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ABSTRACT

There is insufficient research focusing exclusively on how nutrient competition between algae and wetland macrophytes affects the growth of these species. This study examined the relationship between nutrient concentrations (N and P), algal concentrations, and the growth of *Juncus effusus*. *Juncus effusus* growth in the Lake Fayetteville artificial spiral wetland was monitored over a four month period during the prime growing season. Eighteen plants were taken from the wetland and replanted in 1 of 6 treatments: plant-only, algae-only, combined, plant-only +supplement, algae-only +supplement, or combined +supplement. The algae and combined environments received an inoculation of algae, and the +supplement treatments received an infusion of an N and a P supplement. An analysis of variance test was conducted to determine the presence of a significant relationship between *Juncus effusus* growth, nutrient concentrations, and/or algal growth. No significant relationship existed between *Juncus effusus* and nutrient concentrations or between *Juncus effusus* and algal concentrations. There was a significant relationship between algal growth and the presence of *Juncus effusus*, which produced an additive effect causing the greatest algal growth in the combined +supplement treatment. Results indicate that nutrient competition between *Juncus effusus* and algae in the Lake Fayetteville artificial spiral wetland is not the limiting factor in *Juncus effusus* growth in the wetland.

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MEET THE STUDENT-AUTHOR

Toryn Jones

I am from Haskell, Oklahoma where I graduated valedictorian from Haskell High School. I chose to pursue a degree from the University of Arkansas because of its beautiful location and the research opportunities available in the Dale Bumpers College. I graduated sum cum laude with a Bachelor's of Science degree in Environmental, Soil and Water Science.

As an undergraduate, I served on the Dale Bumpers Honors Student Advisory Board and held various positions with the Baptist Collegiate Ministry. I am currently conducting research at the University of Arkansas as a graduate student working under Dr. Thad Scott. I intend to use my experience from Arkansas to acquire a research position at an environmental quality station in Southeast Asia.

I would like to thank my faculty advisor, Dr. Thad Scott for his mentorship. I would also like to thank my other board member, Dr. Lisa Wood and Dr. Curt Rom for their guidance with my thesis and throughout my undergraduate career.

INTRODUCTION

The Lake Fayetteville artificial spiral wetland, which covered approximately 1000 square feet near the Lake Fayetteville dam, was an artificial wetland designed and built by Stacy Levy (http://www.stacylevy.com/). The Spiral Wetland was constructed in the spring of 2013 and was decommissioned in October of 2014. The purpose behind the wetland was to educate the public on the effects of eutrophication and to reduce the impact of nitrogen and phosphorus enrichment on the lake. Also, the artificial wetland provided an aesthetically pleasing view from the lake's park trail, and served as a habitat for many insects, fish, and birds. However, an unanticipated issue arose in that the plant installed in the wetland, *Juncus effuses*, did not appear to grow after being established in Lake Fayetteville.

Eutrophication: Cause and Effect

Eutrophication can be defined as the excessive nutrient enrichment of a water body (Smith et al., 1999). The two most common nutrients involved in eutrophication are nitrogen and phosphorus. Nitrogen and phosphorus are the two limiting agents in plant growth, and, when an excess is introduced into the environment, plants in that environment can begin to grow at a consistent rate until they either reach critical mass or exhaust the excess nutrients (Koottatep and Polprasert, 1997). Unfortunately, nitrogen and phosphorus are highly concentrated in common run-off contaminants such as animal feces, leaf litter, food, and nutrient fertilizers making eutrophication of local water bodies a fairly common occurrence, especially near agricultural areas (Hammer and Knight, 1994).

The most notable effects of eutrophication are algal blooms and hypoxic zones. Algal blooms are the emergence of extremely high concentrations of algae and cyanobacteria (Stevenson et al., 1996). These blooms are characterized by their stench, deep red and green colorings, and high rates of photosynthesis. Hypoxic zones are areas where dissolved oxygen is so low that very few aquatic lifeforms can survive. Hypoxic zones are created once algal blooms die off and detritivores deplete dissolved oxygen through respiration while breaking down the algae for chemical energy (Smith et al., 1999).

