Freezing Plants: Exploring the Effects of Freeze Thaw Cycles on Macrophyte Phosphorus Release

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Abstract:

Every year following the cold winter freeze comes the spring thaw, and with it comes an influx of nutrients that can cause eutrophication problems; even in high nutrient systems. We identified a potential source of nutrients released during the winter freeze; aquatic macrophytes and aimed to determine the quantity of total phosphorus (TP) they release. Specifically, cattails and reeds (*Typha* and *Juncus*, respectively). Stalks were harvested from 3 different sites; a pond with an agriculturally dominated watershed, the beginning of a wetland used to treat effluent waste water, and a site further along in the wetland treatment process. Samples from each site were split into four different sample types: wet freeze, dry freeze, wet control, and dry control. Control and freeze samples were placed in at 1.6^0C and -1^0C respectively, and left for equal amounts of time averaging 31.375 hours (SD 10.25). After treatment, residual water samples were analyzed for total phosphorus concentrations. Results showed that freeze thaw cycles (FTCs) did not induce TP release from macrophytes tested in the lab. These results suggest that the use of macrophytes as a sink for nutrients is a beneficial practice that managers should continue to employ.

Introduction

Excessive loading of phosphorus has been found to be the dominant cause of eutrophication in freshwater lakes; this eutrophication often leads to low dissolved oxygen, and the loss of many aquatic animals (D. L. Correll 1998; Schindler and Vallentyne 2008). Eutrophication is the process by which water bodies are made more eutrophic through an increase in their nutrient supply. An increase in nutrient supply leads to a sudden spike in productivity that would not occur as quickly under natural conditions. While humans have increased the amount of phosphorus released into lakes, termed anthropogenic eutrophication, erosion is also a known source of increased total phosphorus (TP) levels, albeit at a much slower rate. The cost of this water body degradation in the US alone has been estimated at $2.2 billion dollars annually. Primarily caused by the loss of lakefront property value, the money spent recovering endangered species, and protecting drinking water sources (Dodds et al. 2009).

This large economic cost, however, can be mitigated through proper management. It has been shown that wetlands can help reduce nutrient loading to downstream water bodies, as they are especially good at removing nitrogen and phosphorus, the two most limiting nutrients for most water bodies (Juwarkar et al. 1995). Wetland and riparian macrophytes grow in large stands and have a strong ability to absorb and retain nutrients from their environment such as the aforementioned phosphorus and nitrogen (Boyd, 1970). However, nutrients used by the plant for growth are stored in plant tissue and can potentially be harvested as a way to remove even more nutrients than the plants alone (Ciria et al. 2005). Using this technique, all of the nutrients that plants use for growth would be removed from the local nutrient cycle and moved elsewhere. However, in many wetlands harvesting does not occur and winter freezing plays a role in causing a release of nutrients from plant tissue (White 1973). Freeze thaw cycles (FTCs) are an understudied aspect of macrophyte's role in wetland nutrient cycles.

One of the sites chosen for this study is an urban wastewater treatment wetland that serves the city of Columbia, Missouri. This type of wetland uses macrophytes to help with the removal of nutrients to lessen the impact of this water reentering natural ecosystems. In similar wetlands, removal rates of roughly 60% of total phosphorus (TP) were found due to macrophyte nutrient uptake (Haifeng et al. 2014). This uptake rate, however, does not factor in the portion of nutrients that is released back into the system during winter FTCs. The other site was a pond in an agriculturally dominated land use area.

We hypothesize that macrophytes release phosphorus during FTCs. We also hope to give researchers a better understanding of the role macrophytes play in the wetland nutrient cycle through this study. As temperatures change on a global basis, areas that in the past froze and remained frozen until spring may experience a greater number of these FTCs. Through a greater understanding of FTCs, researchers have discovered that some portion of the nutrients absorbed into plant cells are released back into the environment when the cells burst after a freezing event (White 1973). In this experiment, we quantified the amount of phosphorus that macrophytes release during simulated winter FTCs. To achieve this goal, macrophytes were sampled at different locations, in an area dominated by agriculture land use, and another site in a wastewater treatment wetland. These samples underwent FTCs under control, submerged, and dry conditions. Due to cattails typically being found in wetland environments, their stalks can be either submerged in water, or in other scenarios the water dries up and leave the stalks exposed. Dry and submerged treatments were chosen to best simulate these natural field conditions. Through the use of these samples we hope to discover how FTCs impact nutrient release;

especially in regards to the one of the main limiting nutrients, phosphorus, and its implications on downstream water bodies.

