# INTEGRATING SUSTAINABLE PRACTICES: COMPOST TEA AS A NUTRIENT SUPPLEMENT FOR AQUAPONIC PLANT PRODUCTION

by

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# Abstract

**Integrating Sustainable Practices: Compost Tea as a Nutrient Supplement for Aquaponic Plant Production**

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At Stafford Creek Corrections Center, the Sustainability in Prisons Project (SPP), a partnership between The Evergreen State College and The Washington Department of Corrections, grows native emergent wetland vegetation in coconut coir mats for use in Oregon spotted frog (*Rana pretiosa*) habitat restoration. The plants are produced aquaponically, using fish waste as fertilizer. Aquaponics is a symbiotic relationship among plants, fish and beneficial bacteria that convert ammonia and nitrite—both toxic to fish—into less toxic nitrate that plants can use. However, plants require at least 16 nutrients to complete their life cycle, and research shows that fish waste does not adequately provide all those nutrients. This thesis explores the use of compost tea, a brew of compost in water intended to grow beneficial microbial communities and extract dissolved nutrients, as a nutrient supplement. Using a quasi-experimental interrupted time series design, plant tissue samples were taken before and after a compost tea addition to determine changes in tissue concentrations of phosphorus, calcium, magnesium and manganese of two graminoid species as a result of compost tea addition. P, Ca, Mg, and Mn in *Carex obnupta* decreased by 22%, 17%, 18%, and 13% respectively, while *Deschampsia cespitosa* nutrient concentrations did not significantly change. This difference may be due to rapid growth in *C. obnupta* versus slower growth in *D. cespitosa*. Although this experiment was not able to show that compost tea could act as a nutrient supplement in aquaponic water, suggestions are given for improving the research design including improving the compost tea recipe, extending the experimental timeline, measuring plant growth, and analyzing water samples.

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# Introduction

This thesis presents several sustainable practices intended to reduce costs, effort, and pollution while restoring habitat for a threatened species. It grew from a pilot project initiated in 2016 by the Sustainability in Prisons Project (SPP)—a partnership between the Washington State Department of Corrections and the Evergreen State College—that uses an aquaponics system to produce vegetated wetland mats for restoration projects benefiting the federally threatened Oregon spotted frog (*Rana pretiosa*). The project is located at Stafford Creek Corrections Center (SCCC) in Aberdeen, Washington. The vegetated mats are used to both suppress invasive reed canarygrass (*Phalaris arundinacea*) and provide a substrate for native wetland plants that outcompete *P. arundicacea* and provide spawning and rearing habitat for *R. pretiosa*.

*Rana pretiosa* was listed as threatened in 2014 by the United States Fish and Wildlife Service and listed as endangered by the Canadian Species at Risk act in 2003. Historically, *R. pretiosa* was found from southwestern British Columbia, Canada into northern California, and both east and west of the Cascades in Oregon and Washington (WDFW, 2013). *R. pretiosa* has been extirpated from nearly 80% of its range, and now only occurs in Washington on the eastern side of the Puget Trough, and in the Cascades from south central Washington into Oregon's Klamath basin (USFWS, n.d.). In British Columbia, only four populations totaling fewer than 400 individuals remain (Environment Canada, 2015). Recovery efforts have included captive rearing and species reintroduction projects in which SPP was actively involved. *R. pretiosa* captive rearing at Cedar Creek Correctional Facility was the first endangered species project of its kind in a prison; from 2009 through 2015, in partnership with the Oregon Spotted Frog Working

Group, incarcerated technicians raised and released 879 frogs (Sustainability in Prisons Project, 2018). However, reintroduction was not always successful. By late 2012, nearly 5,500 frogs from various captive rearing facilities had been released at Dailman Lake on JBLM, a site previously occupied by *R. pretiosa* (WDFW, 2013). Egg masses were only found in 2011, and from 2012 until 2017, there was no evidence of *R. pretiosa* at Dailman Lake (WDFW, 2013). A single *R. pretiosa* was spotted near the Dailman Lake release site in late 2017, and biologists are uncertain of its origin, since *R. pretiosa* return to the same breeding sites year after year (Hallock, 2013) and had not been observed at this site since 2011 (Amber Martens, personal communication, December 5, 2017).

The greatest threat to *R. pretiosa* is loss of wetland habitat (WDFW, 2013). *Rana pretiosa* is the only fully aquatic frog in the Pacific Northwest; this means it relies on standing water for its entire life cycle (Hallock, 2013). More than 30% of historic wetland habitat in Washington has been lost due to human activities such as development, which leads to habitat fragmentation, water quality degradation, and hydrologic alteration by ditching and damming (USFWS, n.d.).

Another source of *R. pretiosa* habitat loss is reed canarygrass (*P. arundinacea*), an invasive, non-native species that is capable of quickly outcompeting and replacing the native vegetation on which *R. pretiosa* rely (Healy & Zedler, 2010). As a fully aquatic frog, *R. pretiosa* prefer sedge and hardhack dominated wetlands with areas of standing water and tend to avoid the dense thickets created by *P. arundinacea* (Hallock, 2013). However, all sites in Washington with established *R. pretiosa* populations are significantly impacted by *P. arundinacea* (M. Hayes, personal communication, August 7, 2018). SPP and partners are actively researching the emergent vegetated mats'

effectiveness in suppressing *P. arundinacea*. *P. arundinacea* employs multiple strategies to maintain a competitive advantage over other species, resulting in a dense monoculture that is difficult to eradicate. It can reproduce using seeds, rhizomes, and stem fragments; it sprouts early and grows rapidly; it has a long growing season; and although it can invade a wide variety of habitats (Hook et al., 2009), it takes particular advantage of the interaction between shallow water and nutrients (Healy & Zedler, 2010), which can be detrimental to wetland habitats.

Reintroducing native plant diversity into landscapes that have been invaded by *P. arundinacea* requires multiple approaches and can take many years; no single approach is effective (Healy & Zedler, 2010). Even employing multiple approaches can be ineffective. Healy and Zedler (2010) studied the effects of herbicide, burning, and re-seeding with native vegetation over a three-year period. Herbicide was applied each spring, and the remaining slash was burned prior to seeding. Although herbicide and burning effectively increased species richness, there was no difference in seeded and unseeded plots and some species were other non-native weeds. Additionally, these effects were not sustainable; one year after the conclusion of the experiment, *P. arundinacea* averaged 170 cm tall with 88% cover in control plots (no herbicide), while in the experimental plots, reed canary grass averaged 149 cm tall with 81% coverage.

The Sustainability in Prisons Project (SPP) is a partnership between the Washington State Department of Corrections and The Evergreen State College. SPP operates conservation nurseries in several Western Washington prisons that produce plants for restoration projects. A nursery at Stafford Creek Corrections Center (SCCC) is producing vegetated wetland mats in support of *R. pretiosa* habitat restoration efforts in

Washington. Technicians in the nursery grow several species of native wetland emergent plants from wild seed collected on or near restoration sites. When the seedlings have produced ample roots and shoots, they are transferred to coconut coir mats. After the plants are installed in the mats, they continue to grow until the shoots achieve greater than 50% cover. The vegetated mats are delivered to restoration sites where they are staked down using wooden or metal stakes.

The mats, which are made of coconut coir fibers enclosed in coconut twine netting and are about ten centimeters thick, have been used successfully in aquatic restoration projects to suppress weeds and provide a substrate for native plants (Hook et al., 2009). Vegetated coconut coir mats hold promise for restoration of wetland habitats dominated by *P. arundinacea*. One experiment compared seven methods for reestablishing native sedge dominated wetlands in Wyoming (Hook et al., 2009). The least intensive of the seven methods included passive revegetation of a control plot (no additional planting) and broadcast seeding, while more intensive methods compared five different revegetation techniques: direct planting of containerized plants, direct planting of plugs, planting bare root stock, installing salvaged marsh surface from a neighboring wetland, and installing coconut coir mats containing pre-established wetland vegetation. The vegetated mats significantly outperformed all treatments and contained ≤5% non-native or invasive species at the end of the second growing season.

Several potential mechanisms could explain the superior performance of vegetated mats over other methods of competing with *P. arundinacea* and establishing native vegetation. The mats themselves create a physical barrier at the soil surface, while the extensive root systems of the established plants are able to quickly penetrate the soil

below. Well-established plants provide cover that reduces microsites for invasive seedlings, and the coconut fiber in the mats provide a carbon source for denitrifying bacteria that reduce the availability of inorganic nitrogen on which *P. arundinacea* thrives (Hamman, 2016). SPP has noted root penetration of the soil by the plants in the mats as soon as two weeks post installation (Amanda Mintz, personal observation, October 19, 2017). An experiment is currently underway to assess the ability of the mats produced at SCCC to suppress reed canarygrass, increase the diversity of native vegetation, and serve as breeding sites for the Oregon spotted frog. The experiment involves varying levels of *P. arundinacea* eradication, from no treatment to burning, pesticide application, and mechanical removal (Hamman, 2016).

All seeds, plants, and vegetated mats at SCCC are grown in an aquaponics system. Aquaponics combines aquaculture, which refers to fish farming, with hydroponics, which is a method of producing plant crops using nutrient fortified water instead of soil. Combining aquaculture with hydroponics reduces fish waste pollution and the need for chemical fertilizers because the plants use nutrients in the fish waste as fertilizer. In addition to water and fertilizer savings, aquaponics is gaining popularity because it can produce several food crops (fish, plants) in a smaller space throughout the year, and be used in places where conventional agriculture is spatially or environmentally limited (Love et al., 2014). However, several authors have cited the tendency for aquaponics to be deficient in several nutrients vital to plant health (Somerville et al., 2014; Rakocy et al., 2006), particularly iron, potassium and calcium, and recommend adding these nutrients as fertilizers.

In the aquaponics system at SCCC, symptoms of chlorosis, or yellowing of

leaves, have been observed on several different plant species (Fig. 1). Chlorosis can be a symptom of several different nutrient deficiencies, with iron deficiency being most common in aquaponics (Bartelme et al., 2018).

**Figure 1.** Dark green leaves on this *Carex obnupta* are normal; the yellow leaves on the same plant are a sign of chlorosis, a possible nutrient deficiency.

Between 2016 and 2018, compost tea, a fermented brew of compost, was added to the system several times based on anectodal reports that it could improve the health of the plants by increasing their nutrient content, stimulating growth, and improving health, making them more competitive against invasive weeds at restoration sites (Carl Elliott, personal communication [ca. 2016]). In theory, compost tea promotes plant health via beneficial microorganisms that break down organic matter into nutrients available for uptake by plants (mineralization), compete with pathogenic organisms, or otherwise

stimulate the capacity of plants to take up available nutrients (Ingham, 2005). In addition to mineralization of organic matter and protection against pathogens, plant-microorganism interactions in soil-based agriculture are known to promote root growth and fix nitrogen (Bartelme et al., 2018). This increased root surface area can enhance the ability of plants to absorb nutrients. Aquaponics systems provide an underexplored opportunity to evaluate plant-microorganism interactions that eliminate soil matrix complexities and control physical parameters such as temperature, pH and hydraulic retention time (Bartelme et al., 2018). To that end, this thesis investigates the following question: does adding compost tea to the recirculating water in an aquaponics system increase plant tissue nutrient concentration of emergent wetland vegetation grown to enhance *R. pretiosa* habitat and eradicate *P. arundinacea*?

The results of this investigation will be used to update and improve SPP's compost tea protocols for the aquaponics system. Healthier plants will potentially grow and root faster and be more competitive against invasive *P. arundinacea*. Because adding compost tea to aquaponics water has not been previously investigated in the literature, this thesis also makes a significant contribution to the body of knowledge for both aquaponics and compost tea. Aquaponics is an emerging tool for addressing sustainability in food systems (Love et al., 2014). If compost tea increases the nutrient content of plants in aquaponics, it could be a viable alternative to the unsustainable use of chemical fertilizers.

The thesis begins with a literature review presenting the current body of knowledge on plant nutrition in aquaponics, the mechanisms behind compost tea efficacy, and how those two practices may be linked to improve aquaponic plant health.

The literature review provides an overview of how aquaponics works, followed by a discussion of nutrient cycling and nutrient limitations in aquaponics systems, and how aquaponics practitioners address these limitations. It then presents compost tea as an option for nutrient supplementation in aquaponics by reviewing what is known about compost tea as a nutrient supplement and how this knowledge might be applied to an aquaponic system. Following the literature review, the methods describe the aquaponics system parameters at SCCC, followed by SPP's protocols for brewing and adding compost tea. Next, plant tissue collection, storage, preparation, and analysis of tissue nutrient content are described. Results of plant tissue analysis show how plant tissue nutrient content changes over time, before and after compost tea additions. Results are followed by a discussion with an attempt to disinter the effects on plant tissue nutrient content of the compost tea addition, and concluding remarks include suggestions for further research.

# Literature Review

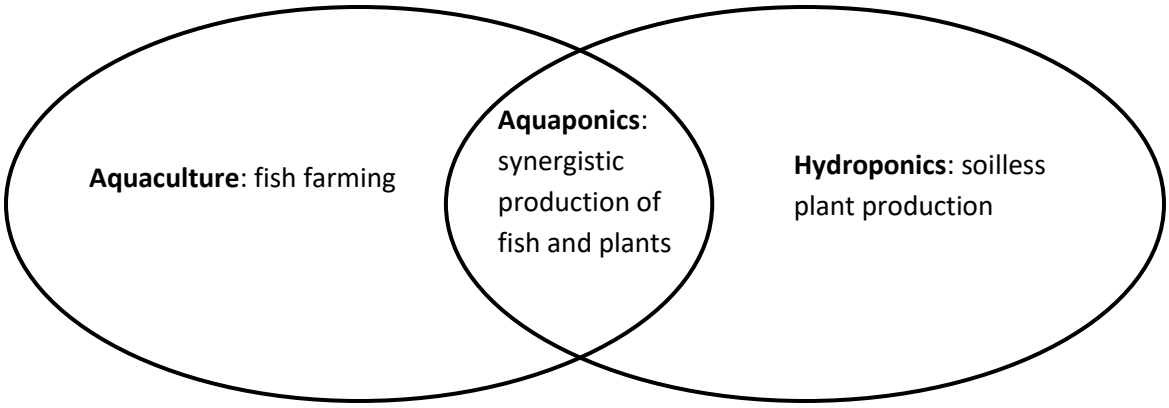
## What is Aquaponics?

