1-1-2014

Variability in the phenolic content of invasive and non-invasive emergent wetland plants

Melissa M. Maurer

R·I·T

Variability in the phenolic content of invasive and non-invasive emergent wetland plants

By:

Melissa M. Maurer

Rochester Institute of Technology
College of Science
Environmental Science Program

A thesis submitted in partial fulfillment of the requirement for the degree of
Master of Science in Environmental Science

Approval Committee:

A. Christina Tyler, PhD
Chair of Committee, Advisor

Elizabeth Hane, PhD
Committee Member

Todd Pagano, PhD
Committee Member

C. Eric Hellquist, PhD
Committee Member

Rochester Institute of Technology
Rochester, NY
May 12, 2014

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

I would like to extend my deepest gratitude to my advisor, Dr. Christy Tyler. I sincerely appreciate all of the time and effort you have devoted to helping me develop this project and see it through to completion. Whether it was editing writing drafts, answering my long lists of questions, giving me several much needed reality checks, catching mistakes before it was too late, helping me with statistical analyses, or teaching me techniques in the lab, you were always there for me in my times of need and I am eternally grateful. I would also like to thank my committee members Dr. Elizabeth Hane, Dr. Todd Pagano, and Dr. Eric Hellquist. You have all helped me in times of crisis and I appreciate you taking on the added responsibility of being on my committee, and making the time to read and edit my writing drafts amidst your busy schedules. In addition, I would like to thank Dr. Susan Smith-Pagano for helping me with statistical analyses and allowing me to use her laboratory equipment, and also Dr. Karl Korfmacher for his input and advice with regard to my soil analysis methods and use of ArcMap. I also extend my thanks to the College of Science and Environmental Science Department for the funding of this project.

However, a project of this caliber would have never been possible without help in the field and in the lab. Therefore, I would like to thank my field and lab volunteers: Lisa Kratzer, Elizabeth Bruen, Kaity Moranz, Brenna DeAngelis, Alyssa Mathews, Benjamin Carson, Benjamin Harrison, Elise Carney, and Caitlin Hanrahan; thank you all for withstanding the elements with me and devoting your free time to help me set up plots, capture snails, collect field data, and complete my final sampling before the growing season concluded. Lastly, I extend many thanks to my friends, family, and future husband for their emotional support and encouragement throughout this entire process; I could not have done this without you, thank you all so much.

TABLES AND FIGURES

ABSTRACT

The colonization of wetlands by invasive plant species negatively impacts vegetation structure, nutrient and organic matter cycling, and ultimately alters native wetland ecosystem functions and services. It is unclear if the spread of invasive species can be attributed to their chemical composition. To further understand mechanisms of plant invasion, it is important to assess secondary chemistry of aggressive invaders. Phenolic compounds are important due to their diverse functionality including pathogen resistance, herbivore deterrence, and allelopathic interference. I conducted a broad field survey and a field experiment to better understand the importance and variability of wetland plant phenolic compounds and the relationship between abiotic and biotic environmental factors. I examined the relationship between leaf phenolic content and environmental conditions for 21 noninvasive and invasive plant species from ten sites. The environmental factors included soil moisture, extractable nitrate and ammonium, and total phosphorus, along with herbivory, and neighboring plant cover. The field experiment targeted two invasive species of cattail (*Typha latifolia*, *T. angustifolia*) in created wetlands at the Rochester Institute of Technology and High Acres Nature Area. I manipulated nutrient availability and herbivore pressure to investigate effects on growth and phenolic content. There was no predictable difference between invasive and noninvasive plants, but there were differences among sites for each species. The difference among sites for invasive species was more pronounced, with significant relationships with different combinations of abiotic and biotic factors, depending on the species. For four of the invasive species examined in detail, season, nutrients and/or herbivory were important factors influencing phenolic content. There were no predictable relationships for noninvasive species. There were no significant differences in growth, phenolic content, or herbivory among treatments in the field experiment suggesting that either the effects tested are unimportant for *Typha* spp., or the threshold was not met for an observable effect. We conclude that interspecific differences in the response of invasive plants to environmental factors preclude drawing general conclusions about the role of total phenol content in invasion success, but that invasive plants may be more responsive to environmental conditions, perhaps enhancing invasion.

1. Introduction

1.1 Mechanisms of species invasion

Wetlands are one of the most important ecosystems on earth; they are responsible for providing an array of vital ecosystem functions and services such as supporting biodiversity, water filtration, and retaining stormwater and nutrients (Zedler and Kercher 2005). Acting as a natural landscape filter, wetlands can accumulate excess debris, nutrients, and sediment which can create disturbance patches that represent ideal conditions for colonization of highly invasive, opportunistic species (Galatowitsch *et al.* 1999, Zelder and Kercher 2004). Creation of wetlands in an effort to mitigate wetland loss also initiates disturbances that enable rapid colonization of invasive species if preventative measures are not taken. Establishment of invasive species such as *Lythrum salicaria* (purple loosestrife), *Phalaris arundinacea* (reed canarygrass), and *Typha* (cattail) spp. in wetlands may change plant communities, increase litter accumulation, and alter nutrient dynamics (Zedler and Kercher 2004).

In this context, an invasive species is defined as either an exotic species that was introduced into the ecosystem ("exotic invader"), or a native species that became aggressively dominant as a result of a disturbance ("native invader"), and that also causes negative economic and environmental impacts (Carey *et al.* 2012, Mack *et al.* 2000). Other species that do not have aggressive spread or negative impacts are considered "noninvasive" throughout this manuscript.

Invasive plants often differ from noninvasive plants in various morphological and functional aspects (Monaco and Sheley 2012, Zedler and Kercher 2004). For example, most wetland invaders have high reproductive potential, rapid growth rates, excessive litter production, and increased biomass compared to their indigenous counterparts (Monaco and Sheley 2012). Aside from their physiological and reproductive differences, there are two hypotheses in particular that predict the mechanisms of invasive species success: the Evolution of Increased Competitive Ability (EICA) hypothesis (Blossey and Nötzold 1995) and the Novel Weapons Hypothesis (NWH) (Callaway and Ridenour 2004).

The EICA hypothesis predicts that when a plant is introduced into an area lacking natural predators, resources are allocated towards growth and reproduction instead of maintenance of herbivore defenses (Blossey and Nötzold 1995). When populations of *L. salicaria* from Europe (native) and from North America (invasive) were grown in a common garden greenhouse experiment and subject to herbivory, the invasive North American populations exhibited faster growth rates than European populations. Plants with low herbivore pressure grew more than those subjected to more intense herbivory. This result suggested that good invaders take advantage of low-herbivory circumstances and allocated resources towards growth and reproduction rather than anti-herbivore defense (Blossey and Nötzold 1995). This ability to respond to changing environmental conditions allows these species to be more competitive in new environments.

As an extension to the EICA hypothesis, Callaway and Ridenour (2004) proposed the Novel Weapons Hypothesis (NWH). This hypothesis states that some species are aggressively invasive by employing phytotoxins that are ineffective against noninvasive neighboring plants, but are powerful allelopathic inhibitors in their introduced environment. These novel chemical inhibitors affect plant-soil feedbacks and are detrimental to surrounding vegetation (Callaway and Ridenour 2004). Similar to the EICA, the NWH suggests that plant communities in the native environment have coevolved. However, plants in the introduced range have not coevolved with the invader and are not accustomed to the unique biochemical characteristics i.e., "novel weapons". Exposure to the invader's chemicals may lead to the reduced competitive ability of noninvasive species, allowing further expansion of invasive species.

Since the NWH was proposed, many studies focusing on different plants and ecosystems have produced controversial results regarding biochemical inhibitors (e.g. Blair *et al.* 2005, Callaway and Aschehoug 2000, Callaway and Ridenour 2004, Callaway *et al.* 2008, Cappuccino and Arnason 2006, Duke *et al.* 2009, Gibson *et al.* 2011, Hierro and Callaway 2003, Inderjit *et al.* 2006, Inderjit *et al.* 2008, Kim and Lee 2011, Perry *et al.* 2007, Thelen *et al.* 2005, Vivanco *et al.* 2004). Some studies have presented evidence supporting the NWH, i.e., some invasive species produce unique chemicals that are absent in noninvasives (Callaway and Aschehoug 2000, Callaway and Ridenour 2004, Callaway *et al.* 2008, Cappuccino and Arnason 2006, Kim and

Lee 2011, Vivanco *et al.* 2004) and exhibit allelopathic effects (Callaway *et al.* 2008, Gibson *et al.* 2011, Hierro and Callaway 2003, Inderjit *et al.*2006, Inderjit *et al.* 2008, Vivanco *et al.*2004). Other studies argue that there is uncertainty with regard to the role of secondary metabolites as they relate to invasion success because of the variable concentrations found in nature; some research indicates that much higher concentrations than used in experiments would be required to have negative effects in the field (e.g. Blair *et al.* 2005, Duke *et al.* 2009, Perry *et al.* 2007).

The conflicting views in the literature support the argument that we need a better understanding of the ecology and defenses of the most pernicious invaders if we are to develop more efficient management strategies (Monaco and Sheley 2012). In particular, some ecologists are examining the chemical defenses of invasive plants, specifically focusing on phytotoxic compounds (Callaway and Ridenour 2004, Hierro and Callaway 2003, Inderjit *et al.* 2006, Vivanco *et al.* 2004). I will examine invasive and noninvasive wetland plant species, using *Typha* spp. as a case study, to explore the differences in potential phytotoxicity and the role of environmental heterogeneity.

1.2 Phytotoxins: Phenolic compounds

Phenolic compounds, which include phenolic acids, flavonoids, tannins, lignins, and coumarins, are among the most common and diverse groups of chemicals found in plants; there are currently over 8,000 known phenolic compounds each differing in structure and chemical composition (Dai and Mumper 2010). Among their many functions, phenolic compounds may provide pathogen resistance, deter herbivory, and influence plant color to attract pollinators (Li *et al.* 2010, Dai and Mumper 2010). Phenolic compounds may also exhibit allelopathic effects - negatively affecting growth and nutrient acquisition of neighboring plants, leaving noninvasive plants more susceptible to herbivory or parasites, and thus making nutrients and space more accessible for the invader (Callaway and Aschehoug 2000, Gibson *et al.* 2011, Kim and Lee 2011).

Production of phenolic compounds by plants is influenced by a complex array of biotic (herbivore presence, phenology, presence of other plant spp.) and abiotic environmental factors

(nutrients, light availability, season), resulting in variability of concentrations within and among species. In addition, differences in phenolic content among species can also vary depending on the plant species, genetic characteristics, and type of plant (aquatic, terrestrial) (Boege 2005, Cronin and Lodge 2003, Li *et al.* 2010, Smolders *et al.* 2000). In freshwater ecosystems, the relationship between these factors and phenolic compound production is unclear, making generalizations about how abiotic and biotic factors impact the concentration of these chemicals problematic (Cronin and Lodge 2003, Gross and Bakker 2012).

The Protein Competition Model (PCM), developed by Jones and Hartley (1999), proposed a biochemical explanation for variation in phenolic concentrations in terrestrial higher plants. The premise of this model is that phenolic compounds and proteins are in constant competition for a common required precursor – phenylalanine. This amino acid can either be directly incorporated into proteins, or deaminated and incorporated into phenolic compounds. These two different pathways that phenylalanine undergoes to be incorporated into either compound results in an inverse relationship between protein and total phenol concentrations. The compound that is in higher demand (proteins or phenolics) determines where the phenylalanine is allocated; protein or phenolic demand is influenced by three categories: growth requirements, genetic characteristics, and environmental factors. Proteins are responsible for growth and carbon fixation, while phenolic compounds are primarily responsible for providing structure and defense. Given these major functions, an example where proteins would be in higher demand than phenolic compounds is if the plant is genetically fast-growing; to maintain a high growth rate and necessary carbon fixation, proteins will be in higher demand than phenolic compounds.

While the production of phenolic compounds confers obvious benefits to the plant, production of these compounds is metabolically costly, leading to potential negative effects on fitness (Boege 2005, Coley *et al.* 1985, Elger and Lemoine 2005, Feeny 1976, Grime 1977). However, some plants, including invasive and naturalized species, have evolved ways to balance the costs and benefits of defense, or produce compounds that serve multiple functions (Cronin and Hay 1996, Feeny 1976, Siemens *et al.* 2002, Thelen *et al.* 2005).

The cost-benefit approach is based on how plant growth and resource allocation responds to variations in the selective pressures of the environment, particularly nutrient availability and herbivore pressure (Grime 1977). When nutrients are limited, plants generally exhibit increased anti-herbivore defense and when nutrients are plentiful, plants produce fewer defense compounds and sustain more herbivore damage. This trend demonstrates the interaction of abiotic and biotic factors, suggesting that in adaptable plants, phenolic compound production will vary to maximize fitness (Boege 2005, Coley 1995, Feeny 1976, Grime 1977). Further, by producing multi-functional phenolic compounds, a variety of plants' individual compounds may protect the plant from herbivore damage and simultaneously impair neighboring plants (e.g. macroalgae [Cronin and Hay 1996], spotted knapweed [Thelen *et al.* 2005] and plants in the mustard family [Siemens *et al.* 2002]). Producing multifunctional phenolic compounds alleviates the metabolic costs of producing multiple compounds, and is an advantageous adaptive strategy for nutrient limited environments.