Both of these results have deleterious effects leading to the slow breakdown of the aquatic ecosystem. Loss of aquatic species and resources leads to further damage by negatively impacting the surrounding terrestrial environment. For humans, eutrophication can lead to the loss of recreational waters, drinking water, food sources, and several benefits from neighboring water bodies and related terrestrial environments (Smith et al., 1999).

Artificial Wetlands: Agents of Eutrophic Remediation

An artificial wetland is a manmade reproduction of a wetland ecosystem that serves the purpose of either replacing a damaged wetland or assisting in the remediation of a polluted waterbody (Kadlec and Wallace, 2009).

There are three primary functions by which artificial wetlands are able to prevent, manage, and reverse eutrophication. These functions are nutrient competition (Reinhardt et al., 2006), light attenuation (Kadlec and Wallace, 2009), and biological oxygen demand (BOD) reduction (Karathanasis, et al., 2003). In a water body experiencing eutrophication, algae and cyanobacteria are often the only organisms capable of using the nitrogen and phosphorus suspended away from the shore (Stevenson et al., 1996). However, when an artificial wetland is constructed and placed over the waterbody, it introduces new hydrophytes capable of utilizing large quantities of nitrogen and phosphorus for growth, thus reducing the amount of nutrients available to algae and cyanobacteria (Vymazal et al., 2007). The competition for nutrients brought on by the introduction of an artificial wetland puts a stress on algae and cyanobacteria which helps to regulate growth and prevent algal blooms (Crumpton and Van Der Valk, 1989).

A somewhat similar effect happens with sunlight. Because algae and cyanobacteria use photosynthesis as a means of energy production, sunlight is crucial in their growth process. By creating a wetland with a solid, opaque layer, light is unable to reach the algae and cyanobacteria under the constructed wetland. In this way, wetlands are able to partially manage the amount of energy available to algae and cyanobacteria (Kadlec and Wallace, 2009).

Wetlands also reduce BOD (Karathanasis, et al., 2003). The BOD is the measure of dissolved oxygen (DO) needed to completely decompose organic matter in a water body (Karathanasis, et al., 2003). High residence time and retention rate in a wetland allows for microbial organisms to break down organic matter from runoff before it enters the primary waterbody (Karathanasis, et al., 1997). This slows the depletion of dissolved oxygen within the waterbody. While the reduction of BOD does not directly remediate eutrophication, it does slow the rate of dissolved oxygen depletion which is a destructive result of eutrophication.

There are several benefits and drawbacks to constructing an artificial wetland as a remediation strategy. The key factors are time, space, money, and personnel. Wetland remediation can take several decades to have a significant effect (Turner et al., 2000). Depending on the waterbody being remediated and the wetland design, remediation by wetlands can also be costly and require heavy maintenance and monitoring for the first several years (Turner et. al, 2000). Yet, due to their resiliency and flexibility, artificial wetlands are an effective long-term and supplemental remediation strategy (Barbier, 1993). This research project seeks to examine the effectiveness of the Lake Fayetteville artificial spiral wetland at reducing the rate of eutrophication in Lake Fayetteville, and to identify the growth limitations of *Juncus effusus*.

Research Hypotheses

There were three hypotheses for this study:

Hypothesis 1. Juncus effusus biomass and algal biomass will be greater where they are grown independently than where they are grown together.

Hypothesis 2. Juncus effusus biomass and algal biomass will increase with increasing nitrogen and phosphorus concentrations in Lake Fayetteville water.

Hypothesis 3. Algae and *Juncus effusus* grown independently from the other and with added nitrogen and phosphorus will display a synergistic effect rather than an additive effect.