### Introduction

Aquaponics and compost tea are both emerging topics of research in the arena of sustainable agriculture. This literature review describes how compost tea may be used to complement nutrients in aquaponics systems. First, aquaponics is defined, and the reasons for its emerging popularity are described. The complexity of nutrient cycling is presented, followed by a review of literature that both supports and discredits the need to supplement nutrients in aquaponics. The next section defines compost tea and how the biological community it comprises may increase nutrient availability to plants, both by breaking down organic material and by releasing plant growth promoting compounds. Further research also indicates that compost tea may contain a dissolved nutrient pool readily available for plant uptake. This chapter concludes with a brief synthesis of the research presented, and justification for exploring the use of compost tea in aquaponics.

### Aquaponics Basics

Aquaponics is a method of growing plants using fish waste as fertilizer. An aquaponic system combines fish farming, or aquaculture, with hydroponic (soilless) plant production (Rakocy et al., 2006; Goddek et al., 2015). In an aquaponics system, nitrogen rich aquaculture water is circulated through the hydroponic portion of the system to fertilize the plants.




Aquaponics combines the benefits of the two systems into a recirculating system that uses fish effluent to fertilize plants. In general, an aquaponics system requires five components (Fig. 2): a fish production unit (aquaculture), a mechanical filter to remove fish manure and uneaten feed, a biological filter containing beneficial bacteria, a pump to move water, and a sump tank to control the amount of water flowing between the aquaculture and hydroponic components (Goddek et al., 2015; Rakocy, 2006). Beyond this basic configuration, aquaponics systems vary widely in size, structure, and species. One of the first and best-known aquaponics systems is located at the University of the Virgin Islands where James Rakocy and colleagues operate a large-scale system stocked with tilapia species that produces vegetables in floating rafts (Konig et al., 2018; Rakocy et al., 2006; Masser et al., 1999). Other systems' hydroponic components include soilless media beds, ebbing and flowing water, or the nutrient film technique in which plants are placed in holes in horizontal pipes, receiving nutrients via a thin film of water flowing through the pipes (Somerville et al., 2014). A variety of fish species, such as carp (*Cyprinus carpio*), common catfish (*Ictalurus punctatus*), rainbow trout (*Oncorhynchus mykiss*), and even giant river prawn (*Macrobrachium rosenbergii*), have been successfully reared in aquaponics (Somerville et al., 2014).

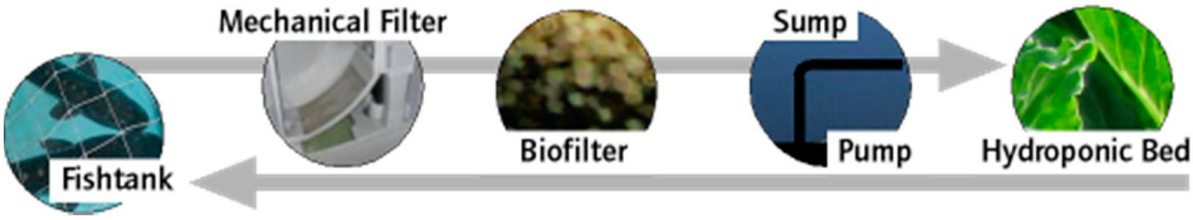



**Figure 1**. Basic components of an aquaponics system (Goddek et al., 2015).

Aquaculture supplies nearly 50% of the global supply of food fish (Turcios and Papenbrock, 2015), because fish can be intensively farmed with less environmental impact than commercial fishing. Hydroponics systems supply nutrients to plants via irrigation water, rather than soil. In a hydroponics system, the water is enriched with nutrient solutions in specific ratios to optimize plant production. Advantages of hydroponics include reduction in soil pathogens, greater water use efficiency, more even distribution of nutrients, and greater crop yields than traditional agriculture (Putra & Yuliando, 2015).

## An Emerging Sustainable Technology

There has been a rapid increase in the use of aquaponics in the last decade. The first modern aquaponics systems were developed in the 1970s to address water quality issues in intensive aquaculture (Bartelme et al., 2018). Since aquaculture produces nearly half of all fish consumed worldwide, it is necessary to reduce its ecological footprint (Turcios & Papenbrock, 2014). Although modern aquaponics technology is still in its infancy, the idea of using fish waste to fertilize plants stems from ancient practices (Goddek et al., 2015). The earliest examples include the 2000-year-old practice of using of fish in rice paddies in China and Thailand, and floating islands called Chinampas built by the Aztecs on shallow lakes in Mexico that date from about 1300 BC (Turcios & Papenbrock, 2014). Fish were cultivated between the islands, and their waste dredged and

used to fertilize the crops. Thus, the knowledge that fish waste contains the elements necessary for plant production has been used for millennia.

Modern aquaponics has emerged over the last four decades from regulatory constraints on fisheries leading to an increase in intensive aquaculture (Tyson et al., 2011) and the need for aquaculture practitioners to reduce toxic buildup of fish waste products (Love et al., 2014). Indeed, aquaculture is the fastest growing sector of worldwide food production; with the exception of poultry, fish consumption has outpaced all other terrestrial animals combined (Goddek, 2015; FAO, 2018). In an aquaculture system, fish produce solid waste and ammonia, which can reach levels toxic to fish if not removed. Removal of these wastes requires water exchanges and expensive treatment of up to ten percent of the total system volume per day to avoid environmentally damaging discharges of nitrates into the environment (Tyson et al., 2011). By using plants to filter out nitrogen wastes, aquaponics can reduce costs while saving water, since the water does not require treatment for nitrates and the clean water can be recirculated back to the fish.

Aquaponics also potentially solves the problem of relying on commercially-produced nutrient solutions and frequent water exchanges on which hydroponics relies (Goddek et al., 2015). In a recirculating aquaponics system, plants, fish, and microbes provide for one another in a mutualistic relationship; fish produce ammonia which can build up to toxic levels, microbes convert the ammonia into nitrate through the process of nitrification, and nitrate fertilizes the plants. Although plants absorb ammonia directly and can help alleviate stress on fish, for most plants, optimal production is achieved when nitrate is the primary nitrogen source (Tyson et al., 2011). By filtering nitrate from the water, the plants act as a biological filter, effectively cleaning the water before returning

it to the fish where the nitrogen is replenished. By recycling water and producing its own fertilizer, an aquaponics system has the potential to address environmental problems such as water and food shortages, soil degradation, and agricultural water pollution (Goddek et al., 2015). Recently, the motivation behind developing new aquaponics technologies has shifted to include issues of food security, environmental sustainability, health and community improvement, climate change adaptation, and education (Love et al., 2014). But many mysteries remain in the general understanding of economic feasibility, nutrient cycling, product yields, and potential for water and energy savings of aquaponics (Goddek et al., 2015; König et al., 2016; Delaide et al., 2017).

## Challenges to Sustainability

Although aquaponics is touted as a more sustainable alternative to aquaculture, agriculture and hydroponics alone, assessing the sustainability of aquaponics systems is complicated by the diversity of system designs and plant-fish combinations. Aquaponics practitioners must account for the unique temperature, salinity, dissolved oxygen and nutrition needs of the fish and plants they produce by designing systems that optimize the balance between the two. For example, the optimal pH range for fish production is 6.5-8.5, and most plants prefer a range of 5.5-7.5 (Somerville et al., 2014). Although there is overlap in this range, some micronutrients essential to plant growth are most mobile below pH 6.0, which can be detrimental to some species of fish (Delaide et al., 2016).

Furthermore, the microbial community vital to a functioning aquaponics system behaves differently with different system parameters. For example, low water recirculation rates can lead to reduced dissolved oxygen, causing reduced rate of oxidation of nitrite to nitrate and accumulation of nitrite to toxic levels (Wongkiew et al., 2017). Nitrifying bacteria function best at pH levels above 7, and many researchers have

reported optimal pH levels for nitrifying bacteria lie between 7.8 and 8.2 (Goddek et al., 2015).

The design and scale of the system affects the skill level required of the practitioner (Goddek et al., 2014); large scale systems may require several pumps, climate control technology, constant system monitoring, and the ability of the practitioner to immediately address problems that could harm fish. Despite these limitations, breakthroughs in the growing body of aquaponics research could lead to better efficiency, sustainability and availability of commercial aquaponics systems.

## Nutrients in Aquaponics

Nitrogen is the third most abundant element in plant tissue after carbon and oxygen, and the most important plant nutrient (Somerville et al., 2014). The primary benefit of coupling hydroponics and aquaponics is to recycle nitrogen into plant tissue, reducing the need for costly remediation or pollution caused by nitrate inputs to the watershed (Tyson et al., 2011). In an aquaponics system, fish produce nitrogen by excreting ammonia as metabolic waste through their gills and feces. If the ammonia is not removed rapidly, it could build to toxic levels and result in fish mortality. Ammonia levels as low as 0.02-0.07 mg/L are proven to have detrimental effects on certain species of fish (Rakocy et al., 2006). Beneficial bacteria grow naturally in aquaponics systems and are responsible for converting ammonia to nitrate, a form of nitrogen far less toxic to fish, through the nitrification process. Nitrification is a two-step process whereby specialized bacteria first convert ammonia into nitrite (also very toxic), then other groups of bacteria convert nitrite to nitrate:

$$NH_3 + 1\ ½\ O_2 \rightarrow NO_2^- + H^+ + H_2O$$ Nitrosomonas species

$$NO_2 + ½\ O_2 \rightarrow NO_3^-$$ Nitrobacter species

Nitrifying bacteria grow naturally in an aquaponics system on the surfaces of tanks, pipes, and even plant roots and growing media; many aquaponics systems also contain biofilter media, which provide significantly more surface area for bacterial growth (Fig. 3).

**Figure 2**. Bio balls are plastic balls that provide extra surface area for bacterial growth in the biofilter tank at SCCC.

Plants need at least 16 macro- and micronutrients for photosynthesis, growth, and reproduction (Somerville et al., 2014). Macronutrients are nutrients plants need in relatively large amounts including nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S). Micronutrients are required by plants, but in much smaller amounts than macronutrients. These include iron (Fe), manganese (Mn), boron (B), zinc (Zn), copper (Cu) and molybdenum (Mo). Plants grown aquaponically receive nutrients through fish effluent. For example, it is estimated that many fish species only

utilize up to 30% of the nitrogen in their food and excrete unincorporated nutrients into the water, which can then be recycled through incorporation into plant tissues (Roosta & Hamidpour, 2011).

Because fish and plants have different nutrient requirements, fish food is not formulated to optimize plant growth and plants may exhibit deficiencies in key nutrients, notably calcium, potassium and iron (Rakocy et al., 2006). Graeber and Junge (2009) noted that K was 45 times lower in aquaponics water than hydroponics, and according to Roosta and Hamidpour (2011), fish do not require K in their diets; therefore, it may not be incorporated into their feed. Many aquaponics practitioners recommend adding calcium carbonate and potassium carbonate as pH buffers against changes in acidity that could harm the fish (Somerville et al., 2014; Rakocy et al., 2006). These buffers have a secondary benefit of providing extra plant nutrition by adding $Ca^{2+}$ and $K^{+}$ ions to the aquaponic water. Aquaponics practitioners also recommend adding iron to aquaponics systems (Rakocy et al., 2006; Somerville et al., 2014). Iron is added to aquaponic systems as ferric ethylenediaminetetraacetic acid (EDTA), also known as chelated iron, which is a form of iron readily available to plants (Somerville et al., 2014; Rakocy et al., 2006). Chelates are soluble organic compounds capable of penetrating plant roots that form complexes with nutrients, such as iron, that would not otherwise be available for uptake by plants (Larcher, 1995).

Studies comparing hydroponic and aquaponic plant production indicate that many nutrients may be deficient in aquaponics systems in which the only nutrient source is fish waste (Bittsanszky, 2016; Roosta & Hamidpour, 2011; Rakocy, 2006). Hydroponics systems are optimized for plant growth based on known plant nutrient requirements.

Hydroponics solution is frequently tested and supplemented as nutrient concentrations are depleted. Aquaponics utilizes fish waste, which contains unpredictable and variable nutrient content. Environmental conditions, bacterial health, plant growth, accumulation of wastes, and fish well-being can all lead to changes in nutrient concentration in aquaponics solution. In a study of three mature aquaponics systems, Bittsanszky et al (2016) showed that most plant nutrients were significantly lower than standard hydroponics solution, except for calcium, sulfate, and copper; iron and manganese concentrations were lowest.

Despite the lower nutrient concentrations of aquaponics, researchers have shown that aquaponic plant production may rival that of hydroponics for certain species (Nozzi et al., 2018; Bittsansky et al., 2016; Delaide et al., 2016). Delaide et al. (2016) compared lettuce growth in hydroponic and aquaponic solutions and found no difference in shoot fresh weight between the two systems. Crops that produce fruits have higher nutrient requirements than leafy greens and may be more susceptible to nutrient stress (Bittsansky et al., 2016); however, studies have shown no differences in tomato production between hydroponics and aquaponics (Roosta & Hamidpour, 2011; Schmautz et al., 2016). Nozzi et al. (2018) compared hydroponic and aquaponic production of three different crops and four different levels of nutrient supplementation: aquaponics without nutrient supplementation, aquaponics with micronutrient supplementation, aquaponics with macro- and micronutrient supplementation, and a hydroponic control. The researchers found that response to nutrient concentrations was crop dependent; one crop responded best to lower nutrient concentrations, while the others preferred higher nutrient concentrations. Although the fully supplemented aquaponics and hydroponics produced

the best overall results for the plants that preferred higher nutrient concentrations, there was no difference in productivity among the treatments by the end of the study, leading the authors to conclude that nutrient supplementation is not always worth the higher costs and level of management (Nozzi et al. 2018).

