high phosphorus retention, such as *P. cordata*, would serve FTWs well. While *I. virginica* displayed a high retention of nitrate, its increased release of phosphorus may limit its usefulness in FTW systems.

The design of this study, utilizing, microcosm trials, was limited due to its small scope. As Schindler (2012)[1] explains, microcosm and mesocosm experiments rarely offer accurate insight into the responses of whole ecosystems to heightened nutrient levels because they tend to be too small to predict full-scale effects. These small-scale experiments are, however, valuable in their ability to isolate specific mechanisms, such as nutrient retention and release, for observation. Thus, while heightened nutrient release of specific plant species were observed in the present study and could indicate that leaving decomposing macrophytes in a water body could contribute to eutrophication, further experiments would be necessary to confirm this prediction.

Further research into this topic should explore the effects of seasonal variations, such as freezing winter conditions and water level fluctuations, on the release of nutrients. Cycles of freezing and thawing can accelerate nutrient release of macrophytes, higher concentrations of nutrients can be observed during cold conditions due to low rates of nutrient uptake by plants (Whitfield et al., 2019)[3]. Water level fluctuations also impact macrophytes, leading to

macrophyte death in dry conditions and causing release of nutrients when dead plant tissues are exposed to water (Lu et al., 2018)[13]. The effects of climate on hydrology, which are predicted to lead to regional changes in precipitation (Schindler, 2012)[1], must also be taken into consideration.

As noted in previous studies, while aquatic plants play an important role in nutrient cycling, they serve only as a temporary sink for phosphorus and nitrogen (Kumwimba et al., 2016[7]; Menon & Holland, 2014[8]). Leaving plant tissues unharvested in FTWs at the end of the growing phase can result in release of nutrients into the water column. The findings of this study concur with previous research that nutrient release rates are highly dependent on species (Lu et al., 2018[13]; Pan et al., 2017[9]). Careful selection of plants for use in FTWs, as well as harvesting strategies to remove plant tissue before decomposition, would be beneficial in mitigating high nutrient levels in freshwater bodies (Bi et al., 2019[2]; Menon & Holland, 2014[8]; Pavlineri et al., 2016[5]). Regardless, further research into the release of nutrients from plants is necessary to improve our understanding of their contributions to eutrophic conditions in water bodies.

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