MATERIALS AND METHODS

Nutrient Competition Experiment

To test the effects of nutrient competition on the growth of *Juncus Effusus*, a two-variable experiment was conducted that compared six varying growth conditions for algae and *Juncus effusus*. The variables were (1) the presence of a competitive organism (algae and/or *Juncus effusus*) and (2) availability of nitrogen and phosphorus. Fifteen *Juncus effusus* plants roughly similar in root and shoot length were selected from the wetland and the roots and shoots were cleaned to minimize contamination. Eighteen 5-gal buckets were laid out in 3, 2 × 3 sets and filled with 5 gal of freshwater. Four buckets in each set had a planter harnessed in just above the water using bailing wire. In each set, the two buckets without planters and two of the buckets with planters were inoculated with 75 mL of algal biomass. This resulted in each set of replicates having two buckets with only algae, two buckets with only *Juncus effusus*, and two buckets with algae and *Juncus effusus* combined. In each set, one bucket from each of these pairs was given 30 mL of trisodium phosphate and 50 mL of potassium nitrate nutrient solutions. These dosages were calculated using maximum concentrations found in Lake Fayetteville. Each set contained one algae-only bucket, one algae-only + supplement bucket, one plant-only bucket, one plant-only + supplement bucket, one combined (algae and plant) bucket, and one combined + supplement bucket (Fig. 1). Each bucket was labelled accordingly and numbered 1, 2 or 3 for the set to which it belonged. To decrease bias, the position of each bucket in a set was chosen at random using a random number generator. The buckets were filled weekly and supplied with aerators. This set-up was monitored for two months, at which time it was disassembled and the plants were harvested to measure biomass and C:N.

Plant Biomass

To measure the weights of the plant roots and shoots, the roots of each plant were removed beginning at the bottommost part of the soil conglomerate and the shoots

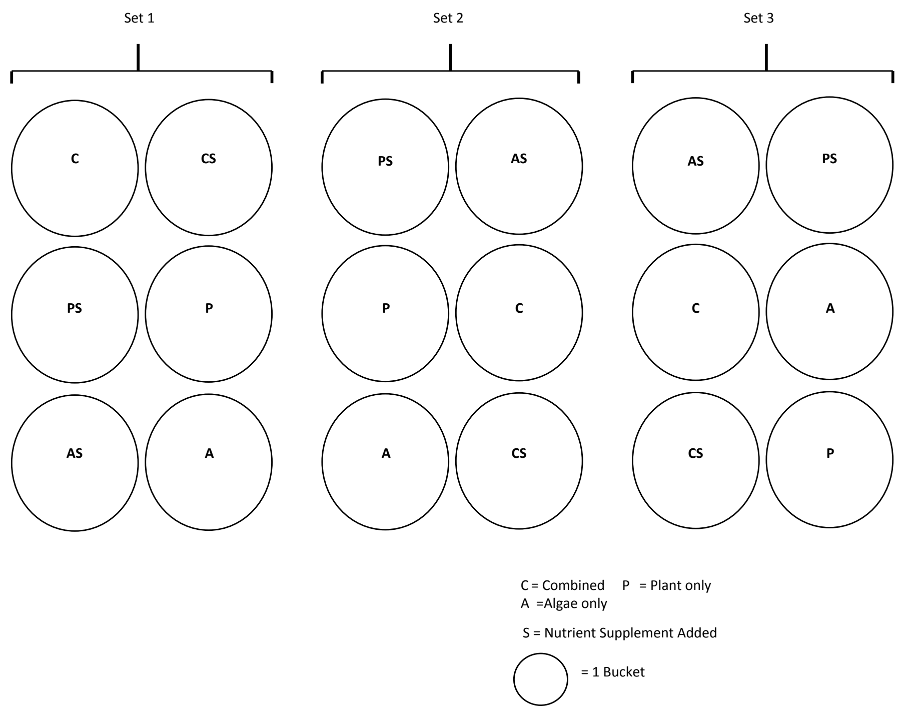

Fig. 1. Visual representation of experimental design.

of the plants were removed beginning at the uppermost part of the soil conglomerate. The roots and shoots were carefully gathered and put into separate bags. Each plant had its own pair of bags. The bags for each set of samples were placed in a drying oven for one week to three weeks. The dry weights of the contents of each bag were measured using a Mettler-Toledo Xs104 balance (Mettler-Toledo LLC, Columbus, Ohio). This process provided root, shoot, and total weight for each plant sample.