In addition to producing multi-functional phenolic compounds, another way that plants can alleviate the costs of producing defense compounds is by only producing compounds in response to sudden changes in the environment; these are termed inducible chemical defenses. Plants utilizing chemical forms of defense, such as phenolic compounds, can either have constitutive or inducible defenses. Constitutive chemical defenses are inherent, inducible defenses are only employed in response to an environmental cue, mainly herbivory. Evolution of inducible defenses, similar to multifunctional compounds, would lessen the metabolic cost and reduce the chance of a negative effect on the plant's fitness if resources were allocated to producing defenses only when necessary (Feeny 1976); Cronin and Hay (1996) and Thelen *et al.* (2005) have shown evidence to support the concept of inducible defenses.

1.3 Wetland plant species phenolics using Typha spp.as a case study

Wetlands, both created and natural, vary spatially and temporally with regard to moisture and nutrient gradients, resulting in diverse, variable communities of plants, animals, and microorganisms (National Research Council Staff 1995). Created wetlands typically have decreased species diversity, different soil characteristics, and may not function as well as a

natural wetland (Campbell *et al.* 2002). Herbivores that reside in wetlands include both specialists (e.g. *L. salicaria* beetles) and generalists (e.g. muskrats, geese, snails, caterpillars) that each have different eating habits and damage the plants in different ways (*pers. obs.*). However, wetlands are also prone to disturbance, i.e. flooding or nutrient loading, causing several invasive species to frequent wetlands (Zedler and Kercher 2004). This abundance of biodiversity, presence of invasive species, naturally variable conditions, and occurrence of disturbances provides a unique opportunity to investigate how different environmental conditions impact phenolic concentrations within and among species.

Due to complex wetland ecosystem interactions, wetland plant biochemistry is understudied compared to terrestrial ecosystems (Ervin and Wetzel 2003, Gross and Bakker 2012, Iason *et al.* 2012, Inderjit 2001, Jarchow and Cook 2009). There are several areas that are in need of further investigation if we are to understand the importance of phenolic compounds and how they impact wetland ecology. It is unclear how environmental conditions influence phenolic compound production, and how concentrations differ among plant species - especially among noninvasive and invasive plants. Whether or not invasion success in wetlands can be attributed to greater or more adaptable production of secondary metabolites, such as phenolic compounds, is also unknown. Based on the benefits of phytotoxin production to terrestrial plant invasion success (Callaway and Ridenour 2004, Cappuccino and Arnason 2006, Gibson *et al.* 2011, Kim and Lee 2011), we suggest that nimble production of these compounds may also play a role in wetland invader success.

One of the most aggressive wetland invaders are species of the genus *Typha*, which are capable of quickly invading wetlands, particularly those subject to recent disturbance (Apfelbaum n.d., Galatowitsch *et al.* 1999, Zelder and Kercher 2004). Following the invasion of a wetland by cattails, monotypic stands are rapidly established, native species are displaced, biodiversity decreases, and litter production increases. These interactions simultaneously alter nutrient cycling as well as trophic interactions (Angeloni *et al.* 2006, Apfelbaum n.d., Houlahan and Findlay 2004, Tuchman *et al*. 2009, Zedler and Kercher 2004). *Typha* spp. make an interesting case study since there is a "native invader" (*T. latifolia*), an "exotic invader" (*T. angustifolia*), and a hybrid invader (*T.*× *glauca*) within the same genus that occur in a similar geographic

range. Examining the phenolic concentrations of closely related *Typha* spp. and how they respond to different environmental conditions may provide insight regarding whether secondary compound concentrations differ among native and exotic invasive species.

The limited work done to date on wetland plants, particularly *Typha* spp., supports the NWH, suggesting that *Typha* spp. are capable of producing and utilizing phenolic compounds to their benefit by impairing neighboring vegetation (Bolser *et al.* 1998, Domènech *et al.* 1997, Jarchow and Cook 2009, Jordan *et al.* 1990, McNaughton 1968, Penko and Pratt 1987, Prindle and Martin 1996). For example, inhibition of germination and growth of other species when grown in proximity to *Typha* has been documented for *T. domingensis* (Prindle and Martin 1996), *T. latifolia* (McNaughton 1968), and *T. angustifolia* (Jarchow and Cook 2009) when soils were inoculated with *Typha* phenolic extracts. In addition to inhibiting establishment of other species, there is potential for persistence of these chemicals in the environment; Domènech *et al.* (1997) found that allelopathic compounds produced by *T. domingensis* were detectable in soils taken from within two meters of the plant.

To further understand the phenolic chemistry of wetland plant species, we must understand, first, how phenolic concentrations are different among noninvasive and invasive species. Second, how are phenolic concentrations affected by various environmental factors? And third, using *Typha* spp. as a model, how do phenolics differ in concentration and response to environmental cues between closely related invasive species? I hypothesize that the phenolic content of invasive species will be higher than noninvasive species, and that phenolic concentration will vary depending on the species and local environmental conditions, as predicted by the PCM (Jones and Hartley 1999). I predict that closely related noninvasive and invasive species will respond differently to environmental cues, as shown for other con-generic plants (Feeny 1976, Lind and Parker 2010, Wolf *et al.* 2011).

2. Methods

2.1 Wetland vegetation phenolic survey

We investigated the variability of total phenolics, with respect to variation in biotic and abiotic factors, for a variety of noninvasive and invasive wetland plant species in emergent freshwater wetlands throughout Central and Western New York State.

Four sites were selected for plant sampling. Three of the four sites were created wetlands - Rochester Institute of Technology (RIT), High Acres Nature Area (HANA), and Rice Creek Field Station (RCFS); the other site was a natural wetland - Camp Rd. RIT, HANA, and RCFS were restored to wetlands after being used for agriculture. RCFS, located in Oswego, NY, is the oldest of the created wetlands. RCFS was created in 1965 after a dam was built for Rice Creek, which then created what is now Rice Pond (Rice Creek Field Station, n.d.). Wetland areas formed around the outskirts of the pond which are primarily dominated by *T.* × *glauca* and *T. angustifolia* but a variety of other freshwater emergent plants are also found where *Typha* spp. are less dominant (*pers. obs.*). RIT, located in Rochester, NY, is the next oldest created wetland created in 2007, and HANA, located in Perinton, NY, was created in 2009 after the landfill expanded to mitigate for the wetlands lost during expansion. The Camp Rd. site is located near a developed area of Hamburg, NY and is presumed to be natural. Satellite imagery dating back to 1995 (Google earth) shows that the wetland has not changed for at least 19 years and does not appear to have been managed for invasives as *T. angustifolia*, *P. australis, L. salicaria*, and *P. arundinacea* are present throughout the site (*pers. obs.*).

Species selected for sampling were representative of dominant noninvasive vegetation and common invasive species; at all sites, *Typha* species were a focus. Because the plant communities differed slightly among sites, different groups of species were sampled at each site. For each species, an individual was randomly selected and a $1m^2$ quadrat was centered over the plant of interest and percent cover of all species in the plot was estimated. The plant height, number of leaves, presence of an inflorescence, and signs of damage (number of broken leaves/stems, senescence) or grazing (number of snail radulations, holes, and chewed edges)

were quantified and recorded. Leaf tissue and rhizospheric soil were then collected from the corresponding plant (n = 5 except for RCFS *C. lupulina* n = 4, and RIT *S. latifolia* n = 2). To increase the cattail sampling effort, six other locations between Buffalo and Oswego were selected specifically for cattail sampling using the USFWS Wetlands Mapper (http://www.fws.gov/wetlands/Data/Mapper.html). All survey locations were recorded using a GPS unit (Figure 1, Appendix A).

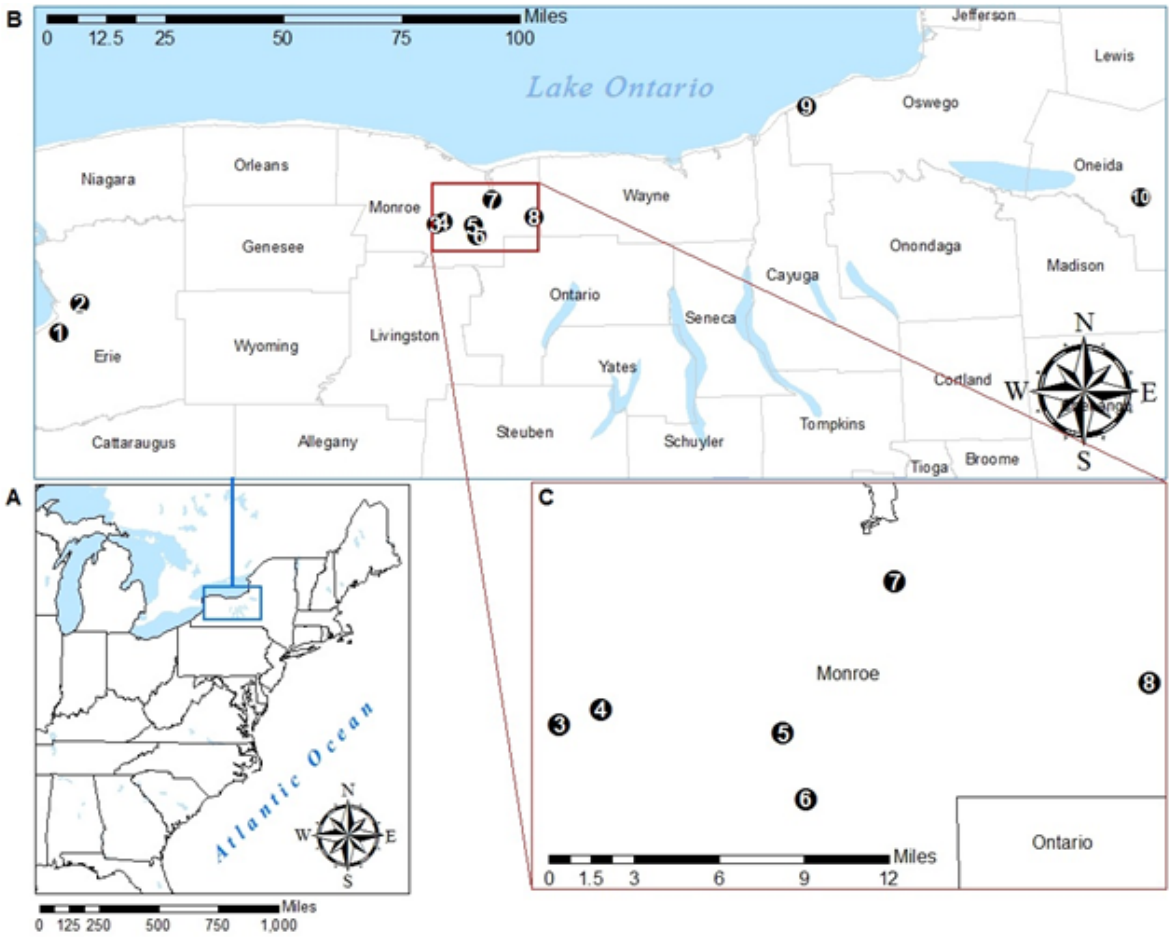


Figure 1. Map of sites in New York used for the wetland vegetation survey and field experiment. (**A**) shows the northeast and in blue is the extent of New York, shown in (**B**), where the sampling sites were located. The red extent is then magnified further in (**C**) to show sites selected in Monroe county more clearly. Numbers correspond to sites listed in Appendix A.

2.2 Manipulative field experiment: Herbivory, nutrients, and phenolic content

A field experiment designed to investigate the effects of herbivory and nutrient availability on phenolic content in *Typha* spp. was conducted in created emergent wetlands at RIT and HANA.

In May 2013, twenty 1 m^2 plots were established within *Typha* zones present at each site where there was at least 80% cover of new cattail shoots over a 9 m^2 area (*T. latifolia* at HANA, *T. latifolia* and *T. angustifolia* at RIT). Plots were arranged in five blocks of four plots spaced 1 m apart; within each block, plots were randomly assigned to one of four treatments: nitrogen addition (N), herbivory (H), N+H, or control (C) (Appendix B). PVC pipe (5' for H and N+H, 2.5' for C and N) was driven into the ground at the plot corners and galvanized hardware cloth (36" tall, ¼ x ¼" mesh) secured around the perimeter of the H and N+H plots with cable ties and pushed down into the substrate. Remaining plots were delineated by securing rope around the PVC to mimic the effect of the cage and to prevent damage to plants near plot edges.

Following plot establishment, ten healthy plants in each plot were tagged and measured. For each plant, we measured the height, number of leaves, noted the presence of an inflorescence, and recorded damage or grazing (number of snail radulations, broken and damaged leaves or stems). For each plot, the water depth, total number of live stems, and total number of plants with an inflorescence was also recorded prior to the start of the field experiment.

Amber snails (*Succinea putris*) are a native species present at both sites and were frequently observed grazing on cattails. To first determine the ambient field density, a 0.25 m^2 quadrat was randomly placed over wetland vegetation at RIT and the number of amber snails within the plot was counted (n = 60). The abundance of snails in each plot was multiplied by four to get an estimate of snails per square meter (Kratzer 2013, *unpublished data*). These estimates were then averaged together for the site to approximate ambient field density (approx. 10 snails/m^2). We then added five times the average field density of snails in an effort to elicit a more pronounced response to herbivory for the field experiment; the same field density was used for both RIT and HANA. Where plots were located at HANA, there were very few, if any amber snails already present because plots were set up along the edge of a pond where average water depth was

approximately 15 cm; amber snails are only partially aquatic and prefer moist to shallow standing water conditions. Snails were collected from the surrounding area and reintroduced into the appropriate herbivory treatment plots (H, N+H). Plots were periodically monitored over the course of the growing season to ensure that caged and strung plots were maintained, and snail densities stayed as consistent as possible. In the N and N+H plots, four perforated 15 mL centrifuge tubes containing nitrate fertilizer (Nitrate of Soda, 15-0-0 NPK) were inserted into the sediment. The fertilization rate was approximately 10 g m^{-2} $month^{-1}$; and tubes were replaced monthly from the start of the experiment (late June) until the end of the growing season (late August) resulting in a total addition of 20 g N·m^2 (Tyler *et al.* 2003, Tyler *et al*. 2007).