Methods

Study Site

Study sites for this project were located in two separate geographical locations in the state of Missouri; one in the northern glacially created plains, and the other in the central floodplain created by the Missouri River (Figure 1). The two floodplain sites were taken from the Eagle Bluffs Conservation area where managers use wetlands as a tertiary treatment for effluent water. Samples taken there were from different cells in the denitrification process which represents a nutrient gradient. Sites were selected to provide a variance in soil medium, and land use. The more northern site being in a livestock dominated agricultural pond. The second site being a nutrient rich effluent treatment wetland.

Three separate sites in Missouri were chosen to sample cattails. The first site was in northern Missouri at the Greenley Research Center (Coordinates: 39°56'56.0832"N; 92°3'22.2156"W), in the clay pan region, the second area was in central Missouri at the Eagle Bluffs Conservation area where pools #4 (Coordinates: 38°51'4.46"N; 92°26'21.8101"W) and #11 (Coordinates: 38°49'57.036"N; 92°25'14.1492"W) were sampled. The Greenley site samples came from a pond whose watershed was dominated by cattle farming, it represented agriculturally dominated land cover with high rates of nutrient rich run off. The Eagle Bluffs sites are a part of a treatment wetland used to treat the City of Columbia's waste water. This continuous wetland starts in pool #2 with effluent water that has been treated dispersing through a series of pools towards pool #12. As water slowly moves through the wetland it reduces in nutrient content creating a nutrient gradient; sample sites were chosen to represent both ends of this gradient.

Field Methods

Each sample was taken a week apart starting with the Greenley site on November 14th, 2016, pool #11 November 19th, and then lastly pool #4 in Eagle Bluffs on November 26th. At the Greenly site reeds (*Juncus*) were the only type of macrophyte present to sample. At Eagle Bluffs one plot of reeds were sampled and two plots of cattails (*Typha*). Three separate one m^2 plots were sampled at each of the Eagle Bluffs sites, and two separate m^2 plots were sampled at Greenly. The bottom 15cm of cattails within these plots were cut and later split into 5 groups for the five different sub samples. Additional samples were taken to help characterize each study site; both soil and water samples were taken for chemical analysis.

Laboratory Methods

Each of these sub samples of cattails were separated out and weighed. Samples were classified as wet freeze, wet control, dry freeze, dry control, (Figure 2.) and an elemental sample was ground up using a pestle and mortar and kept at 4^0C for later analysis. Each sample was placed on a shaker table at 145rpm for one hour in 700ml of deionized (DI) water to extract all available nutrients. Wet samples were placed on the shaker table twice, once after the initial 700ml of DI was added, and again after undergoing a freeze thaw cycle (Figure 2). Dry samples were only shaken after their respective treatments. The control samples were placed in the refrigerator at 1.6^0C; while the freeze treatments were placed in the freezer at -1^0C. Wet and dry samples for each plot remained in the freezer for equal amounts of time.

After the samples were processed on the shaker table a small sample of water was pulled off to be used in total phosphorus (TP) analysis preformed according to the standard method (Prepas & Rigler 1983) and were run in triplicate.

Statistical Methods

For this experiment change in TP release across the different treatment types was the primary independent variable. For the different treatment types an ANOVA test was run to analyze the differences in TP release.

Results

The relationship between freeze-thaw cycles and TP has been found to have no difference between macrophyte types (Figure 3). Concentrations for TP were normalized using the dry weight of the samples and the (estimated) volume of water used for extraction. After normality was tested one and two way ANOVAs were run; results for TP were compared across sample sites, macrophyte [cattails versus reeds] types sampled, and treatment types [wet control, wet freeze, dry control, dry freeze]. Surface water samples taken alongside the macrophyte samples were also analyzed for TP and while a relationship did exist ($F_{2,6}$=4.630, *p* value=0.061, *n*=9), a significant difference was not found (Table 1). The waste water that moves through Eagle Bluffs was thought to have been representing a nutrient gradient due to the assumed removal of nutrients. As the water moved from pool to pool there was no significant drop in nutrients found.