Plant C:N

Dried and weighed plant samples were ground into a powder using a plant mill followed for 45 s by a Wig-L-Bug grinder (International Crystal Labs Inc., Garfield, N.J.). Samples were analyzed for carbon and nitrogen content using a Thermo Flash 2000-C:N analyzer (Thermo Fischer Scientific Inc., Santa Clara, Calif.). Multiplying the dry biomass by the relative proportions of carbon and nitrogen provided the weights of carbon and nitrogen for each plant, shoot, and root.

Chlorophyll-A Concentrations

Water samples (300 mL) were collected after stirring at four irregular intervals. The water was vacuum-filtered and chlorophyll-a in the presence of 90% acetone was measured on a pre-calibrated Turner Fluorometer (Turner Designs Inc., Sunnyvale, Calif.) according to the Turner Design method.

Statistical Methods

Data were analyzed using SAS (SAS Institute, Inc., Cary, N.C.). A two-way analysis of variance (ANOVA) was run on the chlorophyll-a concentrations to test the significance of algae biomass concentrations across the six treatments over time. A one-way ANOVA was run on the carbon, nitrogen, and the CN ratios of each root sample, shoot sample, and whole plant harvested from the experimental phase.

RESULTS AND DISCUSSION

Experiment: Plants

There were no statistically significant differences between the plant (total, root, or shoot) masses or plant C:N ratios across the various competition and/or fertilizer treatments. However, the general patterns observed in the data were worth examination. Plant weight was greatest in plant-only +supplement environments. Conversely, plant weight was least in combined +supplement environments. Plant-only and combined environments without supplements were roughly equal and intermediate in all weight measurements (Fig. 2).

Carbon and nitrogen measurements for the plants were erratic. In plant roots and shoots, plant-only +supplement displayed the greatest concentrations of both carbon and nitrogen with the other three treatments being roughly similar. Plant shoot carbon was similar to the roots in that plant-only +supplement had the greatest concentration. Plants in treatments not receiving nutrients had roughly similar shoot carbon concentrations, while combined +supplement had a relatively low average concentration of shoot carbon. Plant shoot nitrogen was roughly equivalent for all four treatments. Plant carbon mirrored the pattern explained for plant shoot carbon, while plant nitrogen was similar with the exception of combined + supplement being slightly greater than the non-supplement receiving treatments (Fig. 3). Total plant C:N was greatest in the plant-only treatment; however, all treatments were roughly similar with a plant C:N range between 36.26 and 40.60 (Fig. 4).

Experiment: Algal Growth

Algal growth in relation to growing condition (algae, plant, combined) was the only variable measured in this experiment that was found to be statistically significant (P = 0.0154). In all growing conditions (algae, plant, combined), algal concentrations were greater when a nutrient supplement was added. Algal concentrations were greatest in the combined environments with combined +supplement treatments reaching 800 µg chl-a L^{-1}, and combined treatments reaching 345.6 µg chl-a L^{-1}. Algae-only environments displayed the lowest concentrations of algae at 94.5 (with supplement) and 9.7 (without supplement) µg chl-a L^{-1}. The plant-only environments were the intermediate values at 415.7 (with supplement) and 232.3 (without supplement) µg chl-a L^{-1} (Fig. 5).