In late August the plant height, number of leaves, presence of an inflorescence, and damage or grazing was recorded again for all tagged plants. These data were compared to data recorded at the start of the experiment to calculate average growth rate $\left(\frac{Final\ height - Initial\ height}{\#\ Days}\right)$, leaf gain, snail radulations per leaf, and the increase in total number of stems and plants with an inflorescence in the plot. Three sets of three leaves from nine different healthy cattail plants within the plot were collected and stored at -80°C prior to analysis of total phenolic content. Rhizospheric soil was collected to quantify moisture content, extractable nitrate, extractable ammonium, and total phosphorus; samples were stored at -20°C prior to analysis. Herbivore damage was assessed and quantified by calculating the snail radulation (or beetle hole) gain over the course of the experiment $(Final\ \#Rads. - Initial\ \#Rads.)$, and also determining a radulations (or holes) per leaf ratio $\left(\frac{Final\ \#\ Rads.}{Final\ \#\ Leaves}\right)$ to standardize for larger plants with more leaves.

2.3 Laboratory analyses

Total phenolic content was determined by freeze-drying frozen plant tissue with liquid nitrogen and grinding it into a fine powder using a mortar and pestle. Ground plant tissue (0.1 g) was extracted in 60% acetone (10 mL) for 48 hours in the dark at room temperature. Gallic acid (Sigma-Aldrich) was dissolved in 60% acetone and used to make standards of 0.00, 0.25, 0.50, and 0.75 mM. Extractants and gallic acid/60% acetone standards were plated into a 96-well microplate. Folin-Ciocalteu reagent (Sigma-Aldrich, 1:10 v/v) and sodium hydroxide (700 mM)

were added and the absorbance was measured at 765 nm using a Thermo Scientific Varioskan Flash Spectral Scanning Multimode Reader within 5 minutes (adapted from Ainsworth and Gillespie 2007). All phenolic concentrations are reported in gallic acid equivalents (GAE).

Rhizospheric soil moisture content was determined gravimetrically after oven-drying 5 g of moist soil at 105°C for 48 h, and calculating the percent mass lost as water (Topp *et al.* 2008). Inorganic nitrogen was extracted by shaking 5 g moist soil with 50 mL 2M KCl for 16 h. Samples were then centrifuged, the supernatant decanted, filtered (0.45 µm), and placed into whirl-paks. Filtered samples were frozen at -20°C prior to analysis. Extractable nitrate (µg N/L) was quantified on a Lachat QuikChem 8500 Autoanalyzer using the cadmium reduction method (Knepel 2012). Extractable ammonium was quantified using the phenol hypochlorite method (Maynard *et al.* 2008) and sample absorbance was read at 630 nm using a Shimadzu UV 1800 Spectrophotometer.

Soil total phosphorus was determined by adding 50% w/v magnesium nitrate to 0.1 g of oven-dried soil and ashing the sample for 2 h at 550°C in a muffle furnace. Once cool, 10 mL of 1 M HCl was added, samples were shaken for 16 h and allowed to settle overnight. Samples were diluted (10x) and measured at 880 nm using a Shimadzu UV 1800 Spectrophotometer (Kempski n.d., Murphy and Riley 1962).

2.4 Statistical analyses

All statistical analyses were performed using JMP 10 software (SAS Institute Inc., 2012). Data that was not normally distributed was natural log transformed to conform to the assumptions of parametric statistical analyses. The alpha level (α) for all statistical analyses was $\alpha = 0.05$.

Wetland vegetation phenolic survey:

In order to investigate the effects of site and status on the total phenolic content of noninvasive and invasive species, we performed a two-way ANOVA. To understand how environmental conditions influence phenolic concentrations for the same species sampled from different sites,

multiple regression analyses were used. The herbivory data from the wetland vegetation survey was standardized by transforming the leaf-specific herbivory into z-scores and then added together to create a single variable representative of herbivory. Multiple regression analyses were only performed for a few individual species - some replicates had missing data which ultimately excluded them from the analysis; as a result, there were only a few species with a sufficient sample size ($n \geq 5$) to generate a predictive model. In addition to performing multiple regression analyses for individual species, multiple regressions were also generated for all noninvasive and all invasive species. Correlation matrices were used to decide which parameters would be most important based on what was significantly correlated with phenolic content. Using the date samples were collected, % moisture, TP, % cover other spp., *ln* (inorganic N), and herbivory, we performed forward stepwise multiple regression analyses. The best model ($\Delta i = 0$) was chosen using the lowest AIC_c value and the Akaike weights were calculated for all models. Models were compared among species to examine differences in predictors of total phenolic content.

Field experiment:

We used a two-way ANOVA to examine the effects of site and treatment on growth, herbivore damage, soil nutrients, and phenolic content in *T. latifolia* between RIT and HANA. We also performed a two-way ANOVA to investigate the effects of species and treatment on growth, herbivore damage, soil nutrients and phenolic content between *T. latifolia* and *T. angustifolia* at RIT.

3. Results

3.1 Wetland vegetation phenolic survey

There was a significant interaction between the status (noninvasive/invasive) and site when examining the phenolic content of all species sampled in the survey (Table 1). For two of the four sites (Camp Rd. and RCFS) invasive plant species phenolic content was significantly higher than noninvasives ($p < 0.05$), however there was no difference in phenolic content between noninvasive and invasive plant species at RIT and HANA (Table 1). Not all noninvasive plant species were present at the sites sampled, resulting in an unbalanced sampling design. The standard deviation and ranges of phenolic content for species sampled from each site were variable (Table 2, Figure 2). The overall range in phenolic content was greater for invasive than noninvasive species (Min-Max: 0.0 – 28.2 and 0.0 – 23.1, respectively). The average variance in foliar phenolic content was greater for invasive than noninvasive species (24.0 and 17.0, respectively). *L. salicaria* had the highest phenolic content, range, and second greatest variance of all the species sampled during the survey (Mean ± SD: 20.4 ± 7.1 mg·g DW^{-1}, Min-Max: 2.4 - 28.2 mg·g DW^{-1}, Variance: 50.5). *L. salicaria* had the highest phenolic content of all the species sampled at Camp Rd., HANA, and RCFS. *Typha* × *glauca* had the lowest phenolic content (Mean ± SD: 1.1 ± 1.7 mg·g DW^{-1}); while *Scirpus atrovirens* had the lowest range and variance in phenolic content (Min-Max: 5.7 - 8.3 mg·g DW^{-1}, Variance: 1.0).

The multiple regression analysis for all invasive species indicated that a combination of the date the sample was collected, soil moisture, inorganic nitrogen, and herbivory could be used to predict the phenolic content. However, this model only explained 32% of the variance ($p < 0.0001$, Table 3). Native species phenolic content could not be reliably predicted based on the environmental parameters assessed, but the "best" model indicated that the date the samples were collected was the most influential parameter ($R^2 = 0.04$, $p = 0.34$).

Table 1. Results of two-way ANOVA (all sites) and one-way ANOVAs (by site) examining the effect of status (noninvasive or invasive) on phenolic content. *P*-values in boldface indicate significance.

Factor	DF	F	*p*
All Sites			
Site	3	1.27	0.287
Status	1	4.52	**0.035**
Status × Site	3	4.54	**0.004**
Camp Rd.			
Status	1	4.52	**0.039**
HANA			
Status	1	2.15	0.149
RCFS			
Status	1	9.20	**0.005**
RIT			
Status	1	0.02	0.891

Table 2. Mean total phenolic content (mg · g DW^{-1}) ± SD is shown for each species sampled from their respective sites. Ranges and means are shown for individual species as well as for each site in their entirety, and by status (native/invasive). Total ranges and means of bulk phenolic content for all species across all sites and by status are shown towards the bottom right of the table. Additional sites that *T. latifolia* was sampled from were Ellison Park (12.72 ± 5.19), Langner Rd. (4.49 ± 0.77), and Tinker (12.65 ± 3.63). Additional sites that *T. angustifolia* was sampled from were E. River Rd. (12.65 ± 3.58), Lagner Rd. (6.20 ± 0.72), Mendon (10.76 ± 3.88), Tinker (2.66 ± 3.05), and Verona (5.73 ± 2.07).

Family	Scientific Name	Common Name	Camp Rd	HANA	RCFS	RIT	Range	Mean	Variance
Noninvasive Species									
Alismataceae	*Alisma subcordatum*	Water plantain		13.1 ± 5.9		14.5 ± 5.2	5.8 - 20.3	13.8 ± 5.2	27.2
	Sagittaria latifolia	Arrowhead		6.5 ± 5.8		20.6 ± 1.5	0.2 - 21.6	10.5 ± 8.3	69.4
Araceae	*Peltandra virginica*	Arrow arum			5.1 ± 1.7		3.6 - 7.6	5.1 ± 1.7	2.8
Asteraceae	*Eutrochium maculatum*	Spotted Joe-Pye weed			5.9 ± 3.2		2.4 - 10.3	5.9 ± 3.2	10.1
Cyperaceae	*Carex lupulina*	Hop sedge	4.6 ± 6.4		8.6 ± 2.4		0.0 - 14.4	6.4 ± 5.2	27.3
	Carex vulpinoidea	Fox sedge	3.7 ± 0.9			7.9 ± 4.3	2.7 - 13.0	5.8 ± 3.7	13.5
	Cyperus erythrorhizos	Redroot flatsedge	14.7 ± 1.4	17.1 ± 4.7			11.4 - 21.3	15.9 ± 3.5	12.4
	Elocharis congesta	Spike rush	10.8 ± 1.7				7.9 - 12.4	10.8 ± 1.7	2.9
	Schoenoplectus tabernaemontani	Softstem bulrush			4.8 ± 4.8		0.2 - 12.4	4.8 ± 4.8	23.1
	Scirpus atrovirens	Green bulrush				7.4 ± 1.0	5.7 - 8.3	7.4 ± 1.0	1.0
	Scirpus cyperinus	Wool grass				13.6 ± 2.9	11.3 - 18.3	13.6 ± 2.9	8.2
Juncaceae	*Juncus effusus*	Soft rush	12.0 ± 6.8	15.3 ± 3.6		20.4 ± 2.6	0.9 - 23.1	15.6 ± 5.8	33.3
Pontederiaceae	*Pontederia cordata*	Pickerelweed		15.6 ± 3.6			12.7 - 21.3	15.6 ± 3.6	13.1
Scrophulariaceae	*Mimulus ringens*	Monkeyflower				4.6 ± 1.1	3.3 - 6.3	4.6 ± 1.1	1.2
Verbenaceae	*Verbena hastata*	Blue vervain	8.3 ± 2.1				5.1 - 10.5	8.3 ± 2.1	4.4
Invasive Species									
Lythraceae	*Lythrum salicaria*	Purple loosestrife	27.5 ± 0.8	22.7 ± 4.0	19.4 ± 4.9	11.8 ± 6.1	2.4 - 28.2	20.4 ± 7.1	50.5
Poaceae	*Phalaris arundinacea*	Reed canarygrass	11.1 ± 2.5	6.5 ± 1.5	17.5 ± 1.1	17.7 ± 2.4	4.1 - 20.7	13.2 ± 5.1	26.3
	Phragmites australis	Common reed	6.6 ± 5.8	13.2 ± 4.1		12.7 ± 2.6	2.4 - 18.0	10.8 ± 5.1	25.8
Typhaceae	*Typha angustifolia*	Narrowleaf cattail	8.3 ± 2.1	2.7 ± 0.8		7.9 ± 2.1	0.0 - 17.7	7.3 ± 4.0	16.0
	*Typha × glauca***	Hybrid cattail			1.1 ± 1.7		0.0 - 4.1	1.1 ± 1.7	3.0
	*Typha latifolia**	Broadleaf cattail		5.9 ± 2.3		8.9 ± 4.2	3.2 - 17.9	8.9 ± 4.7	22.5
		Range	0.0 - 28.2	0.2 - 27.6	0.0 - 27.4	2.4 - 23.1	0.0 - 28.2		
		Mean	10.8 ± 7.3	12.0 ± 7.0	8.9 ± 7.2	11.7 ± 5.7		10.6 ± 6.5	18.8
		Invasive Range	2.4 - 28.2	1.7 - 27.6	0.0 - 27.4	2.4 - 20.7	0.0 - 28.2		
		Invasive Mean	13.4 ± 9.1	10.5 ± 7.7	12.7 ± 9.0	11.8 ± 4.9		10.9 ± 7.0	24.0
		Native Range	0.0 - 17.2	0.2 - 21.3	0.2 - 12.4	3.3 - 23.1	0.0 - 23.1		
		Native Mean	9.0 ± 5.4	13.5 ± 5.9	5.9 ± 3.3	11.6 ± 6.3		10.2 ± 6.0	17.0

**T. latifolia* is native in origin but can become invasive following a disturbance

***T. × glauca* has both a native and exotic parent (*T. latifolia* and *T. angustifolia* , respectively) and in this instance is classified as an invasive species