Other studies have shown that aquaponic production lags somewhat behind hydroponic production but produces equal or better results than traditional agriculture (Van Ginkel, 2017; Delaide et al., 2016; Graeber & Junge, 2009). Van Ginkel et al. (2017) compared data from three aquaponics systems and one hydroponic grower to California fruit and vegetable production data collected by the University of California-Davis and found that hydroponics and aquaponics had fruit and vegetable areal productivities (unit of plant per square meter) 29 and 10 times higher than the conventionally produced crops. In an experiment comparing aquaponic and hydroponic crop production, Graeber and Junge (2009) found that tomato production in the aquaponic system was comparable to conventional tomato production in soil, but significantly lower than hydroponic production, in which the nutrient solution is carefully controlled. The authors also concluded that tomato quality (measured by taste) in the aquaponic system was compromised by potassium deficiency (Graeber & Junge, 2009).

Aquaponic systems tend to operate at higher pH than hydroponics in order to balance the needs of plants, fish, and microbes. Most importantly, the microbial community converts ammonia to nitrate much more efficiently at pH above 7 (Goddek et al., 2015). Many aquaponics practitioners believe that this compromise between microbial health and plant production is necessary to maintain a healthy and sustainable aquaponics system. Some nutrients, such as nitrate, are available in solution for

immediate uptake by plants. Others are chemically bound or adsorbed to organic matter, which can reduce or prevent their uptake by plants. The pH of an aquaponics system can have a significant effect on nutrient mobility, particularly iron, copper, boron, manganese, and zinc, which become less available for plant uptake above pH 6 (Delaide et al., 2016). Phosphorus is known to bind to calcium as pH increases above 7 creating insoluble calcium phosphates and removing both phosphorus and calcium nutrient pools from the aqueous solutions (Cerozi & Fitzsimmons, 2016). However, Cerozi and Fitzsimmons (2016) investigated the effect of pH on phosphorus availability in water enriched by tilapia effluent and compared the results to a computer model. They found that at phosphorus availability could be maintained at high pH (up to 10.0) due to the presence of dissolved organic carbon (DOC) and carbonates found in aquaponic solution. The DOC and carbonates can bind calcium before it has a chance to react with phosphorus, maintaining phosphorus availability even at higher pH; these results agreed with the computer model when the effects of DOC and water hardness (presence of carbonates) were factored in (Cerozi and Fitzsimmons 2016). In an experiment comparing spinach growth in a hydroponics system operating at pH 5 and supplemented with nutrient solution ideal for spinach growth with an aquaponics system operating at pH 7 and only supplemented with chelated iron, no significant differences were found in root or shoot biomass between treatments (Vandam et al., 2017), although several macronutrients were lower in the aquaponic spinach leaf tissue.

Hydroponics systems rely on nutrient fertilizer solutions for plant growth. Aquaponics systems strive for sustainability by using fish waste as fertilizer, reducing or eliminating the amount of nutrients that need to be added to the system, thereby reducing

the ecological footprint of hydroponic plant production. Apart from chelated iron, potassium, and calcium, some aquaponics practitioners discourage adding hydroponic nutrients to aquaponic systems, warning that adding hydroponic fertilizer could harm the fish or bacteria essential to proper system functioning (Somerville et al., 2014); yet they fail to provide evidence for any harmful direct effects of nutrients with the exception of nitrogen, which is already present in the system. Another, perhaps more important, consideration is the sustainability of adding nutrients to an aquaponics system. Many nutrients that are for agricultural purposes are non-renewable resources; phosphorus depletion is a major concern for global food production (Goddek et al., 2015).

Despite these misgivings, some researchers have discovered that supplementing aquaponics with nutrients produces crops that significantly outperform hydroponics (Nozzi et al., 2018; Delaide et al., 2016). In the first study examining leaf tissue nutrient content of aquaponically grown vegetables, Delaide et al. (2016) compared nutrient uptake of lettuce from a hydroponics system supplemented with nutrient solutions to reach levels specific to lettuce growth, an aquaponics system without supplementation, and a complemented aquaponics (CAP) system supplemented with nutrient solutions to match the concentrations of the hydroponic system. The experiment ran for 36 days, during which nutrient concentrations in the water were tested weekly. Lettuce was harvested on the 36$^{th}$ day and tested for P, K, Ca, Mg, S, Na, Fe, B, Cu, Zn, Mn, and Mo. All leaf tissue macronutrient concentrations (P, K, Ca, Mg, S) were significantly higher in the CAP system, while Fe and Zn were higher in the hydroponic system and Mn and Mo highest in the aquaponics system. Additionally, shoot and root growth were significantly higher in the CAP system in the other two systems, despite aqueous nutrient

concentrations that matched the hydroponics system. The authors concluded that a combination of dissolved organic matter, which could contain plant growth promoting compounds, and plant growth promoting microbes, which are absent in sterile hydroponic systems, must be contributing to the additional growth observed in the CAP (Delaide et al., 2016).

It is clear that adding nutrients to aquaponics can have significant, positive effects on plant growth. However, adding nutrients can be costly and requires a higher level of management (Nozzi et al., 2018). There is still much to be discovered about the composition of dissolved organic matter in aquaponics (Delaide et al., 2016, Goddek et al., 2015), whether such organically derived nutrients are more effective than inorganic nutrient solutions (Nozzi et al., 2018), or whether it is necessary in all cases to supplement nutrients in aquaponics (Bittsanszky et al., 2016). The following section defines compost tea and describes how its interaction with organic matter in aquaponics might make it a viable alternative to adding nutrient solutions

## Compost Tea

### What is Compost Tea?

Compost tea is defined as "A water extract of compost produced to transfer microbial biomass, fine particulate organic matter, and soluble chemical components into an aqueous phase, intending to maintain or increase the living, beneficial microorganisms extracted from the compost" (National Organic Standards Board, 2006). Scheuerell & Mahafee further differentiate compost tea from compost leachate: compost tea is made through a process of fermenting compost in water for a specified amount of time, where fermentation is defined as the production of a microbial community that alters its environment. The brewing process of compost tea that encourages the growth of the

microbial community distinguishes it from compost leachate, which simply requires passing water through compost to extract the existing nutrients and organisms and leach organic matter (Scheuerell & Mahafee, 2002). A common method of brewing compost tea involves filling porous bags with compost and placing them in a vessel of water.

Compost tea can be aerated, requiring the use of constantly recirculating water that introduces oxygen into the tea. Aerated compost teas (ACT) require short brewing times (1-5 days) and promote the growth of aerobic microbes; non-aerated compost teas (NCT) require longer brewing times but are associated with reduced costs, since no energy is required for aeration as compost is simply steeped in water long enough to produce microbes (Scheuerell & Mahafee, 2002). Compost tea has been cited as an option for adding nutrients to both aquaponics (Somerville et al., 2014) and hydroponics systems (Ingham, 2005), but no studies have assessed its efficacy as a water additive in aquaponics.

### Beneficial Biology of Compost Tea

The bulk of compost tea research centers on its ability to suppress plant pathogens; however, similar mechanisms may be responsible for its growth promoting properties (St. Martin, Scheuerell & Mahafee, Dianez). These mechanisms are associated with both chemical and physical properties of the microbial community produced by the compost tea. Some of these properties are due to the microorganisms in the compost tea. Known as plant growth promoting microbes (PGPM), they create a domino effect whereby competition for space, nutrients, and release of hormones and other compounds that stimulate plant growth create unfavorable conditions for pathogens and favorable conditions for plant growth (Bartelme, 2018). For example, saprophytic fungi, which feed on decaying organic matter, are known to suppress plant diseases by colonizing plant

roots, preventing pathogenic fungi from proliferating by occupying all available colonization sites on the roots (Gunnison & Barko, 1989). Since saprophytic fungi obtain nutrition by decomposing organic material, they can mineralize nutrients (convert them into forms available for plant uptake), stimulating the growth of the plant and providing greater root surface area for nutrient uptake (Bartelme, 2018).

Some PGPM are known to release compounds called siderophores that chelate ferric iron, leading to pathogen iron starvation while transporting the iron into plant tissue (Bartelme, 2018; Radzki, 2013; St. Martin). Microbially produced siderophores in compost tea made from wine grapes were able to suppress 100% of pathogenic fungi after two weeks of incubation in vitro by sequestering iron from the pathogens (Dianez et al., 2006). Further research has shown that the siderophore-sequestered iron is then used by plants. For example, Radzki et al. (2013) forced iron chlorosis in tomatoes by feeding them an iron-free nutrient solution for 35 days, then added a mixture of laboratory-cultured siderophores and ferric iron, which is bound and not available for uptake by plants. After the plants were harvested, the researchers found that siderophores significantly increased shoot length and stem diameter compared to a positive control containing fertilizer with iron, and a negative control containing iron-free fertilizer. Dry weight of the siderophore treated plants was not significantly different than that of the positive control, indicating that plants treated with ferric iron and siderophores perform as well as those receiving a full nutrient fertilizer; the siderophores were able to chelate the iron and transport it into the plant tissue. Furthermore, Radzki et al. (2013) found that both iron and phosphorus were highest in plants treated with siderophores; in fact, phosphorus content was significantly higher in plants treated with siderophores. An

explanation for increased phosphorus uptake was not given, but the increase in root mass shown in plants treated with siderohpores may have allowed the plant to take up more P than the plants treated only with fertilizer.

Organic compounds in compost are known to have positive effects on plant growth in soil and are thought to play a role in the plant growth promoting properties of compost tea (Trevisan et al., 2010; Xu et al., 2012). Humic substances, which make up approximately 60% of soil organic matter, stimulate both chemical and physiological responses in plants that contribute to their growth (Trevisan et al., 2010). Their efficacy as a plant growth promoting ingredient in compost tea is uncertain. In a study comparing ACT, NCT, compost leachate, and humic substances derived from each compost tea and leachate (H-ACT, H-NCT, H-leachate), Xu et al. (2010) showed that ACT significantly increased shoot biomass of cucumber by more than 50% compared to other treatments. However, although H-ACT yielded the highest nitrogen and chlorophyll contents in cucumber plants compared to H-NCT and H-leachate, they did not significantly increase root or shoot biomass, indicating that high concentrations of humic substances in soil may inhibit growth (Xu et al., 2012). The role of humic substances as a plant growth promoter in compost tea has not been well explored and warrants further investigation.

### Soluble Minerals in Compost Tea

The mineral content of compost tea can also affect plant growth. Pant et al. (2012) found that soluble nitrogen and gibberellin, a plant hormone, were the ingredients in compost tea responsible for stimulating the growth of pak choi. In this study, applications of compost tea made from food waste vermicompost significantly increased root and shoot length and leaf tissue concentrations of N, P, K, Ca, and Mg. Pant et al. (2012) speculated that some of the increase in mineral concentrations could be attributed to

increased root growth. A multiple regression analysis assessing the effects of active bacteria, fungi, compost tea soluble N concentration, humic acid, and the plant hormone gibberellin-4—produced by fungi typically found in food waste compost—on above ground plant fresh and dry weight, leaf area, N uptake, root dry weight, and root length showed that soluble N and gibberellin-4 were the components in the tea responsible for increased plant growth. However, the effects of soluble N on plant growth were superior to those of gibberellin-4; a one percent increase in gibberellin-4 only increased plant fresh weight by 0.026 g, whereas a 1 mg increase in soluble N increased plant fresh weight by 0.13 grams.

Despite the positive effects of the soluble N in compost tea on plant growth and small but significant effect of gibberellin-4, Edwards et al. (2006) concluded that N concentration of compost tea could not be the only mechanism responsible for its plant growth promoting effects.

### Factors Affecting Efficacy

Much debate exists regarding the efficacy and safety of aerated versus non-aerated compost teas. Non-aerated compost teas have been accused of harboring anaerobic pathogenic microbes that can cause human disease (Ingham, 2005); however, no published research supports these claims (Scheuerell & Mahafee, 2002). In fact, the bulk of compost tea research has proven the pathogen suppression and plant growth promoting capabilities of NCT (Scheuerell & Mahafee, 2002; St. Martin, 2012). Fewer studies have compared NCT to ACT, or focused on ACT alone (Scheuerell & Mahafee, 2002; Hargreaves et al., 2008, 2009). In two separate studies, Hargreaves et al. (2008, 2009) tested the effects of foliar applications of NCT and ACT on strawberries. NCT made from municipal waste (primarily food waste) maintained plant nutrient

concentrations at the same level as inorganic fertilizer (Hargreaves et al., 2008), while ACT made from municipal waste produced equal strawberry yields and similar leaf nutrient content as municipal waste compost directly applied to the soil. Scheuerell and Mahafee (2002) found no difference in performance of ACT versus NCT to control powdery mildew on roses and concluded that compost source was more important that aeration.

Several other studies indicate that the source of compost and brewing parameters of the tea significantly affect the final product (Ingham, 2005; Kim et al., 2015; Islam et al., 2016). The compost tea will only contain the types of microbes, organic matter, and inorganic compounds that were initially present in the source compost. Pant et al. (2012) compared chicken manure, food waste, and vermicomposts and their teas and found significant correlations among both mineral and fungal concentrations in the composts and their corresponding teas. Chicken manure-based composts and teas contained the highest concentrations of all nutrients, while vermicomposts and their teas contained the most active fungi; however, bacterial activity in the compost tea was not correlated with its corresponding compost type, nor was it significantly different among the teas tested, indicating that the brewing process itself produced a similar bacterial community among the teas. Brewing time, temperature, and oxygen will affect the rate at which the microbial communities reproduce, and therefore may affect the function of the compost tea. However, microbial colony formation and activity is expected to continue after the tea is applied, and will depend on environmental conditions (Ingham, 2005). For this reason, the dissolved nutrient concentration in the tea will not necessarily reflect what

nutrients will become available to plants as a result of compost tea addition, as microbial activity is expected to mineralize nutrients bound in organic matter (Ingham, 2005).