There was no significant relationship found between the different macrophytes sampled- reeds and cattails [$F_{1,46}$=3.664 *p* value=0.062 *n*=48]. There was also no significant relationship between the four different treatment types tested; the wet control, wet freeze, dry control, and dry freeze (Figure 2). [$F_{2,45}$=2.222 *p* value=0.120 *n*=48]. There was no significant relationship between the three sites sampled [$F_{5,42}$=1.462 *p* value=0.223 *n*=48].

Discussion

Making sense of it all

It is known that spring snowmelt brings with it an influx of nutrients into aquatic systems; the source of these nutrients, however, is a very complex issue that involves many different variables including land use, substrata, climate, et c. (Elliott 2013). Our study looked to quantify the amount of phosphorus released by the macrophytes, *Typha spp.* and *Juncus spp.*, during winter freeze thaw cycles (FTCs). We found that there was no significant difference between P releases from macrophytes in freeze treatments when compared to a control treatment. This test was run with samples frozen dry and samples frozen while submerged in water to simulate natural conditions. Both of these treatments yielded similar nutrient release concentrations (Table 1). While not statistically significant, a relationship between the type of macrophytes sampled did exist. This relationship suggests that in areas with different macrophyte species present, additional research may be helpful in further developing FTC effects on nutrient release.

There was no difference recorded in nutrient release due to FTCs, which may be due to only one freezing temperature used, -1°C. It has been found that not all freeze treatments are the same; the temperature samples are frozen at plays a role in nutrient release (Elliott 2009). With a colder freeze it is possible that we may have recorded a significant amount of nutrient released. It is also possible that the number of FTCs play a large role in the amount of phosphorus released by macrophytes. Throughout winter in Missouri changing temperatures can freeze and thaw a wetland multiple times and cause increased phosphorus release compared to what was found in this study.

Why this matters

This lack of nutrient release due to more intense freezing may be a good sign for wetlands in more temperate areas going forward. As global temperatures have been increasing over the 1951-1980 thirty year mean (NASA 2017) wetlands will be subjected to lower amounts of intense FTCs. If temperatures continue to follow current trends there will be fewer freezing events each year which suggests a trend of fewer FTCs each year. With reduced FTCs the likelihood of these macrophytes releasing nutrients back into their respective systems should decrease and in turn increase their efficiency of TP removal. Cattails and reeds are commonly used by managers to aid in nutrient uptake in wetlands and other areas prone to runoff such as roadside ditches. Our results suggest that these macrophytes can help to decrease TP in these systems, and can therefore are being used successfully by managers.

Management implications

Macrophytes have been shown to add value to wetland systems through erosion control, filtration, and through adding surface area for attached microorganisms (Brix 1997). Our study addressed the question whether or not FTCs had any direct effect on the amount of nutrients released, with implications for use in constructed wetlands. Based on the results of this study, macrophytes were not found to release a larger amount of nutrients due to FTCs, but they were shown to release nutrients. While it may not be necessary for managers to remove macrophytes from wetland systems due to FTCs, managers may find that removing macrophytes before they die will decrease available nutrients for winter. This study suggests the use of cattails instead of reeds in the removal of phosphorus. Cattails are effective in reducing the overall phosphorus loading in wetland systems.

Conclusion:

We expected that macrophytes harvested in a site used for cattle farming, and a wastewater treatment wetland would yield different amounts of TP release. However, macrophyte TP release remained the same across all sites and treatments. This lack of correlation suggests that macrophytes are not influenced by the level of TP in the surface water they grow in or the land use found in their watersheds. This suggests that cattails are especially good at removing TP in variable environments, and could prove beneficial elsewhere. In both wetland and agricultural dominated areas they did not release TP due to freeze thaw cycles, and will continue to help reduce TP in the water of these environments. Winter's effect on these macrophytes seemed to be less significant than hypothesized. Moving into the future winters in Missouri are going to become shorter and will begin to get wetter (due to a reduction in below freezing temperatures) giving less time for FTCs than before. The use of macrophytes in this type of environment has been shown by our data to not release TP and the impacts of climate change will further reduce the potential negative impacts of macrophytes during the winter months. However, greater research into the complete nutrient cycling of cattails would be beneficial to gather a more complete understanding of their overall impact on TP and other nutrients such as nitrogen.

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Figure 1. Map of Missouri, sample sites marked with yellow pin. Greenly site in northern Missouri, two sites at Eagle Bluffs Conservation Area in central Missouri.