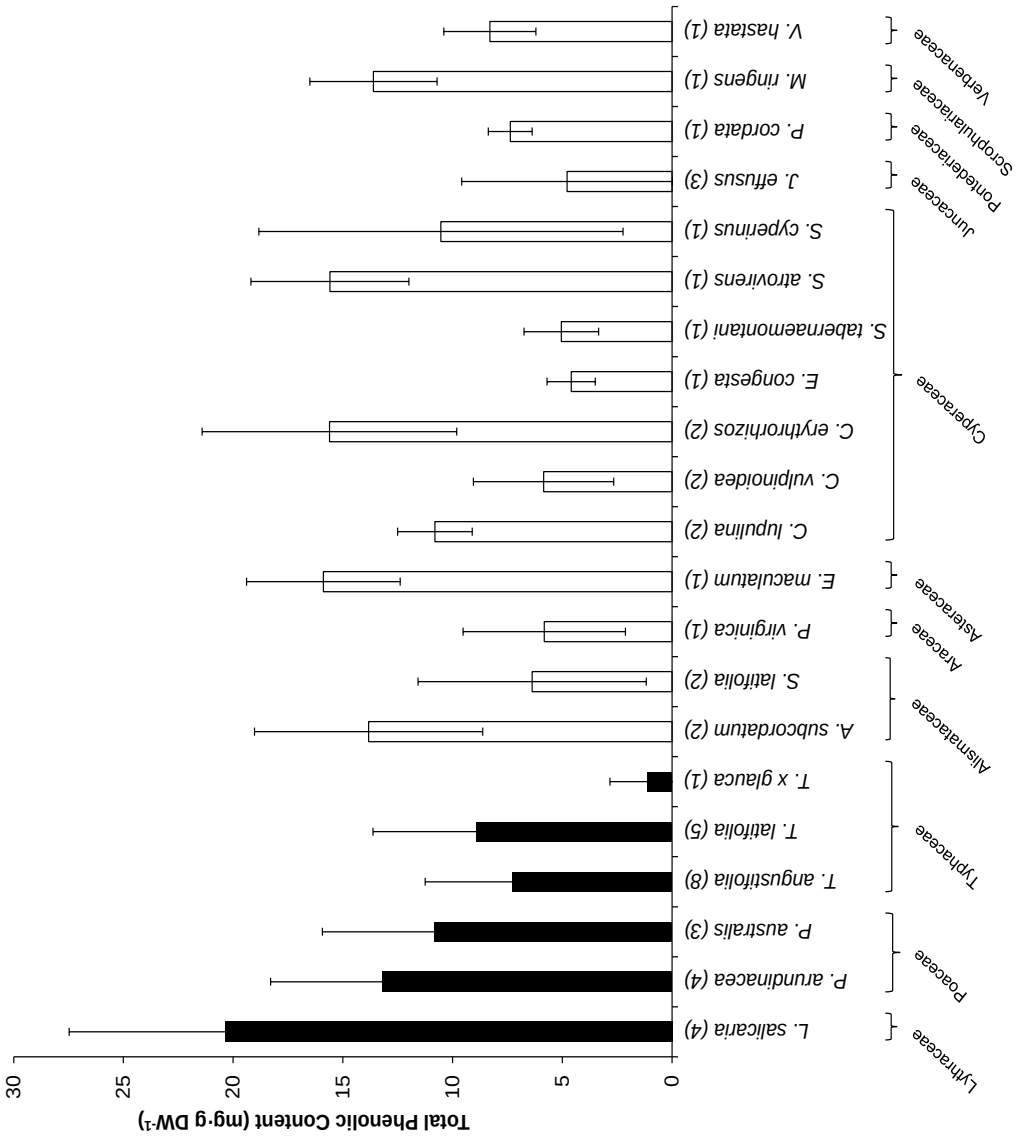


Figure 2. Mean ± SD total phenolic content (GAE) for each species across all sites; numbers in parentheses next to the scientific name correspond to the number of sites species were sampled from (n = 5 for all spp. except RCFS *C. lupulina*: n = 4, and RIT *S. latifolia*: n = 2). Black bars represent invasive species, white bars represent noninvasive species; species are grouped by family.

Table 3. Results of the multiple regression analyses after selecting the best model. *P*-values for the entire model (shown to the right of the species name) along with coefficients and *p*-values for each variable within the respective models are shown. AIC_c, Δi, and w_i values are shown for each model. *P*-values in boldface indicate statistical significance.

Variables	Coefficient	*p*	Model R^2	DF	AIC_c	Δi	w_i
All Noninvasive Species		0.3410	0.043	28	199.15	0.00	0.11
Date	2.36E-06	0.273					
All Invasive Species		**< 0.0001**	0.321	54	380.14	0.00	0.21
Date	-1.45E-06	**0.0001**					
% Moisture	-23.10	**0.004**					
Soil N	2.11	0.075					
Herbivory	2.47	**0.012**					
Sagittaria latifolia		**0.0073**	0.761	4	50.89	0.00	0.53
% Cover Other Spp.	-26.66	**0.023**					
Typha latifolia		**0.0027**	0.975	5	49.04	0.00	0.81
Date	2.21E-06	**0.004**					
% Moisture	-38.35	**< 0.0001**					
TP	0.03	**0.0005**					
Typha angustifolia		**< 0.0001**	0.749	16	95.74	0.00	0.36
TP	-0.01	**< 0.0001**					
% Cover Other Spp.	5.50	**0.003**					
Herbivory	-1.33	0.053					
Lythrum salicaria		**< 0.0001**	0.571	10	78.00	0.00	0.39
Date	9.66E-06	**0.004**					
Phalaris arundinacea		**0.0002**	0.892	8	59.11	0.00	0.60
Date	-6.67E-06	**0.004**					
Herbivory	3.66	**0.021**					

As mentioned in the "*Statistical analyses*" section, missing data for any replicate resulted in exclusion from the multiple regression analysis and as a result, predictive models were only generated for *Sagittaria latifolia* (arrowhead), *T. latifolia*, *T. angustifolia, L. salicaria,* and *P. arundinacea* (Table 3). *S. latifolia* was the only noninvasive species for which a multiple regression could be performed. The results of the regression analysis indicated that phenolic content could be predicted based on the percent cover of other species present within the sampling quadrat ($R^2 = 0.76$, $p = 0.007$, individual relationships shown in Figure 3). *T. latifolia* phenolic content could be reliably predicted using a combination of the date samples were collected, soil moisture, and total phosphorus ($R^2 = 0.96$, $p = 0.003$, individual relationships shown in Figure 4), whereas percent cover of other species, herbivory, and total phosphorus were more important for *T. angustifolia* ($R^2 = 0.75$, $p < 0.0001$, individual relationships shown in Figure 5). *L. salicaria* phenolic content was influenced the most by the date samples were collected ($R^2 = 0.57$, $p < 0.0001$, individual relationships shown in Figure 6) and *P. arundinacea* phenolic content could be predicted using the date samples were collected and herbivory ($R^2 = 0.89$, $p = 0.0002$, individual relationships shown in Figure 7).

3.2 Manipulative field experiment: Herbivory, nutrients, and phenolic content

There were no significant differences in growth, herbivore damage, phenolic content, or soil nutrients among treatments for either species of cattail at RIT and HANA (Figure 6). When comparing *T. latifolia* to *T. angustifolia* at RIT, there were no significant differences in phenolic content, soil nutrients, or herbivore damage; *T. latifolia* did however have a higher growth rate than *T. angustifolia* plots (Table 4a, $p = 0.0005$). There was a significant site difference when comparing *T. latifolia* between RIT and HANA. *T. latifolia* at RIT had a higher growth rate, sustained more herbivore damage, and soil contained higher concentrations of inorganic nitrogen (Table 4b, $p < 0.05$). The average soil moisture was 39% at HANA and 46% at RIT (7% difference), average total phosphorus was 737 mg P·kg DW^{-1} at HANA and 958 mg P·kg DW^{-1} at RIT, (221 mg P·kg DW^{-1} difference); despite site differences, there was no difference in *T. latifolia* phenolic content between RIT and HANA.

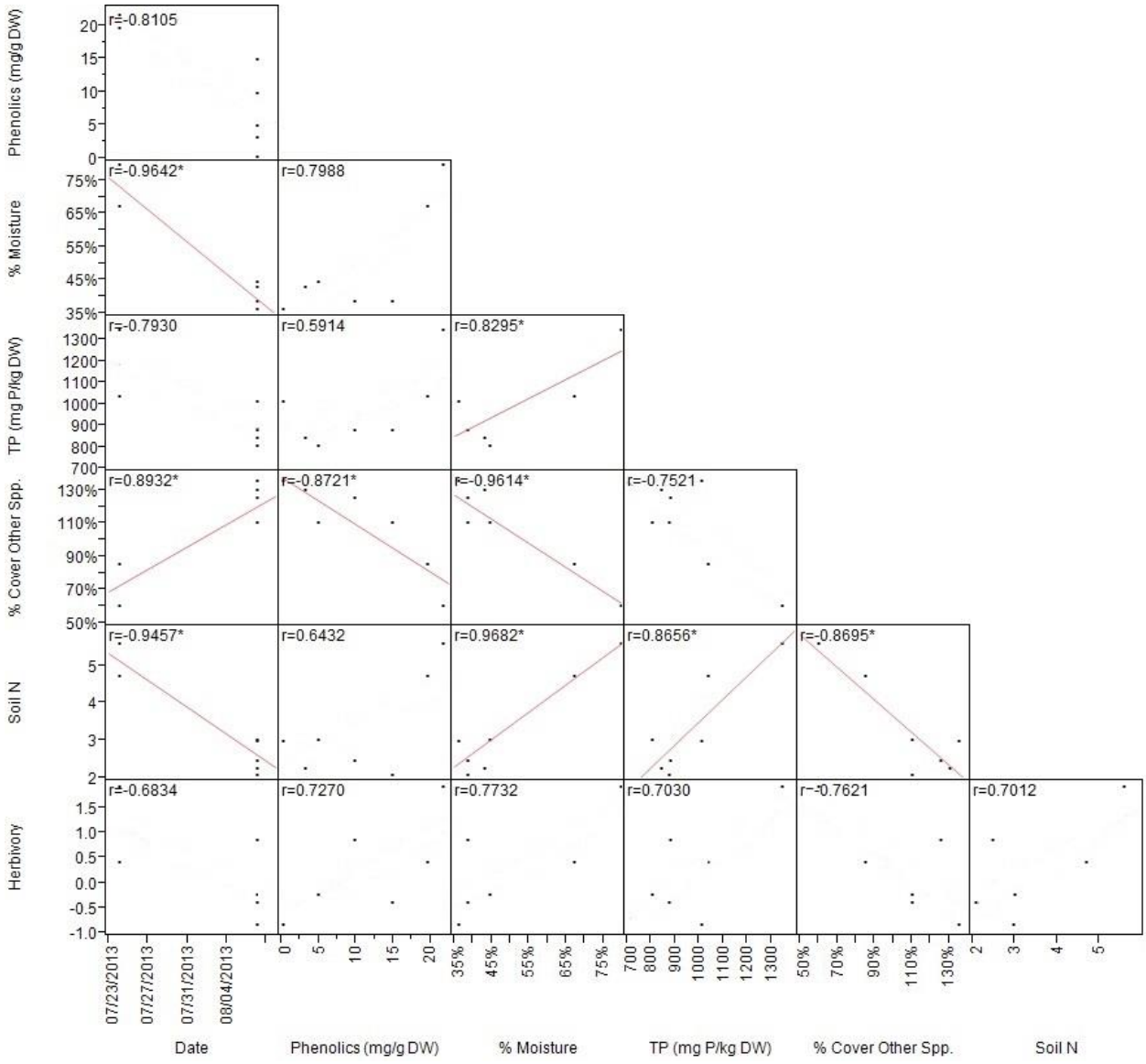


Figure 3. Linear regression matrix showing relationships between variables to be used for multiple regression analysis for *S. latifolia*. Pearson correlation coefficients are shown in the upper left corners of the graphs, those with asterisks indicate significance ($p < 0.05$). Linear regression lines are shown for correlations that were statistically significant.