## Conclusion

Although the use of compost tea in aquaponics solution has not been addressed in the literature, there is ample evidence to presume that it could have positive effects on plant growth by releasing nutrients and other growth promoting compounds into the water. Aquaponics systems tend to operate at lower nutrient concentrations than hydroponic systems, which are supplemented with nutrient solutions in amounts specific to the known needs of the crop being produced. Hydroponics systems also tend to operate at lower pH, making nutrients more available to plants. Despite this, aquaponic plant production remains competitive with hydroponics, and when supplemented with nutrients to equal hydroponic levels, can achieve much greater growth—this interaction between nutrient solutions and aquaponics water is presumed to be related to dissolved organic matter that releases nutrients over time. Compost tea is brewed to encourage the growth of a microbial community that acts on organic matter, breaking it down into components that can be taken up by plants. There is also evidence that the microbes in compost tea release compounds that stimulate growth promoting hormones in plants. The growing popularity of aquaponics and the need to integrate more sustainable methods of fertilizing crops that do less environmental harm creates an opportunity to explore a relationship between these two emerging research topics. The plants in the aquaponics system at Stafford Creek Corrections Center have shown signs of nutrient stress, including chlorosis and whole plant death. This experiment investigates the possibility of using compost tea as an alternative to hydroponic fertilizers as a more sustainable solution to improving plant health.

# Methods

## Introduction

Plant tissue was analyzed for changes in concentration of six nutrients before and after compost tea additions. Nutrients selected for this study have all been cited as deficient in aquaponics (Rakocy et al.,2006; Roosta & Hamidpour, 2011) and spanned a range of macro- and micronutrients. The following six nutrients were selected (Somerville et al., 2014):

**Phosphorus** is a key component of DNA, cell membranes, and adenosine triphosphate (ADP). It also plays a role in photosynthesis.

**Potassium** controls ion flow through membranes and stomata opening.

**Calcium** is a key structural component of cell walls, cell membranes, and stems.

**Magnesium, iron, and manganese** are all critical components of photosynthesis.

Tissue samples were collected at Stafford Creek Corrections Center in Aberdeen, WA between Februray 5 and February 20, 2018 on a total of seven days, three times per day. Compost tea was brewed on site and added to the system on February 12. Samples were taken back to the laboratory at The Evergreen State College, Olympia, WA where they were washed, desiccated, ground, digested, and analyzed for nutrient concentration on a PerkinElmer Elan DRC-e ICP-MS. Microsoft Excel was used to calculate plant tissue concentrations from ICP-MS output, visualize data, and calculate summary statistics. JMP 12 and JMP 14 (SAS) were used for statistical analysis. The following methods section provides a site description and detailed information about plant collection, compost tea brewing, laboratory methods, and statistical analysis.

## Site Description

The Sustainability in Prisons Project (SPP) aquaponics greenhouse is located at Stafford Creek Corrections Center (SCCC) in Aberdeen, Washington. The greenhouse formerly operated an expensive tilapia aquaculture system that was not maintained. In late winter/early spring 2016, hydroponic beds were built adjacent to the existing tanks, pumps and plumbing, and tilapia were replaced with koi. Plants were growing in the hydroponic beds by summer 2016.

SCCC operates a continuously recirculating system that moves ammonia-enriched water from a fish tank, through a solid filter to remove fish solid waste and uneaten food, into a biofilter where ammonia is nitrified (converted to nitrate), and into two holding sump tanks, from which water is pumped into seven, 13.7 m long by 1 m wide planting beds lined with heavy duty pond liner fabric (Fig. 4). The plant beds receive water four times a day for 45 minutes on a staggered schedule, so that only two beds fill at one time; for example, beds 1A and 1B. This schedule ensures that the fish tanks won't become depleted. If the plant sumps become too full, water is redirected into the return sump and returned to the fish tank. After the water stops flowing, the beds take approximately another 30 minutes to drain completely. The beds drain into the return sump from which water is pumped back into the fish tank. The ambient air temperature in the greenhouse is controlled by a heater set at approximately 21° C and fluctuates with the weather +/- 5° C. Water is also heated and maintained between 21-23° C.

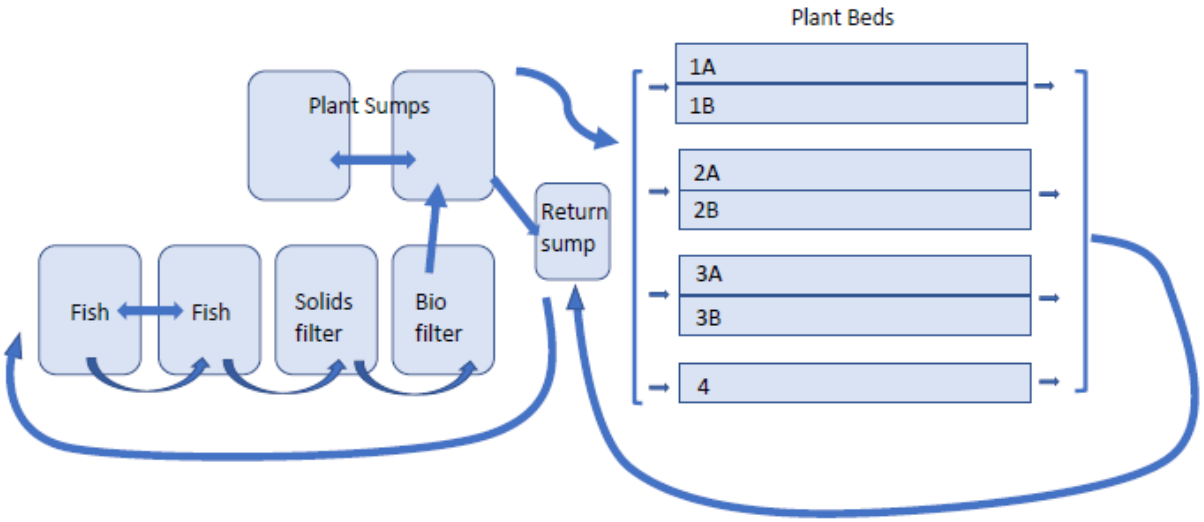



**Figure 3**. Schematic of the aquaponics system at Stafford Creek Corrections Center. Blue arrows denote the flow of water. Water moves between tanks and plant beds via a series of pumps controlled by float valves.

Incarcerated technicians regularly monitor pH, ammonia, nitrite, nitrate, general hardness and carbonate hardness daily using aquarium test kits (API/Mars Fishcare North America, Inc.). Temperature and salinity are monitored using continuously submerged alcohol thermometers and a KoiMedic Salinity Concentration Tester (Trans Instruments, Singapore), respectively. Daily water quality monitoring is necessary to ensure proper system functioning. Changes in nitrite or ammonia concentrations above 0.25 mg/L could indicate a problem with the biofilter and harm the fish (Somerville et al., 2014). Although pH tends to decrease with increasing nitrate, large fluctuations in pH could lead to changes in plant nutrient uptake rates, nitrogen conversion rates in the biofilter, and compromise fish health (Delaide et al., 2016; Somerville et al., 2014). Carbonate hardness (alkalinity) measures the pH buffering capacity of the system, while general hardness measures the concentrations of divalent cations, primarily the essential plant nutrients $Ca^{2+}$ and $Mg^{2+}$ (Somerville et al., 2014).

## Quasi-Experimental Design

Sampling followed a quasi-experimental interrupted time-series design. A quasi-experimental design was necessary since it was not possible to analyze a control group that received no treatment. A control treatment would require separate sump connected to beds that did not receive compost tea. This research was undertaken with full awareness of the limitations and threats to validity of the design. In the encouraging words of Campbell and Stanley (1963):

> [S]he should deliberately seek out those artificial and natural laboratories which provide the best opportunities for control. But beyond that [s]he should go ahead with experiment and interpretation, fully aware of the points on which the results are equivocal. While this awareness is important for experiments in which "full" control has not been exercised, it is crucial for quasi-experimental designs.

## Plant Sowing and Selection

Several species of wetland graminoid (grasslike; sedges, rushes, and grasses) species were sown in late October in a hoop house at SCCC. Plants were selected from a list provided by the Oregon Spotted Frog Working Group identified as common in *R. pretiosa* habitat. In mid-December, plants were evaluated for germination and growth, and decisions made about which plants to use. Two species were selected: *Deschampsia cespitosa*, a perennial, cool season grass, and *Carex obnupta*, a perennial, evergreen sedge. These two plants were selected because they exhibited the fastest and most abundant growth and were considered winter hardy. *C. obnupta* had been successfully grown though the winter the previous year in the aquaponics greenhouse at SCCC. Both

*C. obnupta* and *D. cespitosa* are commonly used in ecological restoration in the Pacific Northwest. *C. obnupta* is used for erosion control, streambank stabilization, and provides food and cover for many wildlife species (Stevens & Hoag, 2006). *D. cespitosa* grows in cool, moist habitats and has been used for restoration of wet meadows (St. John et al., 2011). Both have the potential to become invasive without proper management (Stevens & Hoag, 2006; St. John et al., 2011), which could give them a competitive advantage over invasive *P. arundinacea*.

Plants were installed in 5 ft by 3 ft mats, in alternating rows of four and five plants, 20 cm apart. This is the density used for the mats SPP grows for Oregon spotted frog restoration projects and reflects existing protocols for the aquaponics greenhouse at SCCC, which were developed through experimentation and consultation with the Washington State Department of Fish and Wildlife during the preceding year. Sixteen mats of each species were sown containing 32 plants each, for a total of 512 plants of each species. Eight mats of alternating species composition were placed in each of four beds: 1A, 1B, 2A, and 2B (Fig. 5). The aquaponic water was set to flow through each bed for 45 minutes at 6-hour intervals. Plants grew in mats for approximately six weeks prior to tissue sampling in order to establish roots and ensure survival.

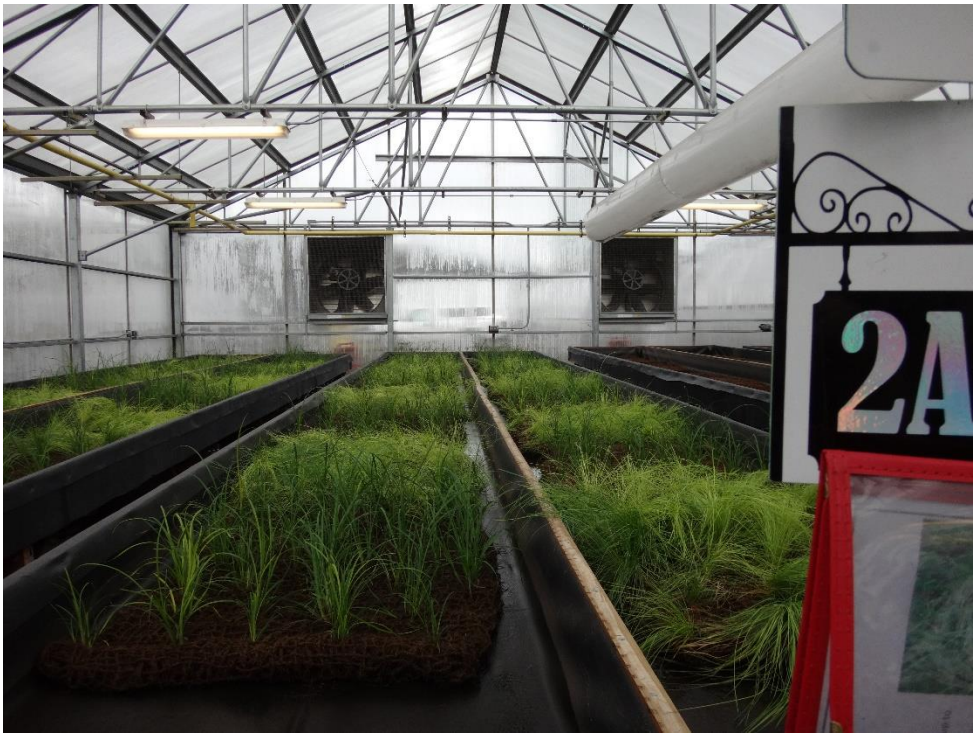



**Figure 4**. Alternating mats of *C. obnupta* (left foreground) and *D. cespitosa* (right foreground, behind sign). Plants were installed 20 cm apart for a total of 32 plants per mat.

## Compost Tea Brewing

Compost was made on site using food waste from the prison facility. SCCC composts up to 800 pounds of food waste per day. Using a rotating drum, the compost is heated to 130° for three days before sitting in a passive pile where it continues heating to about 165°. The compost is turned once per week. The process takes approximately six weeks to complete. The ratio of compost tea to water in a hydroponic system (plants growing in soilless media without fish) is 1:50 by volume (Ingham, 2005). This ratio was used to determine the volume of tea to brew. Our system is approximately 7000 gallons, requiring 140 gallons of compost tea. To make compost tea, finished compost was sifted and placed in four, 19.1” x 16.7” 400-micron nylon mesh bags (Compost TeaLab, Eureka, CA). Each bag contained approximately 12 cups of compost, enough to brew 150 gallons of tea (TeaLab, n.d.). Prior to brewing, 140 gallons of tap water was aerated in a

150-gallon tub for 24 hours to eliminate any residual chlorine. The filled tea bags were submerged in the tub and an aquarium pump continuously pushed water through a PVC pipe around the bags to aerate and extract the tea (Fig. 6). Compost tea was brewed at ambient temperature for approximately 72 hours (Ingham, 2005). One pint of Maxicrop soluble seaweed powder were added at the end of the brewing cycle to feed the bacterial and fungal community in the compost tea. Maxicrop is made from dried *Ascophyllum nodosum* and has a guaranteed N-P-K content of 0-0-17 (Ohstrom's Maxicrop, n.d.). In addition to adding potassium, Maxicrop provides a food source for fungi and bacteria (Ingham, 2005). Maxicrop was mixed with compost tea for approximately 15 minutes. After the Maxicrop was thoroughly dissolved, the pump was turned off and solids allowed to settle for approximately 30 minutes. The compost tea was pumped into the biofilter of the aquaponics system.

**Figure 5**. Finished compost tea. Tea bags were suspended in water from the bamboo pole. A pump, pictured sitting on top of a blue crate to the left of the tub, blew air into the tea through a pipe.

## Field Sampling

This design required sampling during a constrained time period to reduce the influence of plant growth or seasonal changes on leaf tissue concentration. Plant tissue sampling and analysis follows the methods outlined in Jones, Jr. (2001) and Kalra (1998). Plant tissue samples were collected, air dried, and placed in paper bags to be transported to the laboratory. Each sample was a composite of mature leaves taken from four plants, and each plant was sampled only once (Fig. 7). Samples were collected on a total of seven days: February 5, 6, 8, 12, 13, 15, and 20, 2018. Samples were collected at three different times each day, 9:00, 11:00 and 1:00 pm, to account for diurnal variation in nutrient uptake rates.