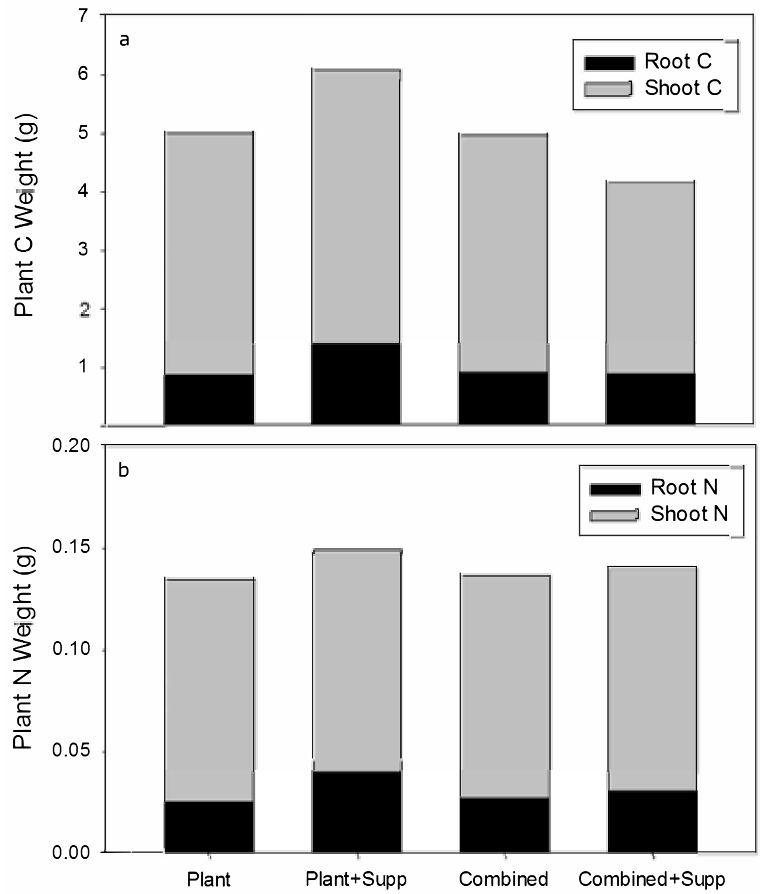

Fig. 3. (a) Proportions of root (P = 0.491), shoot (P = 0.113), and total plant carbon weight (P = 0.109) in relation to competition presence and nutrient availability. (b) Proportions of root (P = 0.615), shoot (P = 0.247), and total plant nitrogen weight (P = 0.378) in relation to competition presence and nutrient availability.

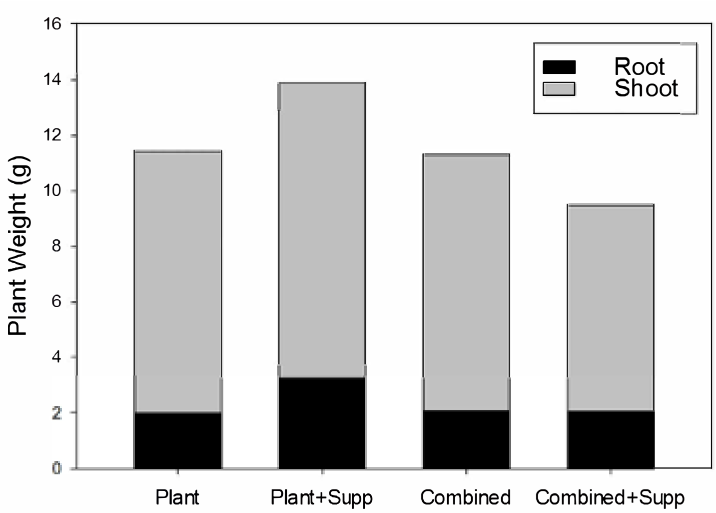

Fig. 2. Proportional root (P = 0.478), shoot (P = 0.130), and total plant weight (P = 0.127) in relation to the presence of competition and nutrient availability. (Combined = plant and algae, Supp = addition of N and P supplements).