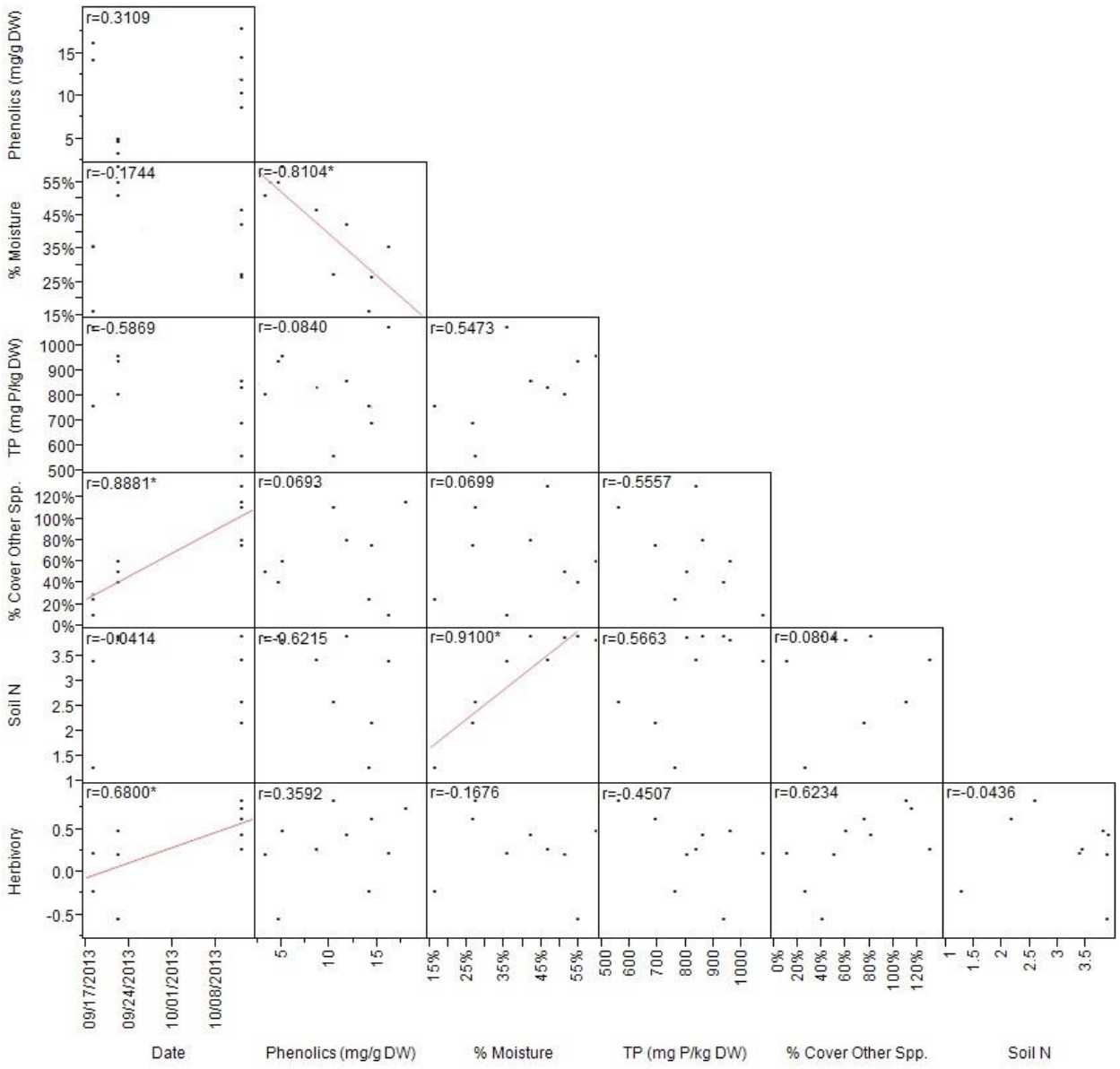


Figure 4. Linear regression matrix showing relationships between variables to be used for multiple regression analysis for *T. latifolia*. Pearson correlation coefficients are shown in the upper left corners of the graphs, those with asterisks indicate significance ($p < 0.05$). Linear regression lines are shown for correlations that were statistically significant.

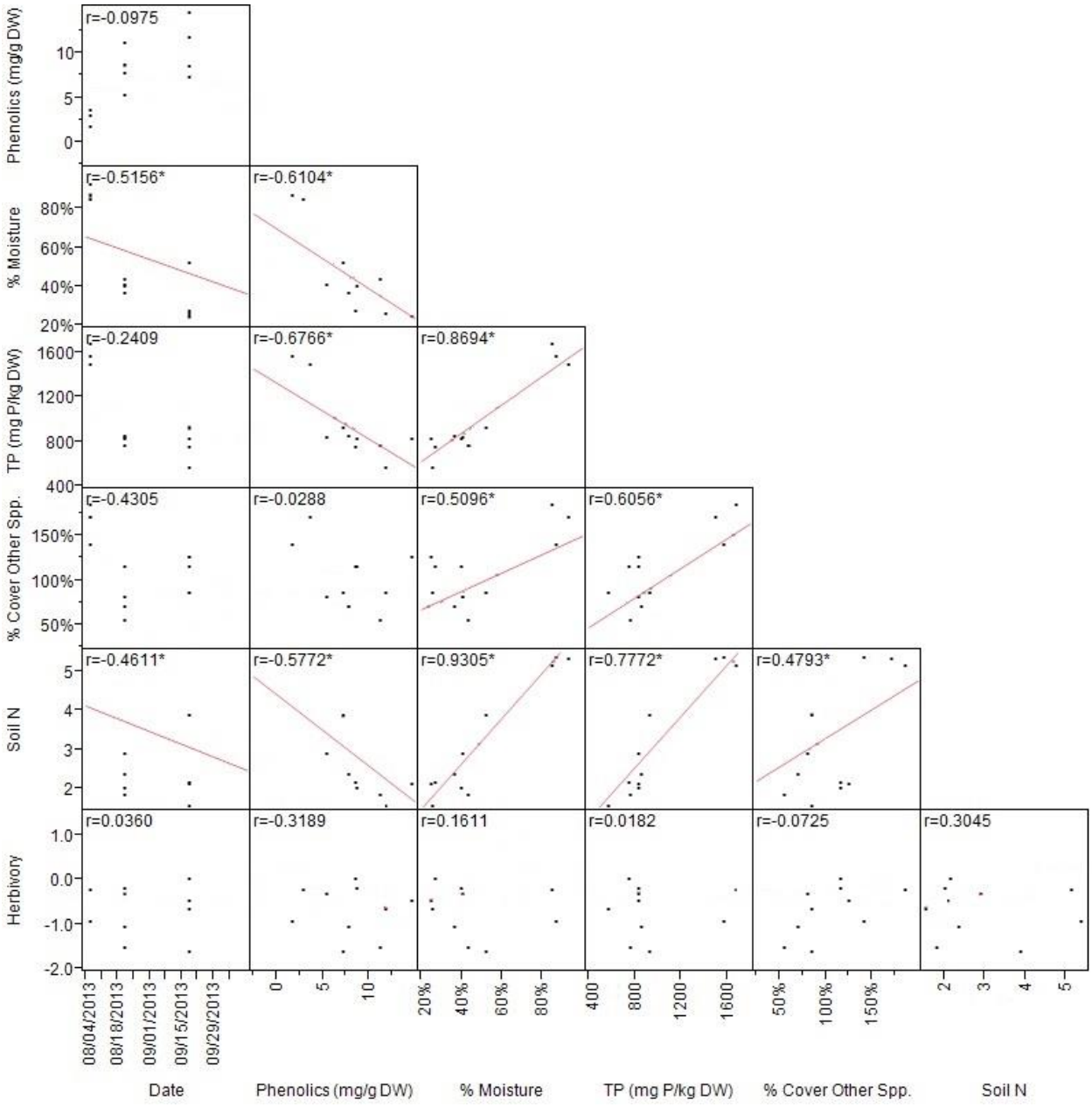


Figure 5. Linear regression matrix showing relationships between variables to be used for multiple regression analysis for *T. angustifolia*. Pearson correlation coefficients are shown in the upper left corners of the graphs, those with asterisks indicate significance ($p < 0.05$). Linear regression lines are shown for correlations that were statistically significant.

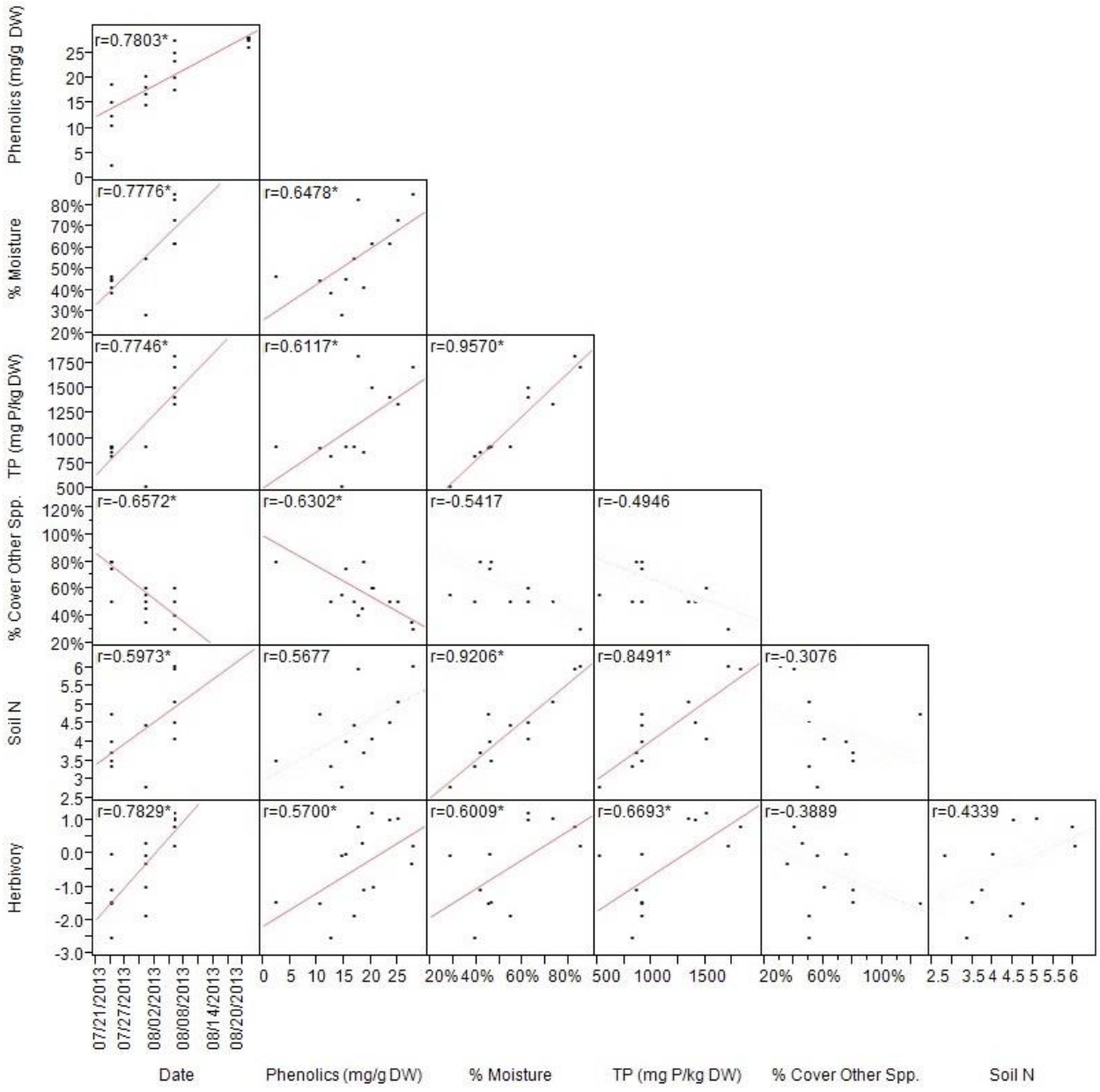


Figure 6. Linear regression matrix showing relationships between variables to be used for multiple regression analysis for *L. salicaria*. Pearson correlation coefficients are shown in the upper left corners of the graphs, those with asterisks indicate significance ($p < 0.05$). Linear regression lines are shown for correlations that were statistically significant.

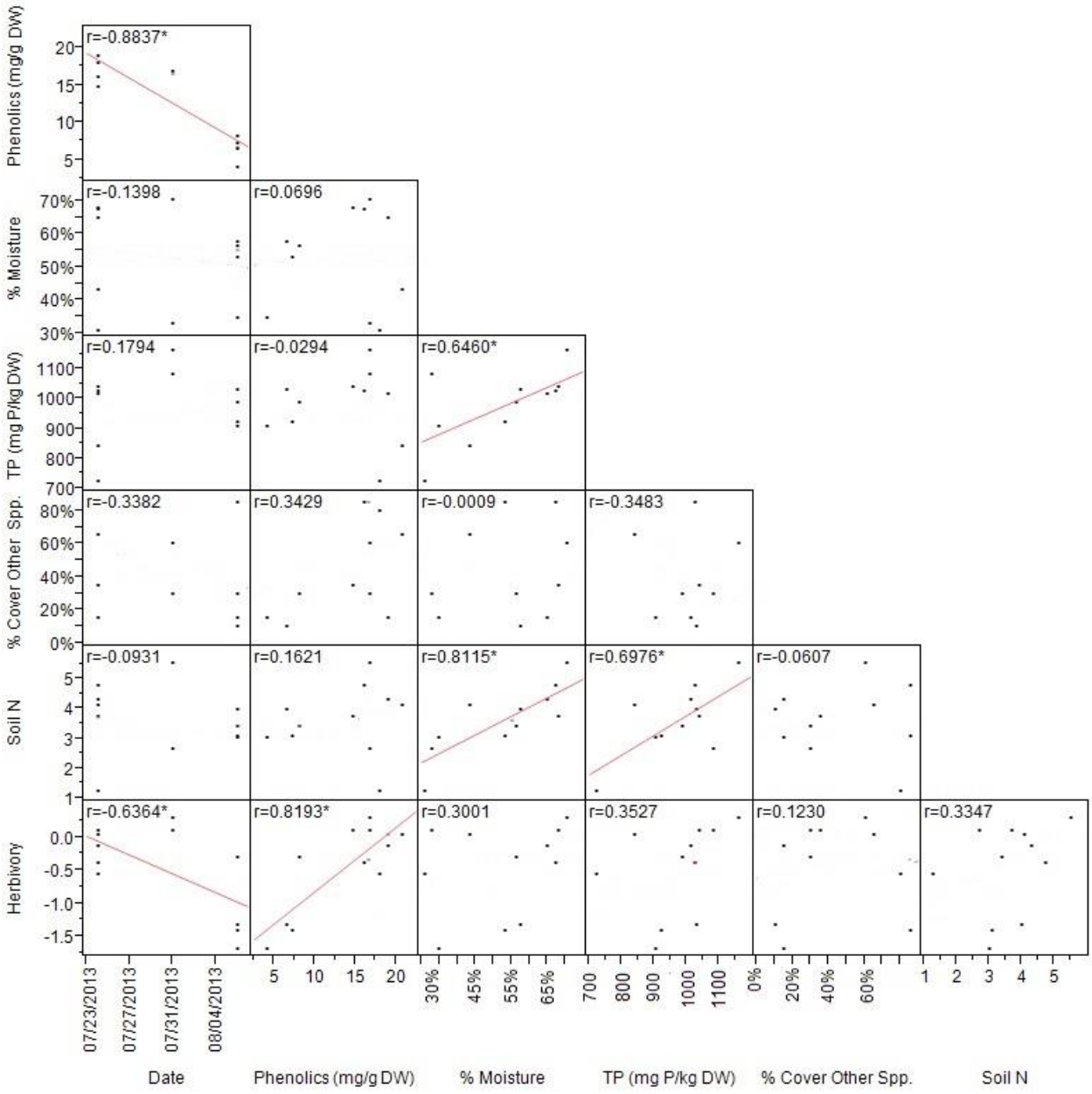


Figure 7. Linear regression matrix showing relationships between variables to be used for multiple regression analysis for *P. arundinacea*. Pearson correlation coefficients are shown in the upper left corners of the graphs, those with asterisks indicate significance ($p < 0.05$). Linear regression lines are shown for correlations that were statistically significant.