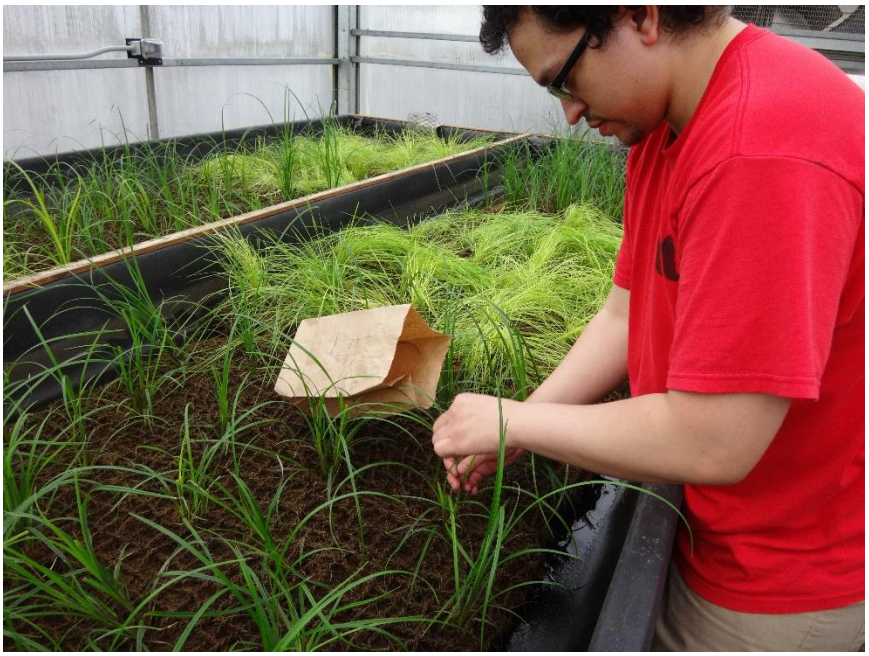


**Figure 6.** Technician Danyl Herringshaw collects the newest leaves from *C. obnupta*. For each composite sample, five leaves from four plants were collected and placed in paper bags for transport to the laboratory.

## Laboratory Methods

### Cleaning, Drying, and Particle Size Reduction

All glassware and plastic used for sample preparation and analysis was washed with soap and water, rinsed in warm water, then rinsed in deionized water. Clean hardware was acid washed in a 1.5 M hydrochloric acid bath for at least 12 hours, then triple rinsed with deionized water.

After transport to the laboratory, plant samples were decontaminated in 1-2% phosphate free detergent solution to remove dust or soil contamination and rinsed with deionized water, then dried for at least 24 hours between 55° and 70° C. Dried samples were cryogenically ground using liquid nitrogen and mortar and pestle. Ground samples were placed in labeled paper coin envelopes and stored in a desiccator until digestion.

### Digestion Procedures

Samples were digested on a hot plate using nitric acid and hydrogen peroxide open digestion methods (U.S. EPA, 1996; Hansen et al., 2012). 0.25 grams of ground sample were weighed and placed in 50 ml beakers. Six millilters of concentrated metals grade nitric acid was added to each sample. Samples were heated to approximately 80° C and refluxed for 15 minutes. Samples were removed from heat and cooled, and two more milliliters nitric acid added to each sample. Samples were brought back up to approximately 80° C and digested for up to two hours, or until reduced to 5 ml. After digestion in nitric acid, samples were removed from heat and cooled, and two milliliters of 30% hydrogen peroxide were added to each sample. Samples were reheated to 80° C for the peroxide reaction to take place. After the peroxide reaction was complete (samples turned clear and bubbled for about 1 minute) and samples allowed to cool, an additional 1

ml of hydrogen peroxide was added, and samples were reheated and allowed to reduce to 5 ml—up to two hours. Samples were cooled, gravity filtered into acid-washed polypropylene or HDPE bottles through Whatman grade 41 110 mm filter papers and diluted with deionized water to 50 ml. Digested samples were stored at 4°C until analysis.

## ICP-MS Elemental Analysis

### *Semi-Quantitative Mode*

Plant tissue samples were analyzed for P, K, Ca, Mg, and Fe using a Perkin Elmer Inductively Coupled Plasma Mass Spectrometer (PerkinElmer Elan DRC-e ICP-MS). Semi-quantitative mode was used to determine appropriate sample dilutions and expected ranges of nutrient concentrations. Semi-quantitative mode uses a range of internal standards to provide an approximate analysis (within 20%) of more than 80 elements in a sample (PerkinElmer, 2011). Samples were diluted in deionized water 2.5, 5, 10, or 100 times in 15 ml centrifuge tubes. Gallium, scandium, indium, and thulium were added to each tube in concentrations of 70 ppb, 50 ppb, 20 ppb, and 20 ppb respectively. Samples were run on the ICP-MS in semi quantitative mode from most to least dilute until the correct dilution factor could be determined. A sample dilution factor of 25 was calculated to reduce contamination due to dissolved solids and bring concentrations to within the detection limits of the ICP-MS.

### *Full-Quantitative Mode*

Samples were analyzed in full quantitative mode to determine precise amounts of each element in the dilute sample. External standards were used to calibrate the machine and bracketed the range of expected concentrations for all six elements being measured,

using three standards per order of magnitude:

- Low standard: 0.1 ppb Fe, Mn/10 ppb Mg, P, K, Ca
- Medium-low standard: 0.5 ppb Fe, Mn/50 ppb Mg, P, K, Ca
- Medium standard: 1 ppb Fe, Mn/100 ppb Mg, P, K, Ca
- Medium-high standard: 5 ppb Fe, Mn/500 ppb Mg, P, K, Ca
- High standard: 10 ppb Fe, Mn/1000 ppb Mg, P, K, Ca

A quality control sample containing 3 ppb Fe, Mn/300 ppb Mg, P, K, Ca was made from different stock solutions than the external standards. Blanks were made with deionized water and analyzed as unknown samples. Gallium internal standard was added to each sample, standard, QC, and blank at the rate of 60 ppb. Blanks were run first to calibrate the machine to zero. Standards were evaluated to make sure that the $R^2$ for each element on the standard curve was at least 0.9998. The QC was evaluated prior to running samples and was expected to fall within ± 10% of the calculated value for each element. Samples were run ten at a time, followed by a blank and QC to evaluate instrument drift. Concentrations of each element in the dilute samples were back calculated to determine how much of each element was in the original plant tissue sample.

## Data Analysis

All ICP-MS data were entered into Microsoft Excel which was used to back-calculate nutrient concentrations in plant tissue, calculate summary statistics, and organize, sort, and visualize data. Statistical analysis was performed using JMP Pro 12 (SAS). Two-way ANOVA and post-hoc Tukey tests were used to evaluate main effects and interactions of time of day and date for each species. Data with no time of day effects were pooled into before and after categories and t-tests performed. Non-parametric Wilcoxon and post-hoc Dunn's tests were used for each element that did not fit a normal

distribution, and one-way ANOVA was used for elements that did follow a normal distribution.

# Results

## Data Analysis Overview

With the exception of phosphorus and calcium in *D. cespitosa*, plant nutrient data were not normally distributed. For this reason, two-way ANOVA was only used to initially assess main and interacting effects of time-of-day and date. One-way Kruskal-Wallis/Wilcoxon tests and, where necessary, post-hoc Dunn's tests with Bonferroni correction were used to assess changes in all plant tissue nutrients in *C. obnutpa*, as well as magnesium and manganese in *D. cespitosa* over time and to determine any time-of-day differences in plant tissue nutrient concentration. One-way ANOVA was used to examine changes over time and time-of-day differences for phosphorus and calcium in *D. cespitosa*, since those data fit a normal distribution.

Iron and potassium data fell out of the range of the standards used for ICP-MS analysis, and were deemed invalid. Approximately one third of the magnesium data were discarded due to a bad stock standard that made interpolating results based on a standard curve impossible. Magnesium in samples run after the stock standard was replaced are included in this analysis.

## Routine Water Quality Monitoring

Water parameters collected each weekday between February 5 and 20th are shown in Table 1. All routine water quality parameters were collected prior to the first plant tissue collections; values through February 12$^{th}$ reflect pre-compost tea conditions. There were negligible variations in temperature, dissolved oxygen, and salinity, and no

variation in ammonia and nitrite throughout the course of the experiment. The highest temperature (74° F) was observed on February 5, and the lowest temperature was observed on February 12, 13, and 14 (70° F).

There were no changes in ammonia and nitrite concentrations throughout the course of this experiment, indicating a properly functioning biological filter. Continuous aeration contributed to low variation in dissolved oxygen. Water was added to the system to make up for loss due to evaporation on February 5 (300 gallons), 6 (200 gallons), 7 (500 gallons), and 14 (500 gallons), which did not correlate with any changes in other water parameters.

Large variations in nitrate were observed. It is notable that the accuracy and precision of the API water quality test kits are very coarse; for nitrate, the lowest readable value is 5 ppm and values double thereafter (10, 20, 40, etc.) until they reach the highest reading, 160 ppm. Therefore, a nitrate reading of 40 ppm using an API test kit could indicate nitrate levels anywhere between 20 and 40 ppm, and a reading of 20 ppm could indicate levels between 10 and 20 ppm. On February 8, seven fish were moved from the fish tank into quarantine to maintain nitrate levels at or below 40 ppm; 1000 gallons of water was transferred from the aquaponics system into a quarantine tank, and the water removed from the system was replaced with 1000 gallons of well water. The following day, nitrate values appeared to drop by half, to 20 ppm. The highest nitrate readings occurred on February 8, after which nitrate dropped to its lowest level (20 ppm) on February 9 following the 1000-gallon water exchange. No other water additions appeared to affect nitrate levels. Nitrate had increased back to 40 ppm by Monday, February 12 and remained at that level for the rest of the experiment.

Highest pH (7.8) was observed on February 13, the day following compost tea additions. The lowest pH values were observed on February 9, 14, 15, and 16. The largest pH change occurred between February 13 and 14, when pH dropped from 7.8 to 7.2. The pH remained at 7.2 from the 14th until the end of the experiment.

Carbonate hardness (known as alkalinity or KH) and general hardness (GH) were tested daily between February 5-9, and weekly thereafter. Average GH between February 5-9 was 82.3 ± 9.8 ppm. KH was 53.7 ppm on all days between February 5-9. GH on February 12 and 20 was 89.5 ppm, and KH on February 12 and 20 was 71.6 ppm. The target range for KH is between 70-100 ppm (Somerville et al., 2014).

**Table 1.** Water quality parameters tested each weekday between February 5 and 20, 2018 compared to target ranges (Somerville et al., 2014).

| | Average | Range | SD | Target range (Somerville et al., 2014) |
|---|---|---|---|---|
| Temperature (°F) | 71.6 | 70-74 | 1.4 | 65-86 |
| Dissolved oxygen (mg/L) | 8.1 | 7.7-8.5 | 0.28 | 5-8 |
| Ammonia (ppm) | 0.25 | 0.25-0.25 | 0 | 0 |
| Nitrite (ppm) | 0 | 0 | 0 | 0 |
| Nitrate (ppm) | 34 | 20-40 | 8.1 | 5-150 |
| pH | 7.4 | 7.2-7.8 | 0.21 | 6-7 |

## Plant Tissue Nutrients

For both *C. obnupta* and *D. cespitosa*, the range of values varied considerably for all nutrients. Mean (SD) values in grams per kilogram for P, Ca, Mg, and Mn, respectively in *C. obnupta* were 3.02 (0.995), 1.59 (0.540), 1.58 (0.532), and 0.0596 (0.0241) respectively. Mean (SD) values for P, Ca, and Mg and Mn were generally higher in *D. cespitosa*, 4.55 (1.59), 2.81 (0.970), 2.38 (0.716), and 0.0448 (0.0164) respectively.

Compost tea addition did not increase plant tissue nutrient concentration. Factorial ANOVA revealed a significant main effect of date on phosphorus concentration

for *C. obnupta* ($F_{[6, 39]} = 3.9961$; $p = 0.0033$). No other main or interacting effects of time-of-day and date were observed for either *C. obnupta* or *D. cespitosa* ($p > 0.05$; Table 2), indicating that only phosphorus in C. obnupta changed over time, and time-of-day did not affect nutrient concentrations for any nutrient in either plant. Non-parametric, one-way tests were run in place of post-hoc tests on all non-normal data, including all *C. obnupta* nutrients, and Mg and Mn in *D. cespitosa*. Data are presented separately for each plant.

**Table 2**. Results of factorial ANOVA assessing main and interacting effects of time-of-day and date on plant tissue nutrient concentration. Red values with one * are significant at $p < 0.05$.

| | Time-of-day | | | Date | | | Time-of-day*Date | | |
|---|---|---|---|---|---|---|---|---|---|
| ***C. obnupta*** | **F** | **df** | **p** | **F** | **df** | **P** | **F** | **df** | **p** |
| **P** | 0.0613 | 2 | 0.9406 | 3.9961 | 6 | 0.0033* | 1.6372 | 12 | 0.1210 |
| **Ca** | 0.0963 | 2 | 0.9084 | 2.0772 | 6 | 0.0782 | 1.1839 | 12 | 0.3279 |
| **Mg** | 0.0424 | 2 | 0.1027 | 1.5359 | 4 | 0.1027 | 1.5359 | 8 | 0.1951 |
| **Mn** | 0.4662 | 2 | 0.6308 | 1.7153 | 6 | 0.1432 | 1.1855 | 12 | 0.3268 |
| | **Time-of-day** | | | **Date** | | | **Time-of-day*Date** | | |
| ***D. cepitosa*** | **F** | **df** | **p** | **F** | **df** | **P** | **F** | **df** | **p** |
| **P** | 0.9357 | 2 | 0.4003 | 0.3483 | 6 | 0.9069 | 0.9716 | 12 | 0.4901 |
| **Ca** | 0.8779 | 2 | 0.4231 | 1.4759 | 6 | 0.2098 | 1.2211 | 12 | 0.3012 |
| **Mg** | 0.0708 | 2 | 0.9319 | 0.5865 | 4 | 0.5865 | 0.2897 | 9 | 0.2897 |
| **Mn** | 1.7228 | 2 | 0.1909 | 0.4652 | 6 | 0.8301 | 1.7241 | 12 | 0.0958 |

### *C. obnupta*

Although factorial ANOVA only showed differences in phosphorus for *C. obnupta*, One-way Wilcoxon/Kruskal-Wallis tests revealed significant changes by date in plant tissue nutrient concentrations for all nutrients in *C. obnupta* (Fig. 9), and confirmed no time-of-day differences in concentration for any nutrient ($p > 0.05$; data not shown). Specifically, nutrient concentrations decreased over time, with post-hoc tests showing

significant pairwise differences between dates before and after the compost tea addition for all nutrients except Mn (Fig. 9).