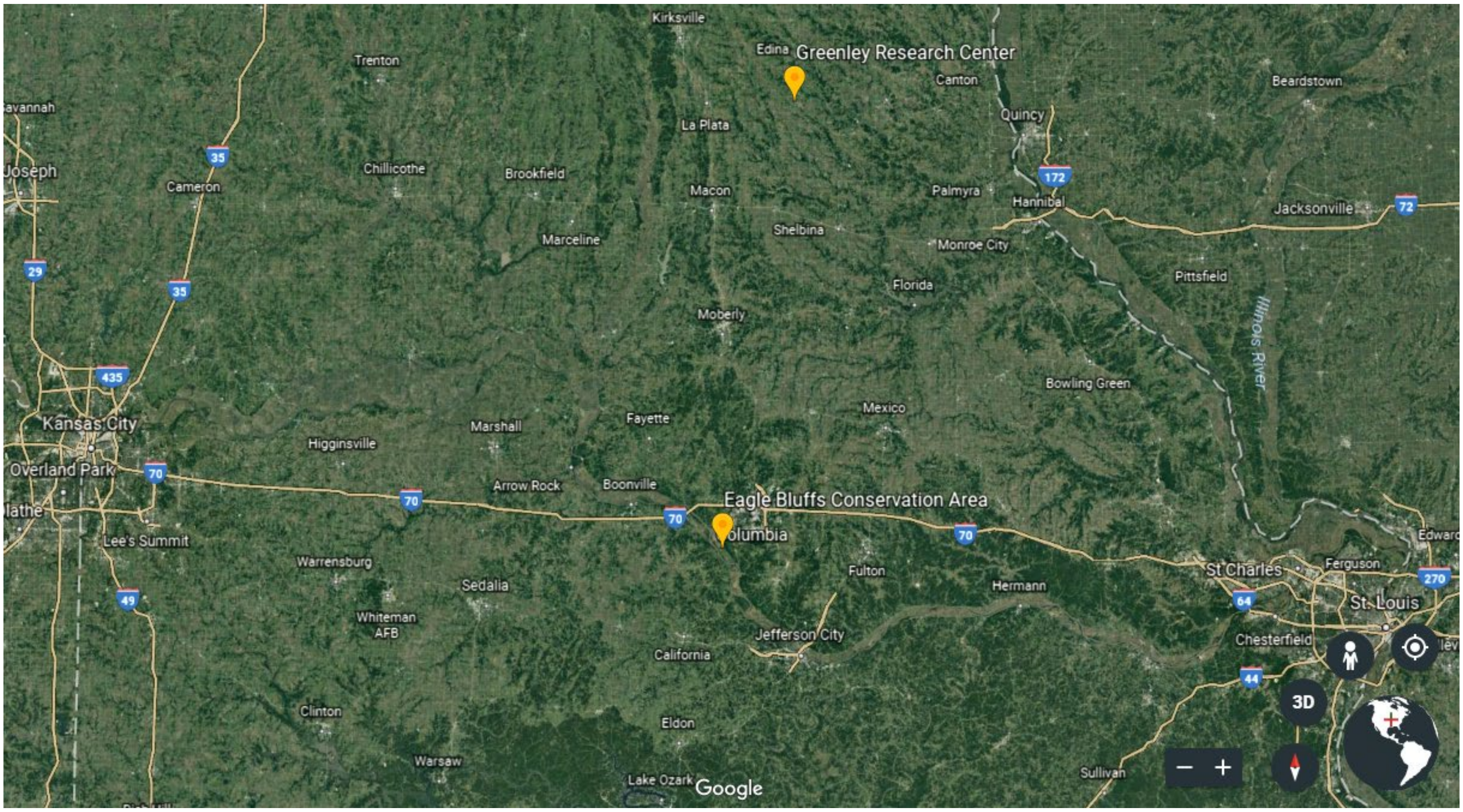

Figure 2. Visual depiction of laboratory protocol for sample distribution.