Analysis of *Juncus effusus* Growth Limitations

Data collected for plant growth in the various treatments supported the rejection of the hypotheses that *Juncus effusus* growth in the Lake Fayetteville artificial spiral wetland is being limited by insufficient nutrient concentrations and/or competition with algae. However, the patterns in the data support the general idea that algae outcompetes emergent plants for nutrients. The lack of statistical significance could be a function of the limited replication in this experiment.

It was hypothesized that *Juncus effusus* placed in an environment with a higher nutrient concen-

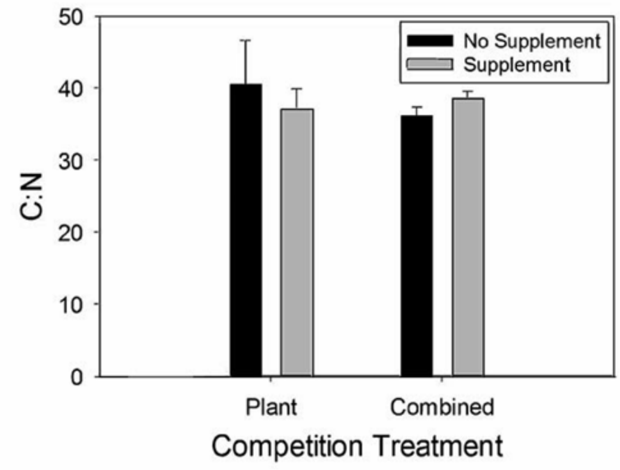

Fig. 4. Average carbon-nitrogen ratio of whole plants across various treatments ($P = 0.32$, $\alpha = 0.05$).

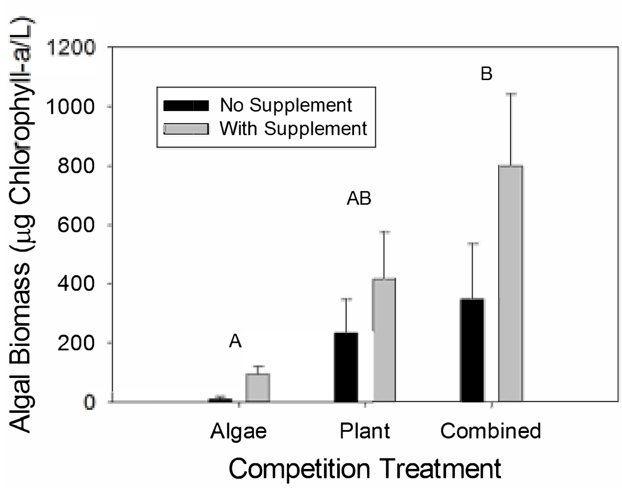

Fig. 5. Average, final algal concentrations across various treatments ($P = 0.0154$, $\alpha = 0.05$).

tration would experience accelerated growth in comparison to the plant without a supplement. This hypothesis was based on data by Gusewell and Koerselman (2002). However, data from the two treatments determined that there was no significant relationship between *Juncus effusus* mass and nutrient concentration. Therefore, nutrient availability alone is not a limiting factor for plant growth in the Lake Fayetteville artificial spiral wetland.

It was further hypothesized that *Juncus effusus* placed in an environment with higher algal concentrations would display less mass and lower nutrient contents due to stress as a result of competition. This hypothesis was molded from data from Engelhardt and Ritchie (2002). Yet, ANOVA showed there was no significant relationship between algal concentrations and mass of *Juncus effusus*. Thus, algal concentration alone is not a significantly limiting factor for plant growth in the Lake Fayetteville artificial wetland.

The last variable tested as a limiting factor for *Juncus effusus* growth was a synergistic effect forged by the combination of algal and nutrient concentrations. The idea behind this comparison was that the presence of excess nutrients could accelerate the growth of algae, which would cause rapid eutrophication in the small environment and exert the greatest stress of any treatment. Despite the combined-only treatment having the highest concentration of algae, lowest mass of *Juncus effusus*, and lowest whole-plant carbon to nitrogen ratio, the ANOVA results were the same in that no significant relationship was apparent. Hence, no synergistic effect of nutrient and algal concentrations is present.