Because there were no significant time-of-day effects or interaction effects between time-of-day and date, data were combined into two groups per nutrient per plant: before and after compost tea addition. The *before* data included all samples collected through 9:00 on February 12, and the *after* data included all samples collected 11:00 on February 12 and after. When the data are combined, there are significant reductions in plant tissue nutrient concentration for all four nutrients in *C. obnupta* after compost tea addition, including Mn (Fig. 10). Median (interquartile range) concentrations before and after compost tea additions for nutrients in *C. obnupt*a are shown in Table 3. P, Ca, Mg, and MN decreased by 22%, 17%, 18%, and 13% respectively.

**Table 3**. Median (interquartile range) of plant tissue nutrient concentrations (g/kg) in *C. obnupta* before and after compost tea additions. All before and after pairs were significantly different (P > 0.05).

| | Before | After |
|---|---|---|
| **P** | 3.21 (2.98-3.50) | 2.50 (2.32-2.64) |
| **Ca** | 1.63 (1.45-1.83) | 1.35 (1.26-1.45) |
| **Mg** | 1.59 (1.48-1.69) | 1.31 (1.21-1.39) |
| **Mn** | 0.0599 (0.0538-0.0736) | 0.0520 (0.0453-0.0570) |

### *D. cespitosa*

There were no significant changes in P ($F = 0.332$, $p = 0.9174$, $df = 6$), Ca ($F = 1.40$, $p = 0.2303$, $df = 6$), Mg ($\chi^2 = 3.49$, $p = 0.6248$, $df = 5$), and Mn ($\chi^2 = 3.27$, $p = 0.7738$, $df = 6$) concentrations in *D. cespitosa* (Fig. 11), and no significant differences in time-of-day for P, Ca, or Mg ($p = 0.0717$, 0.3523, and 0.3883 respectively); however, there was a significant time-of-day effect on Mn. Post-hoc Dunn's tests show differences between samples collected at 9:00 and samples collected at 1:00 ($p = 0.0128$). Separate

one-way analyses of the 9:00, 11:00, and 1:00 data show no significant changes in Mn for any time of day ($p = 0.8787$, 0.0843, and 0.2419 respectively).

Like the *C. obnupta* data, samples for P, Ca, and Mg were pooled to represent plant tissue nutrient concentrations before and after compost tea additions. One-way ANOVA (P, Ca) and Wilcoxon/Kruskal-Wallis tests (Mg) show no significant differences between plant tissue nutrients before and after compost tea was added (Fig. 12).

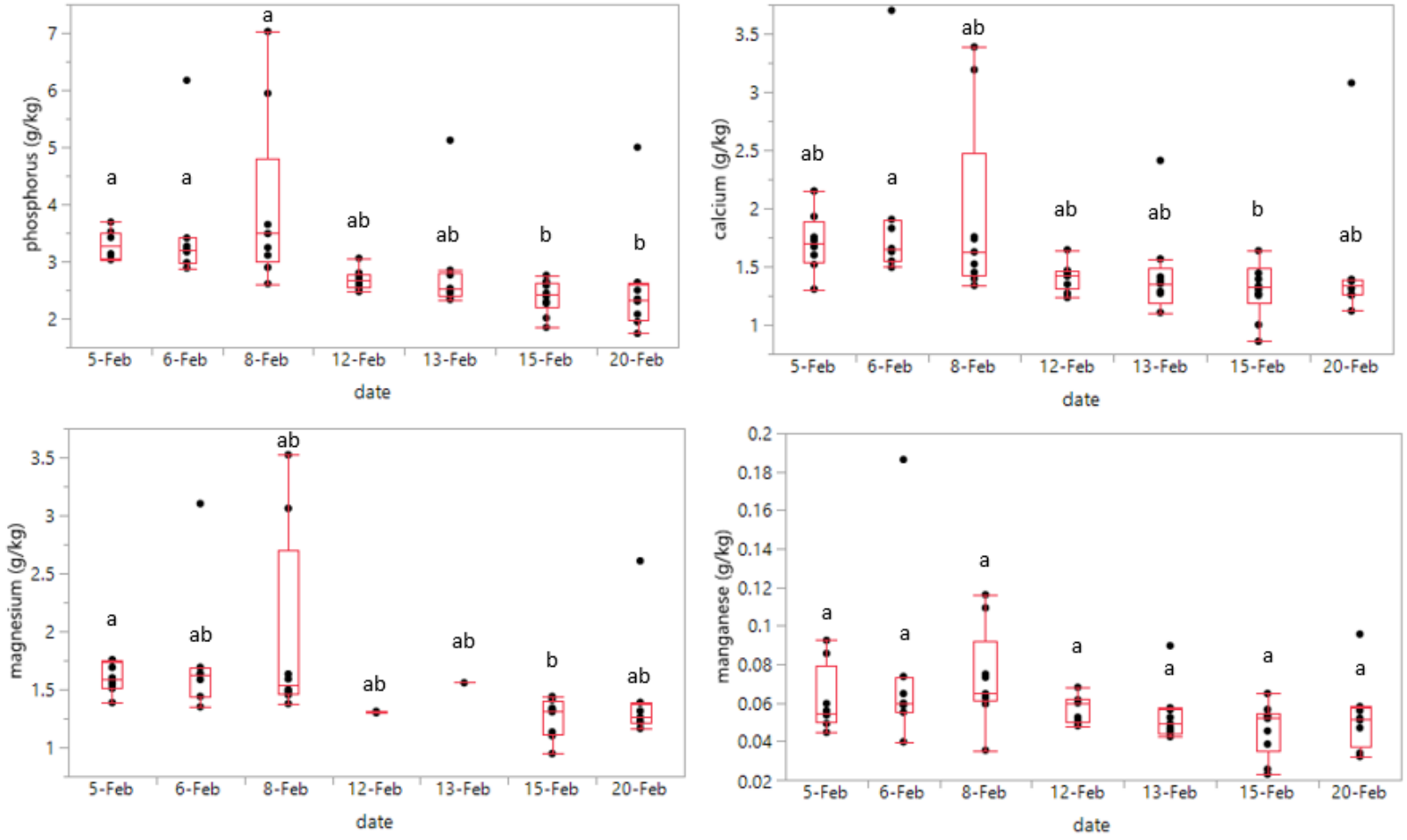



**Figure 8**. Plant tissue nutrient concentrations in *C. obnupta* on seven non-consecutive days. Boxes show medians and interquartile ranges (IQR); whiskers show 1.5*IQR, with outliers shown outside of the whiskers. Different letters represent significant difference (Dunn's Multiple Comparisons, p>0.05).

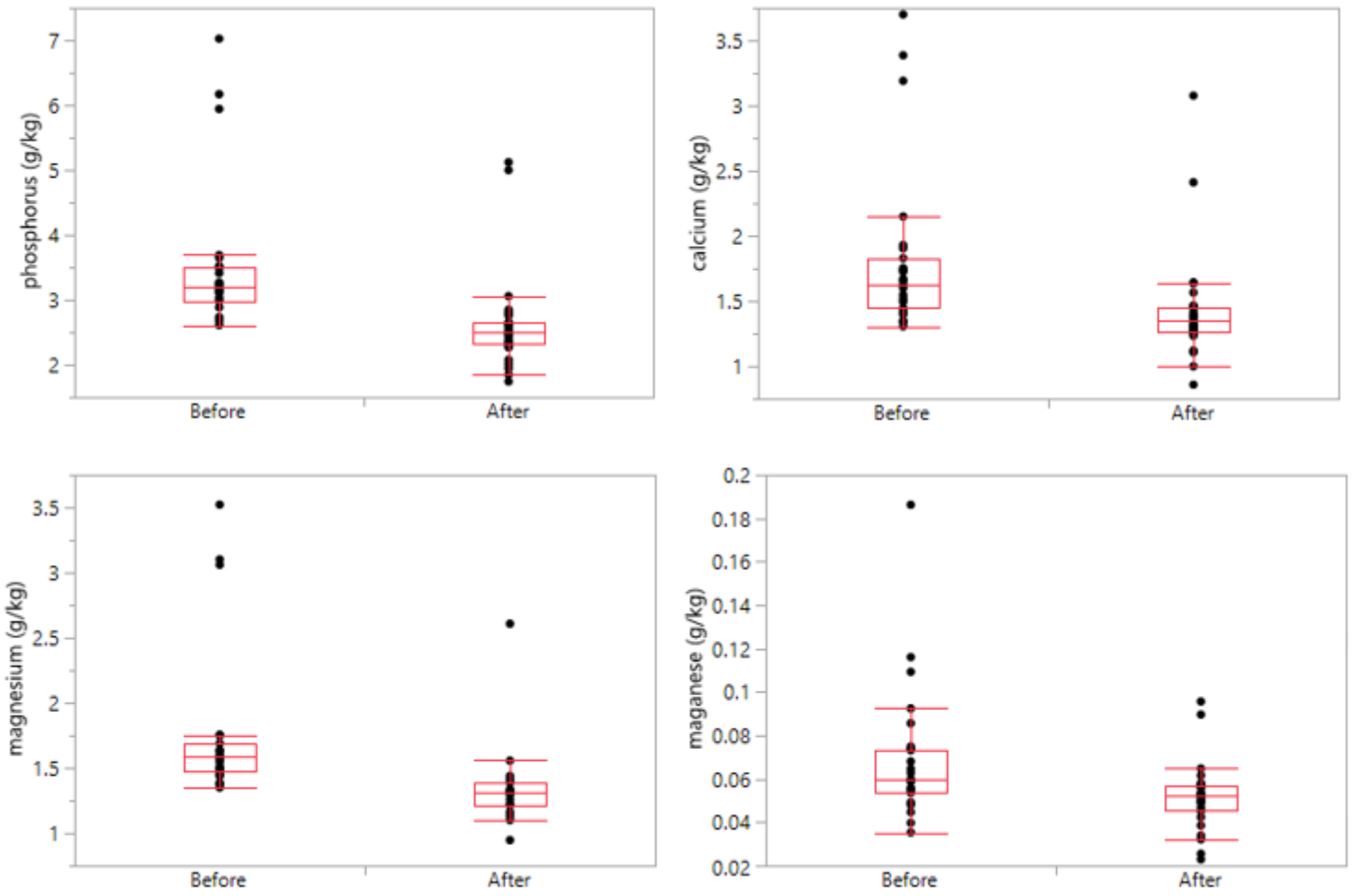



**Figure 97**. Comparison of plant tissue nutrient concentrations in *C. obnupta* before and after compost tea addition. Differences were significant for all nutrients (Wilcoxon/Kruskal-Wallis, p-values shown on graphs).

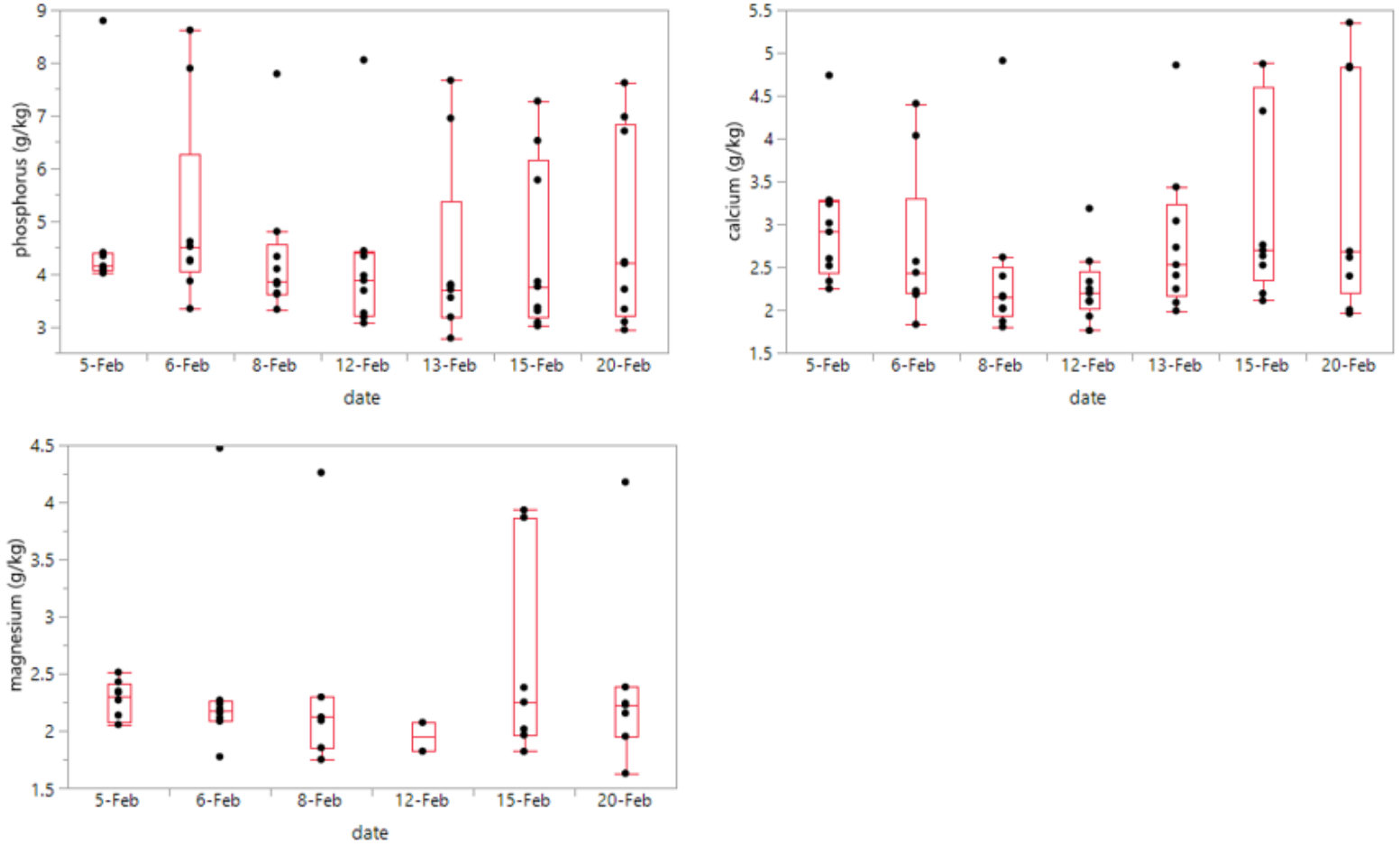



**Figure 80**. Plant tissue nutrient concentrations in *D. cespitosa* on seven non-consecutive days. Boxes show medians and interquartile ranges (IQR); whiskers show 1.5*IQR, with outliers shown outside of the whiskers. There were no significant differences between dates for any nutrient (P, Ca: Student's T, $p > 0.05$; Mg: Wilcoxon/Kruskal-Wallis, $p > 0.05$).