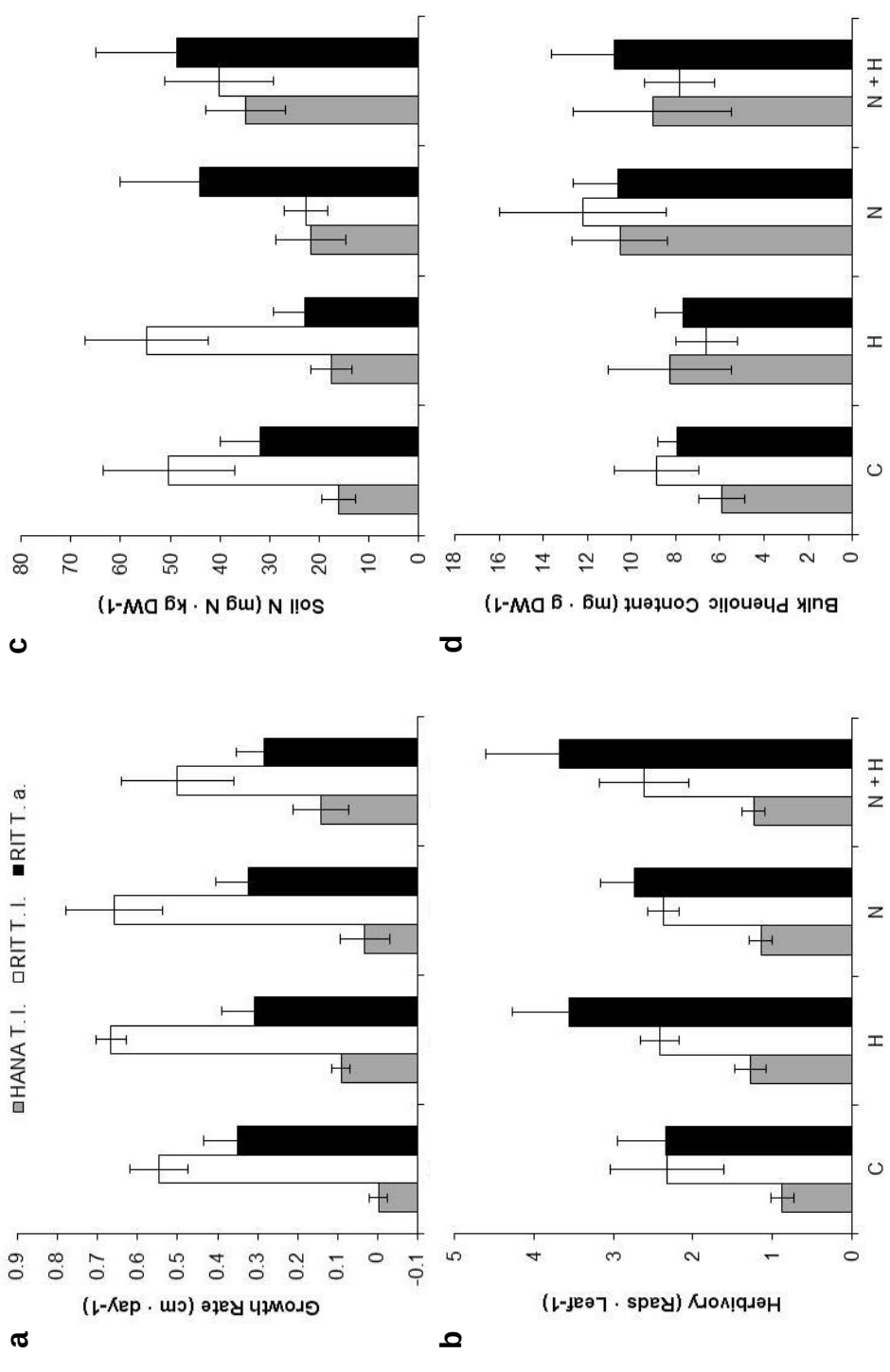


Figure 8. Graphs showing (a) growth rates, (b) herbivory (radulations), (c) inorganic soil nitrogen, and (d) phenolic content among treatments for *T. latifolia* at HANA and RIT, and *T. angustifolia* at RIT. Error bars are ± SEM.

Table 4. Results of two-way ANOVAs examining the factors of growth, herbivory, phenolic content, soil inorganic nitrogen, and total phosphorus at (a) RIT for *T. latifolia* and *T. angustifolia*, and (b) for *T. latifolia* between RIT and HANA. *P*-values in boldface indicate significance.

(a) RIT *T. latifolia* and *T. angustifolia*

Factor	DF	F	*p*
Growth Rate			
Treatment	3	0.48	0.70
Species	1	18.04	**0.0002**
Treatment × Species	3	0.41	0.75
Herbivory			
Treatment	3	0.62	0.61
Species	1	0.51	0.48
Treatment × Species	3	0.09	0.96
Phenolic Content			
Treatment	3	1.00	0.41
Species	1	0.04	0.84
Treatment × Species	3	0.12	0.95
Soil N			
Treatment	3	0.23	0.88
Species	1	1.82	0.19
Treatment × Species	3	1.01	0.40
Soil P			
Treatment	3	0.94	0.43
Species	1	0.01	0.93
Treatment × Species	3	1.54	0.22

(b) HANA and RIT *T. latifolia*

Factor	DF	F	*p*
Growth Rate			
Treatment	3	0.82	0.50
Site	1	103.21	< **0.0001**
Site × Treatment	3	1.19	0.33
Herbivory			
Treatment	3	1.11	0.36
Site	1	33.33	< **0.0001**
Site × Treatment	3	0.11	0.96
Phenolic Content			
Treatment	3	0.79	0.51
Site	1	0.47	0.50
Site × Treatment	3	0.16	0.92
Soil N			
Treatment	3	1.57	0.22
Site	1	13.16	**0.0012**
Site × Treatment	3	2.48	0.08
Soil P			
Treatment	3	2.35	0.10
Site	1	33.38	< **0.0001**
Site × Treatment	3	0.94	0.44

4. Discussion

To date, wetlands and other aquatic ecosystems are understudied and deserve further inquiry with regard to understanding phenolic chemistry and the environmental factors that affect phenolic concentrations (Gross and Bakker 2012, Iason *et al.* 2012, Jarchow and Cook 2009). The increased complexity in wetland ecosystems stems from the added element of water in comparison to terrestrial environments, adding additional environmental factors that could impact phenolic concentrations. In spite of environmental variability among wetlands, I was able to demonstrate predictable patterns in wetland plant phenolic content.

Wetland vegetation phenolic survey:

In this study, we investigated inter- and intraspecific species relationships between abiotic and biotic environmental factors and phenolic compound production, in addition to comparing noninvasive and invasive wetland plant species phenolics. We would like to caution, however, that quantitative comparison is difficult as results can vary with extraction methodology, choice of standard, solvents used, time allowed for extraction, temperature, and Folin reagent batch (Blair *et al.* 2005, Box 1983, Dai and Mumper 2010, Gallo *et al.* 2010, Li *et al.* 2010, Lou *et al.* 2012, Pan *et al.* 2003, Proestos and Komaitis 2008, Rispail *et al.* 2005, Torti *et al.* 1995, Trabelsi *et al.* 2010). As a result, the phenolic concentrations in this study are relative, and the trends I observed are qualitatively compared.

Overall, we found variable concentrations of phenolics that were site and species dependent. The variation among sites could have been a product of sampling both created and natural wetlands, as created wetlands generally have different soil characteristics, hydrology, plant communities, and tend to have increased abundance of invasive species compared to natural wetlands (Campbell *et al.* 2002, National Research Council Staff 1995). As a result of having different plant communities, the variability in phenolics can also be due to the characteristics and growth habits of the plants present. Boutin and Keddy (1993) address the different types of wetland plants and explain in detail that depending on the genetic traits and adaptability of the plant species, within a diverse community, plants can have different growth rates, delayed flowering,

different growth forms (tall and narrow or short and wide crown area), variable reproductive strategies (seed dispersal, clonal growth, vegetative spread), and also vary in their tolerance to disturbances. All of these different characteristics could cause phenolics to be influenced in different ways when the plants are responding to variable environmental conditions. The PCM also supports the contribution of diverse plant traits to variable phenolic concentrations, as the basis of the model is that production of phenolic compounds as opposed to proteins is dependent upon the plant's individual growth requirements, genetic characteristics, and environmental factors (Jones and Hartley 1999).

At two of the four sampling sites, the phenolic content of invasive species was higher than that of noninvasives, supporting the findings of Kim and Lee (2011) and Wolf *et al.* (2011). The concentration ranges and variance for invasive species was greater than noninvasive species, similar to the findings of Kim and Lee (2011), which could be indicative of increased phenotypic plasticity in response to environmental conditions. In addition to having more variable phenolic content, the invasive species' multiple regression models indicated that species responded differently to the environmental parameters measured. Again, this can be related to the PCM, the different genetic characteristics, plant growth requirements, and versatility of different invasive plant species could result in different factors influencing phenolic compound production (Jones and Hartley 1999).

The lack of a suitable model to predict total phenolic content in noninvasive species suggests that either the phenolic concentrations did not have a pronounced response to environmental conditions, or are affected by parameters not examined here such as soil microbiota, pH, light availability, or phenology (Boege 2005, Cronin and Lodge 2003, Gross and Bakker 2012, Li *et al*. 2010, Smolders *et al*. 2000). The presence of other species was most important in predicting the total phenolic content for *S. latifolia*. It is possible that the negative influence of other species on phenolic content could be unrelated, and happened to be correlated by chance. The PCM explains that phenolic allocation varies based on phenology; concentrations are typically high when the leaves are either very young, or when they are old. Otherwise, the phenolic concentrations should be lowest in the intermediate growth stage (Jones and Hartley 1999). *S. latifolia* leaves may have been in different growth stages when they were sampled. Leaves

sampled earlier in the season (RIT, mid-July) when other plants were also younger and less developed (lower % cover) had higher phenolic content than those sampled later (HANA, early August), when other plants were more mature as well (increased % cover); in this case, it would appear as though the percent cover of other species was negatively correlated with total phenolic content.

The best multiple regression model that was generated for all invasive species shows that there is not consistent support for the EICA or NWH among species. Herbivory and soil nitrogen were important factors for this model, having a positive correlation with phenolic content, contradicting the EICA hypothesis (Blossey and Nötzold 1995) and the concept of a cost-benefit trade off (Coley *et al.*1985,Grime 1977). In addition to herbivory and soil nitrogen, the date samples were collected and soil moisture also were important parameters in predicting phenolic content in the all invasive species model. Both date and soil moisture were negatively correlated with phenolic content; the inverse relationship between phenolics and date could be a result of the phenolic concentrations decreasing with maturation of leaves, as the PCM would suggest. The decrease in phenolics with increasing soil moisture could potentially be related to changes in water salinity as more moisture accumulates and dilutes the effects; some studies have shown evidence that salinity could be an important factor when investigating plant growth responses, and could then reasonably be extrapolated to phenolic compound production (Ervin and Wetzel 2003, Jordan *et al.* 1990).

Examining the invasive species models individually, however, shows evidence to support different invasion hypotheses. In the case of *T. angustifolia*, important predictors of phenolic content were soil phosphorus, other species present, and herbivory. Although it is difficult to assess causality, there is evidence to suggest that when growing in nutrient-poor conditions, resources were allocated towards producing phenolics; as phenolic concentrations increased, herbivore damage decreased. These results support the EICA hypothesis and the concept of a cost-benefit trade off (Blossey and Nötzold 1995, Boege 2005, Coley et al. 1985, Elger and Lemoine 2005, Grime 1977). The model predicting the phenolic content of *T. latifolia*, which is native in origin but can become invasive following a disturbance, was negatively influenced by

soil moisture and reflected a positive correlation with soil phosphorus and the date samples were collected – unlike the *T. angustifolia* model.

This distinctive difference between the native-invasive model (*T. latifolia*) and the exotic-invasive model (*T. angustifolia*) could potentially be explained by examining the differences between *T. latifolia* and *T. angustifolia* in the context of the PCM (Jones and Hartley 1999). *T. latifolia* inherently has different genetic and developmental traits than *T. angustifolia*. First, these two *Typha* species will be genetically different because they originate from two different continents. *T. latifolia* has coevolved with the environment in which it resides, *T. angustifolia* has not and will need to adapt quickly and efficiently in order to succeed, and as a result, exhibits increased phenotypic plasticity in comparison to *T. latifolia* in some respects. For example, *T. angustifolia* flowers earlier, can tolerate deeper water depths and higher saline/alkaline conditions, and also devotes more resources towards reproduction in comparison to *T. latifolia* (Grace and Harrison 1986). The PCM would predict that *T. angustifolia*'s increased plasticity could relate to a differential response in phenolic compound production to environmental factors compared to *T. latifolia* (Jones and Hartley 1999).