Analysis of Algal Growth

While no relationship was established between the presence of algae and *Juncus effusus* growth, ANOVA revealed a significant relationship between the presence of *Juncus effusus* and algal growth.

Assuming *Juncus effusus* failed to exert a significant competitive stress on algae, the concentrations in the combined + supplement replicates should be roughly equal to the concentrations in the algae-only +supplement replicates. One possible explanation is that adding the *Juncus effusus* plants to the experimental treatments also transferred algae attached to these plants. In other words, the plants seeded the experimental units with extra algae.

IMPLICATIONS

The Lake Fayetteville artificial spiral wetland was designed to raise awareness regarding eutrophication and how nutrient enrichment in aquatic systems can be disastrous. The designers expressed a desire for the wetland to ecologically benefit the lake by reducing the rate of eutrophication. However, in the wetland's first year, there was minimal plant growth and *Juncus effusus*, which were expected to grow to their full 4 ft, scarcely reached 6 inches. The initial concern was that *Juncus effusus* was not sufficiently capable of competing with algae for nutrients needed for growth (N and P). However, after monitoring the wetland during its second summer (data not shown) and conducting this study, it is apparent that competition with aquatic vegetation was not a limiting factor in *Juncus effusus* growth. Alternative limitations which could have hindered *Juncus effusus* growth are: growing season, plant anatomy, and physical disturbance/stress.

Construction began on the artificial spiral wetland in the late spring of 2013. It is likely that, when the wetland was finished and seeds fully planted, *Juncus effusus* had missed part of its growing season and was not able to reach expected heights. *Juncus effusus* anatomy could also have an impact on why the plants grew poorly in the first growing season. According to a *Juncus effusus* fact sheet provided by the Natural Resource Conservation Service, *Juncus effusus* seeds need to over-winter near the surface of the soil in order to grow properly (NRCS, 2002). Furthermore, *Juncus effusus* grows naturally in moist soils 6 inches or less below water (NRCS, 2002). The *Juncus effusus* growing in the spiral wetland were in moist, shallow planters sitting above the lake. This means, rather than the plants being rooted in the soil and growing up through the water as they do naturally, they were grown in soil near the surface and their roots extended down into the open water.

The last credible option for limiting *Juncus effusus* growth is the constant physical disturbance endured by the *Juncus effusus* during the course of the spiral wetland installation. While situated in Lake Fayetteville, the spiral wetland suffered ice, wind, and hail damage. Due to lack of a skeletal structure, high winds easily flipped the wetland over on itself leaving dozens of plants at a time submerged underwater with their roots in the air. The wetland displayed damage caused by large birds roosting on the soft, foam body and even tears left by boaters. Physical disturbance at that level could explain the stunted growth exhibited by the *Juncus effusus*. Due to research rejecting the hypothesis that nutrient competition was a primary factor in limiting *Juncus effusus* growth, it is likely that one (or a combination) of the aforementioned factors are responsible for limiting *Juncus effusus* growth.

CONCLUSION

Results from this study revealed that *Juncus effusus* in the Lake Fayetteville artificial spiral wetland was not limited in growth by nutrient availability or competition with algae. The masses of plants grown in treatments containing algal inoculations and/or nutrient supplements were not significantly different from plant masses produced in the control treatments (plant-only). Similarly, there was no significant variation in the C:N ratios or the nutrient weights across the various treatments. Little research exists to evaluate the direct competition between wetland plants and algae. The results of this study were not statistically significant and could therefore not fully explain the patterns observed; however, this could have been the result of poor replication. A more comprehensive study with greater replication could show that the biological patterns observed in this study were meaningful, which could influence the future construction and maintenance of floating wetlands for aesthetic and water quality improvement purposes.

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