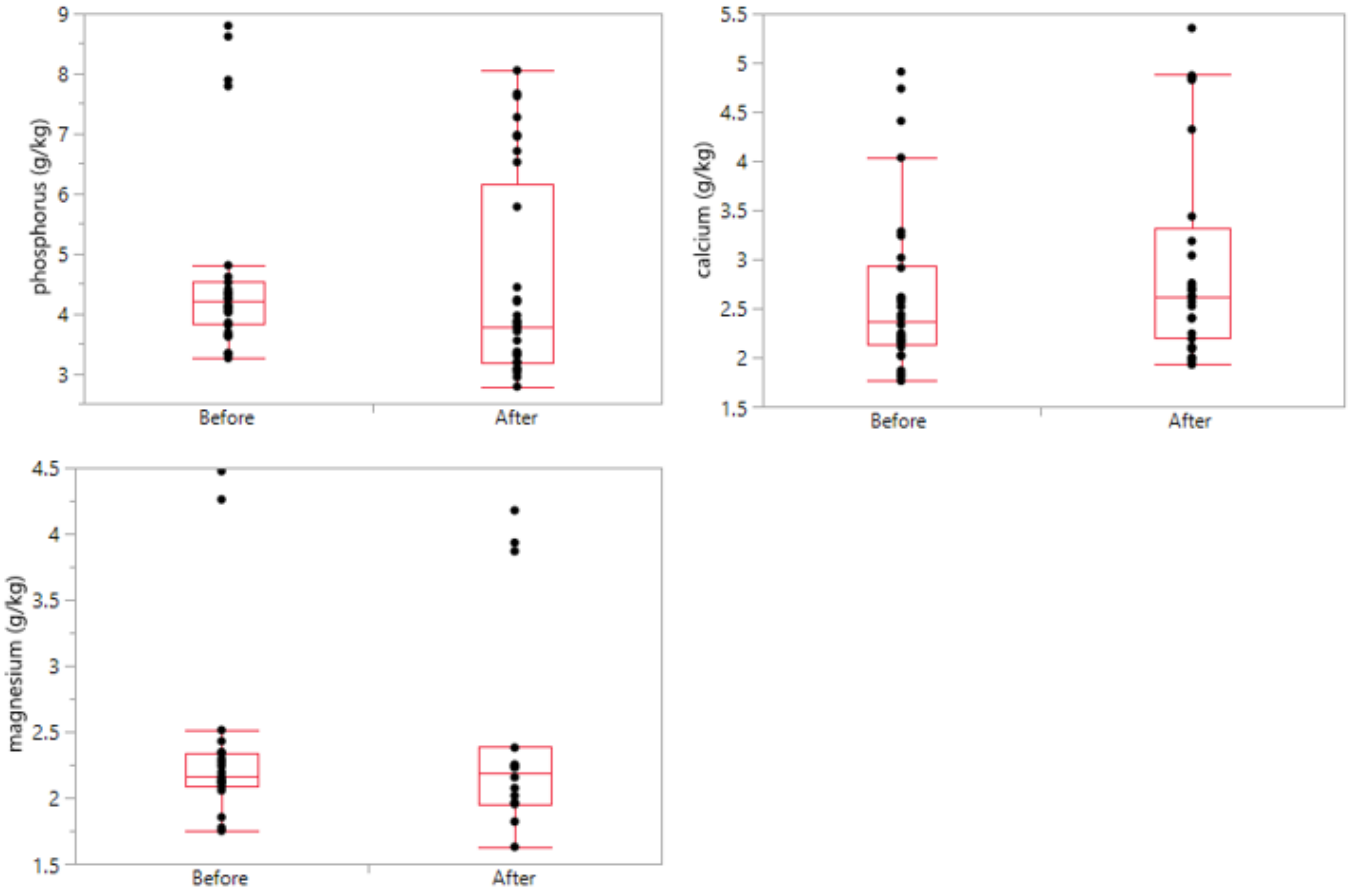



**Figure 91**. Comparison of plant tissue nutrient concentrations in *D. cespitosa* before and after compost tea addition. No significant differences were found between means for P and Ca (Student's T, $p > 0.05$), or Mg (Wilcoxon/Kruskal-Wallis, $p > 0.05$). Manganese data could not be pooled due to effects of time of day on nutrient concentration.

# Discussion

The data analysis addressed the research question: does adding compost tea to the recirculating water in an aquaponics system increase plant tissue nutrient concentration of emergent wetland vegetation grown to enhance *R. pretiosa* habitat and eradicate *P. arundinacea*? A quasi-experimental interrupted time series design was used to evaluate changes over time in plant tissue nutrient concentration resulting from the addition of compost tea by first creating a baseline, then collecting additional samples post-addition. The results of this study did not support the hypothesis that compost tea added to aquaponics water would increase plant tissue nutrient concentration. In fact, for *Carex obnupta*, leaf tissue nutrient concentration decreased significantly before and after compost tea addition for the four nutrients evaluated, while for *Deschampsia cespitosa*, tissue nutrient concentrations before and after compost tea additions were not significantly different. This discussion outlines the limitations of the study design and data collected. Results of tissue nutrient analysis for each plant are discussed separately, followed by a discussion of the limitations of the compost tea brewing process and a summary of recommendations for future research.

## Quasi-Experimental Design

This study used a quasi-experimental interrupted time series (ITS) design for which there was no precedent in the literature for evaluating aquaponics systems. ITS designs are often used in public health studies where real-world settings preclude random selection of control or experimental groups for ethical, practical, social, or logistical reasons (Handley, Lyles, Mcculloch, & Cattamanchi, 2018). Such studies are conducted with full awareness of threats to internal validity, for which the design of the study

attempts to compensate. For example, an ITS design requires a large number of measurements to establish a baseline prior to the intervention, essentially creating a control scenario in lieu of a separate group (Handley et al., 2018). A basic ITS assumes that any trend in the pre-intervention data will continue regardless of the intervention, and any change in the trend can be attributed to the intervention (Handley et al., 2018).

For this thesis project, the ITS approach was applied because of the inability to assign a control group that would not receive compost tea. The hydroponic component of the system could not be partitioned to separate beds such that one part received aquaculture water plus compost tea and the other part only received aquaculture water. In other aquaponics studies assessing the effects of nutrient treatments on plant growth, researchers have constructed adjacent systems and assigned one as a control, leaving all measurements of plant growth and tissue elemental composition till the end of the experiment (Delaide, Goddek, Gott, Soyeurt, & Jijakli, 2016). Because it lacked a control group, this project was designed to control for as many other factors as possible. To control for plant growth, the plant tissue was collected during a constrained time period; seven sample dates distributed within the 16-day period between February 5-20. The compost tea was added on February 12, allowing for the creation of a baseline using samples taken between February 5 and 12. Samples were collected at different times of day and evaluated for the effects of time of day on nutrient uptake rates. At each time of day, three replicates were collected, each containing tissue from four different plants to account for plant-to-plant variation. Finally, no plant was sampled more than once, creating independence among all samples.

## Ongoing Water Quality Monitoring

In order to maintain healthy conditions for fish and monitor the system for equipment failures, technicians at SCCC test for dissolved oxygen (DO), pH, salinity, temperature, ammonia, nitrite, and nitrate each weekday morning, and for the purpose of this study, also collected data on general hardness and alkalinity. Large variations in nitrate were observed. It is notable that the accuracy and precision of the API water quality test kits are very coarse; for nitrate, the lowest readable value is 5 ppm and values double thereafter (10, 20, 40, etc.) until they reach the highest reading, 160 ppm. Therefore, a nitrate reading of 40 ppm using an API test kit could indicate nitrate levels anywhere between 20 and 40 ppm, and a reading of 20 ppm could indicate levels between 10 and 20 ppm. On February 8, seven fish were moved from the fish tank into quarantine to maintain nitrate levels at or below 40 ppm; 1000 gallons of water was transferred from the aquaponics system into a quarantine tank, and the water removed from the system was replaced with 1000 gallons of well water. The following day, nitrate values appeared to drop by half, to 20 ppm. The highest nitrate readings occurred on February 8, after which nitrate dropped to its lowest level (20 ppm) on February 9 following the 1000-gallon water exchange. No other water additions appeared to affect nitrate levels. According to the API kits, nitrate had increased back to 40 ppm by Monday, February 12 and remained at that level for the rest of the experiment. Future research should use more precise tests to determine ammonia, nitrite, and nitrate values to get a clearer picture of actual changes in concentrations.

## ICP-MS Challenges and Data Purge

This research set out to examine changes in six different macro- and micronutrients: potassium, phosphorus, calcium, magnesium, manganese, and iron. The typical range of values of each of these nutrients in terrestrial plant tissue is shown in Table 4. ICP-MS is a sensitive instrument that can detect elements in the low parts per billion range. Typically, samples are diluted to prevent clogging of the instrument's tubes. Standard dilutions are used to create a standard curve that calculates concentration based on "counts": the number of individual atoms of the element that are detected by the mass spectrometer. The standards must bracket the expected concentrations for all nutrients, with at least two standards per order of magnitude. The highest and lowest standards should be one order of magnitude higher and lower than the expected range of concentrations.

The standard operating procedure for this study used a single set of five standards to determine the concentrations of all six elements based on the expected concentrations and subsequent dilutions determined by the initial qualitative analysis. Unfortunately, the amount of potassium in most of the samples exceeded the range of concentrations, while the iron in the lowest two standards and samples was not different from the amount of iron in the blanks, which calibrate the zero value for each element. These two elements were eliminated from the study. Because iron and potassium are both considered deficient enough in aquaponics to require supplementation (Rakocy, Masser, & Losordo, 2006), future research should consider separating the macro- and micronutrients into separate analyses, using a lower dilution factor for the micronutrients and adding higher standards for the macronutrients.

For magnesium, the concentration of the quality control sample was consistently higher than the required range of its calculated value for approximately half of the samples run. After obtaining a new stock standard and remaking the standards and quality control samples, the quality control sample was within the required range. Therefore, approximately half the magnesium data had to be discarded. This resulted in some dates and times (primarily on February 12 and 13) having few to no replicates. Regardless, the pooled data for magnesium contained enough replicates for a statistical analysis.

**Table 4**. Average range of P, Ca, Mg, and Mn (g/kg) in dry mass of terrestrial plants (Kalra, 1998) compared to average (SD) concentration (g/kg) in dry mass of *C. obnupta* and *D. cespitosa* in the aquaponics system at SCCC.

| | Range (Kalra, 1998) | *C. obnupta* | *D. cespitosa* |
|---|---|---|---|
| **P** | 0.1-10 | 3.02 (0.995) | 4.55 (1.59) |
| **Ca** | 0.4-15 | 1.59 (0.540) | 2.81 (0.970) |
| **Mg** | 0.7-9 | 1.58 (0.532) | 2.38 (0.716) |
| **Mn** | 0.003-1 | 0.0596 (0.0241) | 0.0448 (0.0164) |

## Leaf Tissue Nutrient Analysis

### Leaf Tissue Sampling

Where possible, the youngest mature leaves were collected from each plant per Kalra (1998). For graminoids that grow uniformly from the base, such as *C. obnupta* and *D. cespitosa*, it can be challenging to collect leaves of uniform age or to determine which leaves are the correct age. Leaf age is important in plant tissue analysis because certain nutrients preferentially accumulate in different plant tissues. For example, calcium will accumulate in the tips of older leaves (Larcher, 1995). Mistakenly collecting older leaf tissue could result in calculating plant tissue concentrations higher than those actually being absorbed.

For each plant, leaf tissue data were examined in two different ways. First, samples taken on each of seven dates were compared pairwise in case the compost tea addition caused subtle changes that would not have been detected by pooling samples before and after compost tea additions, and also to note any significant differences in tissue nutrient concentrations that could not be explained by compost tea addition (e.g. significant pairwise differences in dates prior to compost tea addition). Second, because there were no time-of-day effects for all nutrients except Mn in *D. cespitosa*, all samples taken before compost tea addition were treated as replicates and pooled, and all samples taken after compost tea addition were treated the same (with the exception of Mn in *D. cespitosa*). The pooled samples were then compared to one another for each nutrient to determine the overall effect of compost tea addition. The following sections discuss results for each of the plant species analyzed.

### *C. obnupta*

Results of pairwise comparisons showed significant decreases in nutrient concentration for P, Ca, and Mg, but not for Mn, in *C. obnupta*. Each of the significant decreases occurred between samples taken before compost tea addition and samples taken after compost tea addition. Visual examination of the data indicates a gradual reduction in concentration (Fig. 9). For Mn, when the data was pooled, there was a significant difference between samples taken before and samples taken after compost tea addition, despite no pairwise differences among dates. Although a constrained time period was used, growth was visually observed during the 16-day duration of this study; therefore, it can be inferred that the gradual reductions in plant nutrient concentration for *C. obnupta* were due to plant growth that outpaced the absorption of nutrients from the

hydroponics system. Regardless, the concentrations of all four nutrients remained within an acceptable range for leaf tissue (Table 4). The same pattern was not observed for *D. cespitosa*.