P. arundinacea appears to be utilizing a different strategy with regard to phenolic production; phenolic content was positively correlated with herbivory, unlike what the cost-benefit trade off posits - that herbivory decreases with increasing phenolics (Coley *et al.* 1985, Grime 1977). Instead, this trend may be indicative of an instance where production of phenolic compounds is induced following herbivore damage (Cipollini *et al.* 2005, Cronin and Hay 1996, Dicke and Baldwin 2010, Thelen *et al*. 2005). *P. arundinacea* has historically been reintroduced on several occasions for use as a forage crop and potentially was bred to have decreased anti-herbivore defenses; perhaps over time, as its invasiveness increased, *P. arundinacea* evolved inducible chemical defenses. In addition to herbivory, the date the samples were collected was an important predictor of phenolic content, suggesting that foliar concentrations vary seasonally.

Similar to *P. arundinacea*, according to the best model generated for *L. salicaria*, phenolic content was most strongly influenced by the date that the samples were collected. It is possible that no other factors appeared to significantly influence the model predicting the phenolic

content because *L. salicaria* is such a versatile, tolerant species. *L. salicaria* is partially tolerant to shade, grows in a variety of soils, and is capable of directly responding to changing ecological conditions (Mal *et al.*1992). *L. salicaria* also displays phenotypic plasticity with different growth stages; seedlings germinate quickly and exhibit high growth rates, as *L. salicaria* matures it then begins to spread vegetatively and produces copious amounts of seeds – a single mature plant is capable of potentially releasing upwards of 2,700,000 seeds (Mal *et al.* 1992). One factor that we would have predicted to be an important predictor of phenolic content for *L. salicaria* was herbivory. The EICA was originally hypothesized using *L. salicaria* and showed that increased herbivory affected *L. salicaria* growth, and if the assumptions of the EICA are correct, then resources should instead be allocated towards herbivore defense. We observed evidence of herbivory to varying degrees on all of the *L. salicaria* plants that we sampled, so it was interesting that the total phenolic content was unaffected by the severity of herbivory.

Field experiment: Herbivory, nutrients, and phenolic content

The results of the field experiment focusing on *Typha* spp. showed that neither species responded physically or chemically to nitrogen fertilization or herbivory treatments. It is possible that differences in growth were not evident with nitrogen addition because the field experiment was started later than anticipated and critical, beginning growth was missed. Based on these results, it is also possible that herbivory does not affect growth of *Typha* spp., or that herbivore density was not high enough to elicit a pronounced response in growth and phenolic content. In addition, the responses – or lack thereof – of *T. latifolia* and *T. angustifolia* support the trends in the predictive models generated from the survey data, neither the *T. latifolia* nor *T. angustifolia* phenolic concentrations were significantly influenced by soil nitrogen.

When comparing *T. latifolia* between sites, there were significant differences in environmental factors that were found to be important predictors of phenolic content. Despite the differences between sites, the phenolic content remained unchanged. A potential explanation for why the phenolic content was similar for *T. latifolia* between RIT and HANA may be a matter of threshold. Based on the correlation coefficients for TP and % Moisture in the *T. latifolia* predictive model, the slight increase in phenolic content could have been a result of RIT having

much higher soil phosphrous than HANA even though RIT's soil moisture was slightly greater. Since soil phosphorus had a much smaller coefficient – and therefore had a lesser effect on the model than soil moisture, and soil moisture was only slightly greater at RIT, the phenolic concentration was minimally affected. Perhaps in order to observe a more pronounced effect, greater differences in environmental conditions, particularly moisture, would be required to significantly decrease the phenolic content. This is further illustrated by comparing the ranges in soil moisture for *T. latifolia* samples from the survey to samples from the field experiment; the range in moisture for the survey (used to generate the model) was 16-60%, whereas it was 28-56% for the field experiment.

However, it is important to note that the overall concentration of phenolic compounds may be just as significant as the chemical composition of the total phenolic content. Jarchow and Cook (2009), when examining root phenolics of *Typha*, found that *T. angustifolia* produced different, not more, phenolic compounds than *T. latifolia.* In support of the NWH, studies have shown that some invasives produce unique chemicals that are not found in native plant species; overall total phenolic content may appear similar among species, but the chemical composition may be entirely different. Examining specific compounds present may provide additional insight with regard to species invasion and allelopathic interference, as certain chemicals may behave differently and have variable effects. Some chemicals may severely inhibit microbial activity and processes that noninvasive plants depend on, resulting in inhibition of plant growth, decreased fecundity, or mortality in extreme cases (Callaway *et al.* 2008, Vivanco *et al.* 2004).

5. Conclusions

This study further explored the natural variation of phenolic concentrations and examined several environmental factors to help understand phenolic compound production in noninvasive and invasive wetland plant species. However, the drawback to solely examining the total phenolic content for different species is that a very important aspect of secondary chemistry is omitted. The specific chemical make-up of the secondary metabolites can be equally or more important than the total concentrations with regard to understanding mechanisms of invasion (Coley 1985, Jarchow and Cook 2009, Wolf *et al.* 2011). While exploring the chemical make-up for each species was outside the realm of this study, this warrants further investigation to determine if invasive wetland plant species have unique chemicals that are not present in noninvasive species (Cappuccino and Arnason 2006, Jarchow and Cook 2009).

The results of this study demonstrate that the response of total foliar phenolic content to environmental factors varies among wetland plant species, and that invasive plants may have more plastic phenolic production in response to environmental cues. The heterogeneity among important factors in the predictive models for the individual species, both abiotic and biotic, demonstrates the complexity of studying biochemical interactions in an ecological context and the difficulty in establishing a single paradigm to describe the production of secondary metabolites in plants. There is also uncertainty with regard to how phenolic compounds persist in wet environments and how they are affected by different biochemical interactions in aquatic ecosystems (Ervin and Wetzel 2003, Gross and Bakker 2012, Inderjit 2001). Generalizations about how phenolic compounds are affected by environmental factors and how they impact invasion success are still difficult to formulate. However, based on the results of this study there is the possibility that invasive species producing variable, environmentally responsive phenolic compounds enhance their invasion potential and success.

Literature Cited

Ainsworth, E. A., & Gillespie, K. M. (2007). Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. *Nature protocols*, *2*(4), 875–7.

Angeloni, N. L., Jankowski, K. J., Tuchman, N. C., & Kelly, J. J. (2006). Effects of an invasive cattail species (*Typha* × *glauca*) on sediment nitrogen and microbial community composition in a freshwater wetland. *FEMS Microbiology Letters*, *263*(1), 86–92.

Apfelbaum, S. I. (n.d.). Cattail (*Typha* spp .) management. *Applied Ecological Services*, 1–6.

Blair, A. C., Hanson, B. D., Brunk, G. R., Marrs, R. A., Westra, P., Nissen, S. J., & Hufbauer, R. A. (2005). New techniques and findings in the study of a candidate allelochemical implicated in invasion success. *Ecology Letters*, *8*(10), 1039–1047.

Blossey, B., Nötzold, R. (1995) Evolution of increased competitive ability in invasive nonindigenous plants: A hypothesis. *Journal of Ecology, 83*(5), 887-889.

Boege, K. (2005). Influence of plant ontogeny on compensation to leaf damage. *American Journal of Botany*, *92*(10), 1632–1640.

Bolser, R. C., Hay, M. E., Lindquist, N., Fenical, W., & Wilson, D. (1998). Chemical defenses of freshwater macrophytes against crayfish herbivory. *Journal of Chemical Ecology*, *24*(10), 1639–1658.

Boutin, C., & Keddy, P. A. (1993). A functional classification of wetland plants. *Journal of Vegetation Science, 4*, 591–600.

Box, J. D. (1983). Investigation of the Folin-Ciocalteau phenol reagent for the determination of polyphenolic substances in natural waters. *Water Research*, *17*(5), 511–525. doi:10.1016/0043-1354(83)90111-2

Callaway, R. M., & Aschehoug, E. T. (2000). Invasive plants versus their new and old neighbors: A mechanism for exotic invasion. *Science*, *290*(5491), 521–523.

Callaway, R. M., & Ridenour, W. M. (2004). Novel weapons: Invasive success and the evolution of increased competitive ability. *Frontiers in Ecology and the Environment*, *2*(8), 436–443.

Callaway, R. M., Cipollini, D., Barto, K., Thelen, G. C., Hallett, S. G., Prati, D., Stinson, K., Klironomos, J. (2008). Novel weapons : Invasive plant suppresses fungal mutualists in America but not in its native Europe. *Ecology*, *89*(4), 1043–1055.

Campbell, D. A., Cole, C. A., & Brooks, R. P. (2002). A comparison of created and natural wetlands in Pennsylvania , USA. *Wetlands Ecology and Management*, *10*, 41–49.

Cappuccino, N., & Arnason, J. T. (2006). Novel chemistry of invasive exotic plants. *Biology Letters*, *2*, 189–193.

Carey, M. P., Sanderson, B. L., Barnas, K. A., & Olden, J. D. (2012). Native invaders – challenges for science, management, policy, and society. *Frontiers in Ecology and the Environment*, *10*(7), 373–381. doi:10.1890/110060

Cipollini, D., Mbagwu, J., Barto, K., Hillstrom, C., & Enright, S. (2005). Expression of constitutive and inducible chemical defenses in native and invasive populations of Alliaria petiolata. *Journal of Chemical Ecology*, *31*(6), 1255–1267. doi:10.1007/s10886-005-5284-3

Coley, P. D., Bryant, J. P., & Chapin, F. S. (1985). Resource availability and plant antiherbivore defense. *Science*, *230*(4728), 895–899.

Cronin, G., & Hay, M. E. (1996). Induction of seaweed chemical defenses by amphipod grazing. *Ecology*, *77*(8), 2287–2301.

Cronin, G., & Lodge, D. M. (2003). Effects of light and nutrient availability on the growth, allocation, carbon/nitrogen balance, phenolic chemistry, and resistance to herbivory of two freshwater macrophytes. *Oecologia*, *137*(1), 32–41.

Dai, J., & Mumper, R. J. (2010). Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. *Molecules*, *15*(10), 7313–7352.

Dicke, M., & Baldwin, I. T. (2010). The evolutionary context for herbivore-induced plant volatiles: beyond the "cry for help". *Trends in Plant Science*, *15*(3), 167–175. doi:10.1016/j.tplants.2009.12.002

Domènech, J. A., Perez, E., Gallardo, M. T., & Martin, D. F. (1997). Identification of allelopathic chemicals in a pond of cattails. *Florida Scientist*, *60*(3), 202–203.

Duke, S. O., Dayan, F. E., Bajsa, J., Meepagala, K. M., Hufbauer, R. a, & Blair, A. C. (2009). The case against (-)-catechin involvement in allelopathy of *Centaurea stoebe* (spotted knapweed). *Plant Signaling & Behavior*, *4*(5), 422–4.

Elger, A., & Lemoine, D. (2005). Determinants of macrophyte palatability to the pond snail *Lymnaea stagnalis*. *Freshwater Biology*, *50*, 86–95.

Ervin, G. N., & Wetzel, R. G. (2003). An ecological perspective of allelochemical interference in land–water interface communities. *Plant and Soil*, *256*(1), 13–28. doi:10.1023/A:1026253128812

Feeny, P. (1976). Plant apparency and chemical defense. *Recent Advances in Phytochemistry*, *10*, 1–40.

Galatowitsch, S. M., Anderson, N. O., & Ascher, P. D. (1999). Invasiveness in wetland plants in temperate north america. *Wetlands*, *19*(4), 733–755.

Gallo, M., Ferracane, R., Graziani, G., Ritieni, A., & Fogliano, V. (2010). Microwave assisted extraction of phenolic compounds from four different spices. *Molecules (Basel, Switzerland)*, *15*(9), 6365–74. doi:10.3390/molecules15096365

Gibson, D. M., Krasnoff, S. B., Biazzo, J., & Milbrath, L. (2011). Phytotoxicity of antofine from invasive swallow-worts. *Journal of Chemical Ecology*, *37*, 871–879.

Grace, J. B., & Harrison, J. S. (1986). The biology of canadian weeds.73. *Typha latifolia*, *Typha angustifolia*, and *Typha* × *glauca*. *Canadian Journal of Plant Science*, *66*, 361–379.

Grime, J. P. (1977). Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *The American Naturalist*, *111*(982), 1169–1194.

Gross, E. M., & Bakker, E. S. (2012). The role of plant secondary metabolites in freshwater macrophyte-herbivore interactions: Limited or unexplored chemical defences? In G. R. Iason, M. Dicke, & S. E. Hartley (Eds.), *The ecology of plant secondary metabolites: From genes to global processes* (pp. 154-169). Cambridge, UK: Cambridge University Press.

Hierro, J. L., & Callaway, R. M. (2003). Allelopathy and exotic plant invasion. *Plant and Soil*, *256*(1), 29–39.

Houlahan, J. E., & Findlay, C. S. (2004). Effect of invasive plant species on temperate wetland plant diversity. *Conservation Biology*, *18*(4), 1132–1138.

Iason, G. R., Dicke M., Hartley S. E. (2012) The integrative roles of plant secondary metabolites in natural systems: A synthesis. In G. R. Iason, M. Dicke, & S. E. Hartley (Eds.) *The ecology of plant secondary metabolites: From genes to global processes* (pp. 1-9). Cambridge, UK: Cambridge University Press.

Inderjit. (2001). Soil-Environmental effects on allelochemical activity. *Agronomy Journal*, *93*, 79–84.

Inderjit, Callaway, R. M., & Vivanco, J. M. (2006). Can plant biochemistry contribute to understanding of invasion ecology? *Trends in Plant Science*, *11*(12), 574–580.