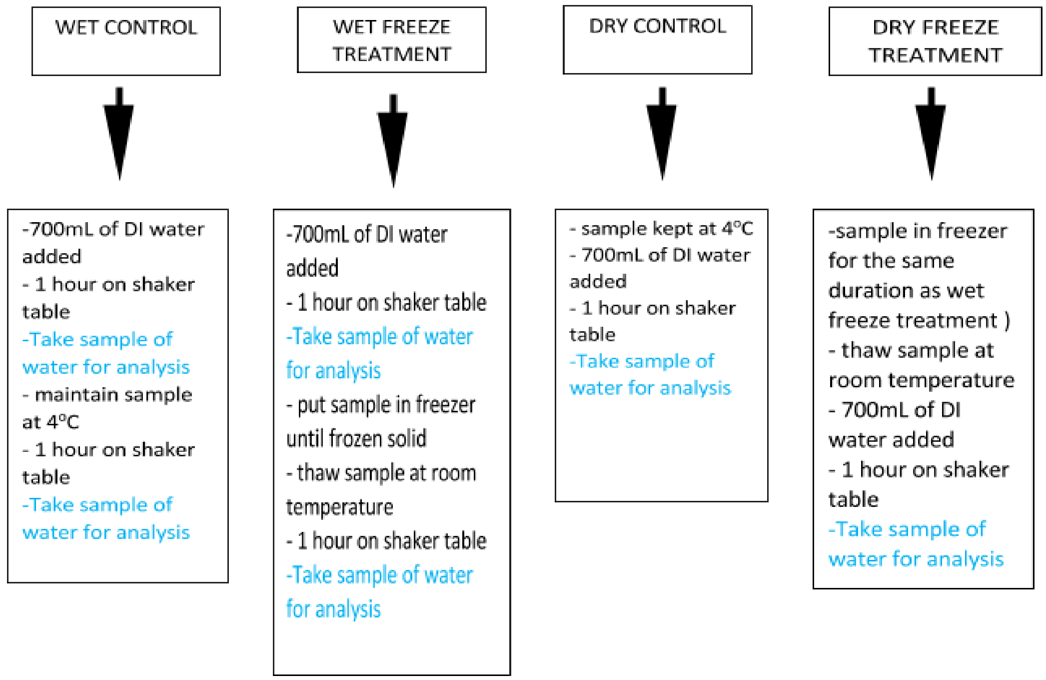

Figure 3. After data normalization relationships between all treatment types were found to have no significant differences. Across all treatments and sample sites the relationships were found to be statistically the same; this relationship is represented by the letter "A". Wet samples were run twice, once pre-treatment and again post treatment.

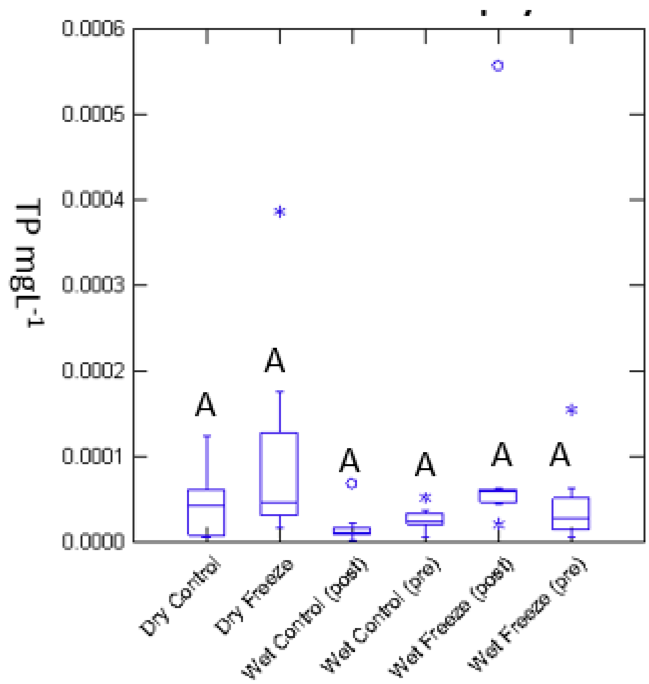

Table 1. A comparison of the three different sites average water chemistry for Total Phosphorus. Using an ANOVA no significant differences were found (Greenly and Eagle Bluffs Pool #4 had a p value = 0.052).

Site Name	Type of macrophyte sampled	GPS Coordinates	Site Description	Mean TP (mgL^{-1}) [min, max]
Greenley	Reed	-92.057, 39.949	Small Agricultural Pond	0.137 [0.089, 0.192]
Eagle Bluffs Pool #11	Reed & Cattails	-92.420, 38.832	Wetland cell farther from effluent discharge	0.756 [0.667, 0.832]
Eagle Bluffs Pool #4	Cattails	-92.439, 38.851	Wetland cell closer to effluent discharge	1.34 [0.697, 1.735]