### *D. cespitosa*

There were no significant differences among sampling dates for *D. cespitosa*, nor were pooled samples taken before and after compost tea additions significantly different. Results of two-way ANOVA showed a significant effect of time of day on Mn concentration, but when data for each time of day was evaluated separately, there were no significant changes in tissue nutrient concentration. *D. cespitosa* is slow to establish, often taking more than one season, and may not have exhibited the same amount of growth over the sampling period as *C. obnupta* (Stevens & Hoag, 2006; St. John et al., 2011). Alternatively, *D. cespitosa* may have grown while also taking up additional nutrients, and the resulting increase in carbon could mask the increase in nutrient content by maintaining the same concentration.

## Conclusion of Tissue Analysis

This is the first time-series study of plant tissue nutrients in aquaponics, and because most research on plant growth and nutrition is conducted using conventional randomized experimental design, other time series studies of plant tissue are absent in the literature. Plant tissue studies are primarily used to detect nutrient deficiencies or presence of soil contaminants (Delaide et al., 2016). Despite the lack of precedent for this study, the constrained time period was considered valid because other studies of aquaponically and hydroponically grown plants have shown fertilizer-induced changes in plant tissue nutrient concentrations over periods of time as short as seven days (Radzki et

al., 2013). Kim et al. (2015) cited research showing that nutrient solutions applied to leaves have appeared in plant roots within one hour of application, indicating that plants can absorb and incorporate available nutrients quickly. Using water quality measurements, Buzby and Shin (2014) found that nasturtium and lettuce removed 63% and 37% of available phosphate respectively from an aquaponic planting bed within a four-hour period. Perhaps a finer sampling regime, with intensive sampling within the first few hours after compost tea addition, would have revealed something that this experiment did not.

However, compost tea differs from fertilizer in that its major benefit is to provide a microbial community that can stimulate plant growth in many ways, including breaking down organic matter into nutrients that plants can use (Ingham, 2005). Perhaps the eight days following compost tea addition was not long enough for the compost tea microbial community to populate the system, break down organic matter, and affect plant tissue nutrients.

It is also feasible that the compost tea stimulated plant growth through the action of plant growth promoting microbes (PGPMs) but did not increase the nutritional value of the aquaponic solution. For example, some PGPMs produce molecules that mimic plant growth hormones, which could stimulate root growth and consequently boost nutrient uptake (Radzki et al., 2013). This increased uptake could lead to additional plant growth, and the increase in carbon would mask the increase in nutrient content of the plant tissue (Larcher, 1995). Future studies could evaluate plant nutrient content and plant growth to determine increases in plant nutrient uptake and disinter how much of that

uptake was due to increased nutrients in the aquaponics water versus increased root surface area for nutrient uptake.

## Compost Tea

### Recipe

There is no standard brewing process or recipe for compost tea (Islam et al., 2016). The tea recipe used in this research was developed by Sustainability in Prisons Project staff prior to the onset of this research, using information from The Compost Tea Brewing Manual (Ingham, 2005). A mistake in the compost tea recipe used for this project called for Maxicrop Kelp Powder to be added at the end of the brewing process (see Appendix). This mistake could have had a significant effect on the final microbial content of the tea; according to Ingham (2005), organisms may not be active or survive long without the addition of food early in the brewing process. In contrast, Scheuerell and Mahafee (2002) noted a loss of disease suppression qualities of compost tea with added nutrients, which would indicate that nutrient additions are not necessary to achieve active microbial communities. Because Maxicrop adds potassium (K), its addition late in the brewing process may have caused the high plant tissue concentrations of K that resulted in its elimination from this analysis.

### Amount

To my knowledge, there have been no published studies on the use of compost tea in aquaponics water at this time. The concentration of compost tea in our system was calculated using a single-sentence reference from The Compost Tea Brewing Manual (Ingham, 2005, p. 32): “In water systems…1 gal of compost tea should be added to 50 gal water…”. This is the only reference in the literature to the amount of compost tea that

should be used in an aquaponics system. For this project, approximately 140 gallons of compost tea was brewed to accommodate a system containing approximately 7,000 gallons of water. Future studies could increase the amount of compost tea added to the system to determine if the concentration used in this study was too low.

### Other Factors Influencing Effectiveness

As noted previously, there is conflicting information regarding the efficacy of aerated (ACT) versus non-aerated compost tea (NCT). This study used compost tea continuously aerated for approximately 72 hours. Ingham (2005) does not provide a method for brewing NCT, claiming that a lack of active organisms will make the tea ineffective. Scheuerell and Mahafee (2002) contradict this claim, however, citing many studies that prove the effectiveness of NCT for disease suppression. The most effective NCTs are brewed for approximately 14 days, while the authors claim that much less in known about the optimal brewing times for ACT (Scheuerell and Mahafee, 2002). Ingham claims that the most active microbial communities can be achieved in 24 hours with commercial brewers (and a bit longer for homemade ones), while others have used brewing times anywhere from one to 14 days (Scheuerell and Mahafee, 2002). Trial and error may be the best way to determine optimal brewing parameters for the aquaponics system at SCCC, particularly due to the dynamic environmental parameters of the system and the fact that the specific nutritional needs of the plants are unknown.

# Conclusion

This thesis intended to provide a basis for the application of compost tea as a sustainable nutrient supplement for aquaponically produced wetland plants grown in coconut coir mats for *Rana pretiosa* habitat restoration. Compost tea has previously been used as a foliar spray on aquaponic and hydroponic crops, but no research exists regarding the efficacy of using compost tea directly in aquaponics water. However, it has been shown that using commercially produced fertilizers in aquaponics water can result in production that rivals or outcompetes soil-based and hydroponic systems that have controlled nutrient levels. It follows that compost tea could provide a similar effect.

Although this experiment failed to reject the null hypothesis that compost tea will not increase plant tissue nutrient concentrations, there are still many avenues to explore to improve the compost tea recipe and experimental design. This research was novel primarily because a lack of experimental control required the use of a quasi-experimental design for which there was no precedent in aquaponics or crop research specific to determining changes in plant tissue nutrient concentration. The following section provides suggestions for future research on compost tea in aquaponics, both emerging topics of research ripe for exploration.

## Summary of Recommendations for Future Research

The timeframe of this experiment was constrained to control for plant growth and seasonal change. It may not have been long enough to capture changes in soluble nutrients due to microbial activity. Running the experiment longer while incorporating some of the following recommendations may paint a better picture of the efficacy of compost tea in the aquaponics system at SCCC while also controlling for threats to validity.

## Water Testing

Previous studies have compared the elemental concentration of water entering plant beds to that of the water leaving the same plant beds to determine plant nutrient uptake in water systems (Buzby & Lin, 2014). This study was less concerned with dissolved nutrients than with the microbial action of the compost tea which can lead to increased plant nutrient incorporation via secondary processes, such as microbial solubilization of organic matter. Furthermore, non-uniformity is often a trait of aquaponics water, making nutrient analysis challenging (Vandam et al., 2017). At SCCC, the water that enters each bed is continuously drained at a slightly slower rate than it enters, causing it to mix as it fills the bed. It is unknown if this mixing is consistent throughout the bed, and subsequently if the water leaving the bed was well mixed enough to contain consistent nutrient concentrations at any point in time. Comparing the nutrient concentrations of water entering the beds to that leaving the beds may not provide accurate information about nutrient uptake by plants. Complicating things further are the microbes vital to the operation of the aquaponics system. Microbes need nutrients too; plants do not have sole responsibility for nutrient uptake rates in aquaponics systems.

The technicians at SCCC are limited in the nature of water quality testing equipment they are able to use in the prison environment. In order to achieve better accuracy and precision of water quality measurements, samples should be collected for laboratory analysis. Because the movement of water in the system is non-uniform, water collection should occur often, in multiple locations throughout the system, to get a better sense of the movement of nutrients and differences in environmental parameters system-wide. Determining changes in water quality parameters and linking those changes with

environmental changes will help evaluators determine the effects of inputs on water quality. Also, understanding the differences in nutrient movement within the system will help determine the best locations to take future water quality measurements to assess change over time.

If another compost tea experiment is carried out at SCCC, evaluators should test water for changes in nutrient concentration over time. This would shed some light on nutrient uptake by plants and how water quality affects leaf tissue nutrient concentration.

### Plant Analysis

Nutrient requirements of most food crop plants have been determined (Kalra, 1998), but the nutrient requirements of *D. cespitosa* and *C. obnupta* are unknown. Understanding the nutrient requirements of plants helps determine whether nutrient inputs are necessary. Nutrient requirements of crops vary by environmental conditions and life stages. It is unlikely that nutrient requirements will be determined for the various graminoids produced at SCCC; however, future research could determine how these plants are typically produced in nurseries, and what inputs they receive for optimal growth.

Plant growth should be evaluated parallel to leaf tissue nutrient content to determine the effect of growth on leaf tissue nutrient concentrations. In addition to whole plant dry weight, roots and shoots should be measured. Roots tend to grow larger in systems with lower nutrient concentrations where they are seeking nutrient pools (Delaide et al., 2016), indicating a nutrient deficiency. But increased root mass can lead to increased shoot mass, as larger root systems can absorb more nutrients (Bartelme et al.,

2018). Therefore, understanding how a plant is growing can inform the researcher about the nutrient status of the media in which it grows.

Future research should determine the iron concentration of plant tissue and system water. Chlorosis is often a symptom of iron deficiency (Kalra, 1998), and can often be seen in the plants at SCCC. Iron has been cited as deficient in aquaponics and is frequently added in plant-available forms (Rakocy et al., 2006). Although the four nutrients evaluated in this study do not appear deficient in plant tissue, they may still be deficient in iron. Potassium was also excluded from this study, but deficiency is unlikely as it is a major component of the soluble kelp added to the compost tea.

### Compost Tea Brewing

Because there are no standard compost tea brewing methods, perhaps the best way to refine the compost tea recipe and brewing process at SCCC is through trial and error. A few parameters with which to experiment are:

- Ratio of compost tea to aquaponics water—the maximum capacity of the brewer is 140 gallons, but two batches of tea could be prepared and added within one week
- Addition of Maxicrop Kelp Powder at the onset of brewing
- Experiment with other "food": molasses, humic acid
- Evaluate microbial communities in compost, tea, water—what is being extracted from the compost to the tea? What is multiplying in the water? Which microbes are surviving, and for how long?

The results of this research did not demonstrate that compost tea could increase plant tissue nutrient concentrations, but compost tea may still be a viable source of nutrition if

prepared and added properly; a method that could be unique to the specific parameters of the aquaponics system at SCCC.

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# Appendix

## Inputs to the System: Compost Tea and Maxicrop (kelp)

**Executive summary:**

Along with nitrogen, plants require many nutrients for healthy growth and mature life cycles. Due to the nature of the EVM Aquaponics Pilot System, external Potassium (K) and Phosphorus (P) will need to be generated and added to the system to maintain nutrient needs of the plants. This will be completed through the addition of highly nutrient rich compost into water that will then be added to the system at on a quarterly or more frequent timetable.

**System description:**

The Input Tea System will be situated on the left side of the biofilter, where the inputs will be added when needed. The system will consist of multiple yet simple components which include:

- A 150 gallon tank
- An aeration unit linked to the air input system
- A measured and sanitized amount compost or other organic nutrient rich substrate
- A 20 ounces of Maxicrop kelp powder to 120 gallons of water
- A water heated to maintain a temperature of 65 degrees F

**System operations and protocols:**

Starting a compost tea cycle:

- Clean all parts of the compost system to remove any bio-film
- Fill tank to the 120 gallon marker; when the bags are added to the system the water level will rise to 150 gallons. No additional water will need to be added if the tea brews no more than 5 days.
- Aerate water for 1 day to ensure high levels of dissolved oxygen/dechlorination. Optimal range of DO is 10-12ppm. The lowest DO reading should be 8ppm.
- Add four full 5 gallon bags of compost in net bags
- On the day of compost tea addition, add 20 ounces of Maxicrop concentrated powder to the compost tea. Allow it to mix for approximately 15 minutes prior to turning off the pump and allowing solids to settle.

Compost tea systems require daily observation and testing to ensure high levels of DO are present within the system. If a compost tea brew becomes too low in oxygen or tea brews too long, anaerobic growth can occur, leading to pathogenic and harmful bacterial and algal population expansion.

Due to the high needs of observation and testing of compost tea, **daily operations include**:

- ❖ Checking the DO levels of the compost tea and documenting the reading in the Compost Tea System Data sheet. Levels of DO will fluctuate based on bacterial population expansion or contraction. It is essential the DO level does not fall below 8ppm.
    - ➢ If DO level falls below 8ppm:
        - ■ Add emergency aeration unit detailed in system training
        - ■ If anaerobic growth is detected via low DO reading and foul smell, we need to discard that brew batch. Do not add compost tea to the full EVM System until SPP staff have deemed it appropriate.
- ❖ A couple of times a day agitate bags to avoid anaerobic bio-film build up within the compost tea bags
- ❖ Checking the temperature of the water to ensure stable water temperature of 65 degrees F is maintained.
- ❖ For troubleshooting refer to *The Compost Tea Brewers Manual* by Elaine Ingham

P and K nutrients will be tested bi-monthly to determine the amount of compost tea needed to add to the system. In the initial stages of the system, testing will be done to calculate the amount of compost tea needed to raise the P and K levels at 0.02-0.1 and 10 to 20 ppm, respectively.

- ❖ Compost tea is complete in 3 to 5 days and can be pumped into the solids separator tank. Do not brew the compost for longer than 5 days.
- ❖ Apply compost tea and kelp in the morning of a Monday or a Tuesday after brewing from Thursday or Friday. This will allow for a more complete monitoring of the aquaponics system during the week after compost tea additions.
    - o Turn off the pump to the compost tea and let particulates settle for 30 minutes; do not add the bottom 5 inches (the sludge) to the bio-ball tank. Send sludge to the compost center at SCCC.
    - o Listen for system pump malfunctions or sounds that would indicate pump wear.
    - o Monitor fish health and pay close attention to DO, pH, and ammonia/nitrite/nitrate for fluctuations.