Inderjit, Pollock, J. L., Callaway, R. M., & Holben, W. (2008). Phytotoxic effects of (+/-)-catechin in vitro, in soil, and in the field. *PloS One*, *3*(7), e2536.

Jarchow, M. E., & Cook, B. J. (2009). Allelopathy as a mechanism for the invasion of *Typha angustifolia*. *Plant Ecology*, *204*(1), 113–124.

Jones, C. G., & Hartley, S. E. (1999). A protein competition model of phenolic allocation. *Oikos*, *86*(1), 27–44.

Jordan, T. E., Whigham, D. F., & Correll, D. L. (1990). Effects of nutrient and litter manipulations on the narrow-leaved cattail, *Typha angustifolia* L. *Aquatic Botany, 36*, 179–191.

Kempski K. (n.d.) [Total phosphorus extraction protocol]. Unpublished protocol.

Kim, Y. O., & Lee, E. J. (2011). Comparison of phenolic compounds and the effects of invasive and native species in East Asia: Support for the novel weapons hypothesis. *Ecological Research*, *26*(1), 87–94.

Knepel, K. (2012). Determination of nitrate in 2M KCl soil extracts by flow injection analysis. *Lachat Instruments QuikChem Method 12-107-04-1-B*.

Li, Z.-H., Wang, Q., Ruan, X., Pan, C.-D., & Jiang, D.-A. (2010). Phenolics and plant allelopathy. *Molecules*, *15*(12), 8933–8952.

Lind, E. M., & Parker, J. D. (2010). Novel weapons testing: are invasive plants more chemically defended than native plants? *PloS One, 5*(5), e10429. doi:10.1371/journal.pone.0010429

Lou, Z., Wang, H., Zhu, S., Chen, S., Zhang, M., & Wang, Z. (2012). Ionic liquids based simultaneous ultrasonic and microwave assisted extraction of phenolic compounds from burdock leaves. *Analytica Chimica Acta*, *716*, 28–33. doi:10.1016/j.aca.2011.03.012

Mack, R. N., Simberloff, D., Lonsdale, W. M., Evans, H., Clout, M., & Bazzaz, F. A. (2000). Biotic Invasions: Causes, epidemiology, global consequences, and control. *Ecological Applications*, *10*(3), 689–710.

Mal, T. K., Lovett-doust, J., Lovett-doustr, L., & Mulligan, G. A. (1992). The biology of Canadian weeds. 100. *Lythrum salicaria*. *Canadian Journal of Plant Science, 72*, 1305–1330.

Maynard, D. G., Kalra, Y. P., Crumbaugh, J. A. (2008). Nitrate and exchangeable ammonium nitrogen. In M. R. Carter & E. G. Gregorich (Eds.), *Soil sampling and methods of analysis* (2nd ed., pp. 71-80). CRC Press.

McNaughton, S. J. (1968). Autotoxic feedback in relation to germination and seedling growth in *Typha latifolia*. *Ecology*, *49*(2), 367–369.

Monaco, T. A., & Sheley, R. L. (Eds.). (2012). CABI Invasive Species: Vol. 2. Invasive plant ecology and management: Linking processes to practice (Vol. 2). CABI.

Murphy, J., & Riley, J. P. (1962). A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta, 27*, 31–36.

Pan, X., Niu, G., & Liu, H. (2003). Microwave-assisted extraction of tea polyphenols and tea caffeine from green tea leaves. *Chemical Engineering and Processing*, *42*(2), 129–133. doi:10.1016/S0255-2701(02)00037-5

Penko, J. M., & Pratt, D. C. (1987). Insect herbivory in Minnesota *Typha* stands. *Journal of Freshwater Ecology*, *4*(2), 235–244.

Perry, L. G., Thelen, G. C., Ridenour, W. M., Callaway, R. M., Paschke, M. W., & Vivanco, J. M. (2007). Concentrations of the allelochemical (+/-)-catechin in *Centaurea maculosa* soils. *Journal of Chemical Ecology*, *33*(12), 2337–2344.

Prindle, V., & Martin, D. F. (1996). Allelopathic properties of cattails, *Typha domingensis*, in Hillsborough County, Florida. *Florida Scientist*, *59*, 155–162.

Proestos, C., & Komaitis, M. (2008). Application of microwave-assisted extraction to the fast extraction of plant phenolic compounds. *LWT - Food Science and Technology*, *41*(4), 652–659. doi:10.1016/j.lwt.2007.04.013

Rispail, N., Morris, P., & Webb, K. J. (2005). Phenolic Compounds: Extraction and Analysis. In *Lotus Japonicus Handbook* (pp. 349–355).

Rice Creek Field Station: Mission and History (n.d.) In *SUNY Oswego*. Retrieved May 20, 2014, from http://www.oswego.edu/academics/opportunities/rice_creek_field_station/about/mission_and_history.html

Siemens, D. H., Garner, S. H., Mitchell-olds, T., & Callaway, R. M. (2002). Cost of defense in the context of plant competition: *Brassica rapa* may grow and defend. *Ecology*, *83*(2), 505–517.

Smolders, A. J. P., Vergeer, L. H. T., Van der Velde, G., & Roelofs, J. G. M. (2000). Phenolic contents of submerged, emergent, and floating leaves of aquatic and semi-aquatic macrophyte species - why do they differ. *Oikos*, *91*(2), 307–310.

Thelen, G. C., Vivanco, J. M., Newingham, B., Good, W., Bais, H. P., Landres, P., Caesar, A., Callaway, R. M. (2005). Insect herbivory stimulates allelopathic exudation by an invasive plant and the suppression of natives. *Ecology Letters*, *8*(2), 209–217.

Topp, G. C., Parkin, G.W., & Ferrè, T. P. A. (2008). Soil water content. In M. R. Carter & E. G. Gregorich (Eds.), *Soil sampling and methods of analysis* (2nd ed., pp. 939-940). CRC Press.

Torti, S. D., Dearing, M. D., & Kursar, T. A. (1995). Extraction of phenolic compounds from fresh leaves: A comparison of methods. *Journal of Chemical Ecology*, *21*(2), 117–125.

Trabelsi, N., Megdiche, W., Ksouri, R., Falleh, H., Oueslati, S., Soumaya, B., Hajlaoui, H., Abdelly, C. (2010). Solvent effects on phenolic contents and biological activities of the halophyte Limoniastrum monopetalum leaves. *LWT - Food Science and Technology*, *43*(4), 632–639. doi:10.1016/j.lwt.2009.11.003

Tuchman, N. C., Larkin, D. J., Geddes, P., Wildova, R., Jankowski, K. J., & Goldberg, D. E. (2009). Patterns of environmental change associated with *Typha* × *glauca* invasion in a great lakes coastal wetland. *Wetlands*, *29*(3), 964–975.

Tyler, A. C., Mastronicola, T. A., & McGlathery, K. J. (2003). Nitrogen fixation and nitrogen limitation of primary production along a natural marsh chronosequence. *Oecologia*, *136*, 431–438. doi:10.1007/s00442-003-1277-5

Tyler, A. C., Lambrinos, J. G., & Grosholz, E. D. (2007). Nitrogen inputs promote the spread of an invasive marsh grass. *Ecological Applications*, *17*(7), 1886–1898.

Vivanco, J. M., Bais, H. P., Stermitz, F. R., Thelen, G. C., & Callaway, R. M. (2004). Biogeographical variation in community response to root allelochemistry: novel weapons and exotic invasion. *Ecology Letters*, *7*(4), 285–292. doi:10.1111/j.1461-0248.2004.00576.x

Wolf, V. C., Berger, U., Gassmann, A., & Müller, C. (2011). High chemical diversity of a plant species is accompanied by increased chemical defence in invasive populations. *Biological Invasions*, *13*(9), 2091–2102. doi:10.1007/s10530-011-0028-5

Zedler, J. B., & Kercher, S. (2004). Causes and consequences of invasive plants in wetlands: Opportunities, opportunists, and outcomes. *Critical Reviews in Plant Sciences*, *23*(5), 431–452.

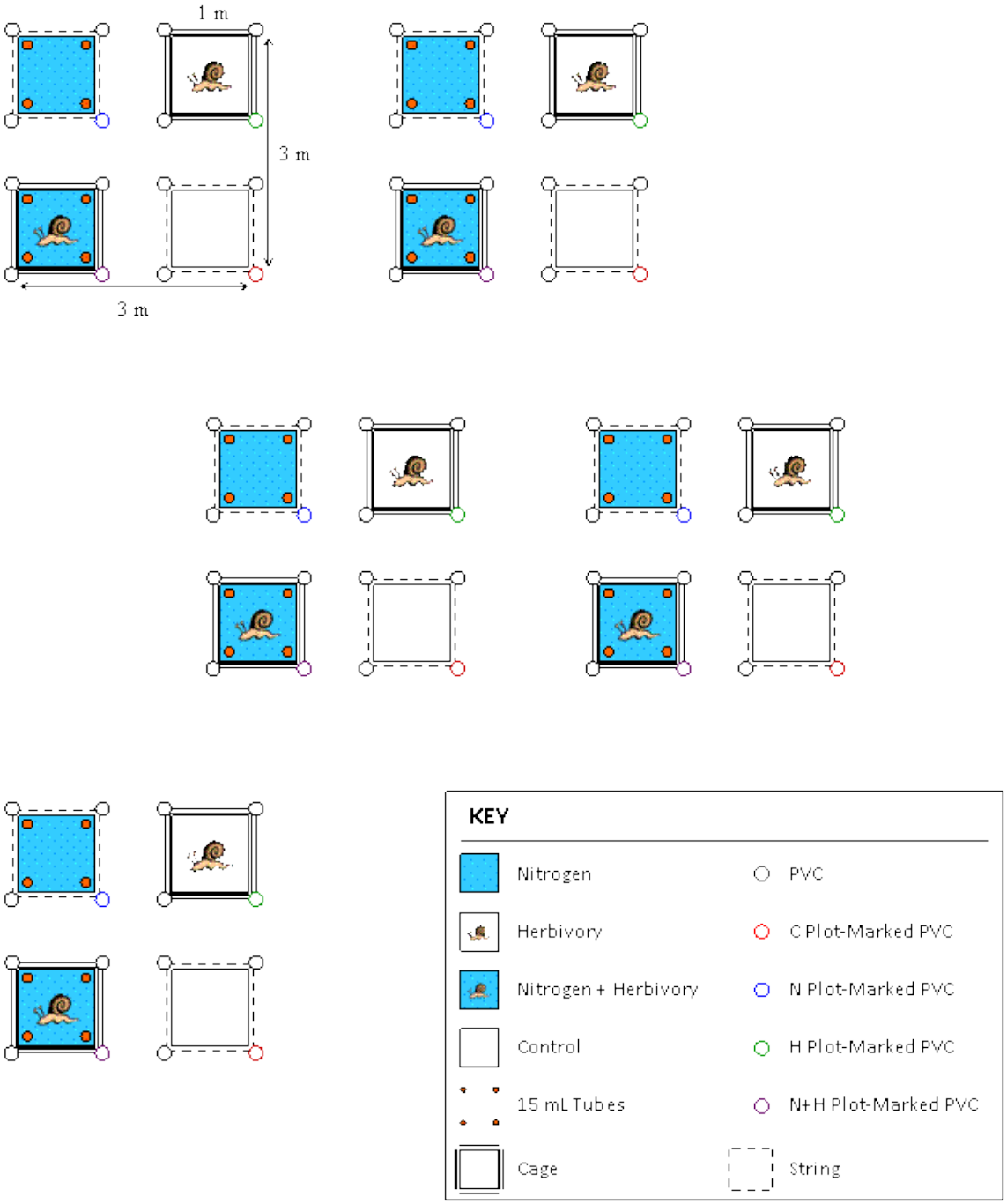
Zedler, J. B., & Kercher, S. (2005). Wetland resources: Status, trends, ecosystem services, and restorability. *Annual Review of Environment and Resources*, *30*(1), 39–74. doi:10.1146/annurev.energy.30.050504.144248

APPENDICIES

Appendix A. Coordinates of sampling sites and whether or not the wetland was created or natural. Site number corresponds to the labeled points on the map in Figure 1.

#	Site	County	Created/Natural	Latitude	Longitude
1	Camp Rd.	Erie	Natural	42 ° 44' 20.31 " N	78 ° 50' 39.89 " W
2	Langner Rd.	Erie	Natural	42 ° 49' 34.15 " N	78 ° 46' 50.77 " W
3	E. River & Bailey	Monroe	Natural	43 ° 4' 18.55 " N	77 ° 41' 18.21 " W
4	RIT	Monroe	Created	43 ° 4' 46.06 " N	77 ° 40' 0.73 " W
5	Tinker Nature Park	Monroe	Natural	43 ° 4' 3.03 " N	77 ° 34' 26.69 " W
6	Mendon Ponds Park	Monroe	Natural	43 ° 2' 0.23 " N	77 ° 33' 45.19 " W
7	Ellison Park	Monroe	Natural	43 ° 8' 41.20 " N	77 ° 31' 0.78 " W
8	HANA	Monroe	Created	43 ° 5' 34.86 " N	77 ° 23' 11.45 " W
9	RCFS	Oswego	Created	43 ° 25' 49.13 " N	76 ° 33' 4.41 " W
10	Verona	Oneida	Natural	43 ° 9' 15.27 " N	75 ° 31' 29.92 " W

Appendix B. Plot diagram showing the randomized block design used for the manipulative experiment.



Appendix C. Species sampled across sites listed by scientific name. X's indicate sites where a particular species was sampled.

Family	Scientific Name	Camp Rd	Ellison	E. River	HANA	Langner	Mendon	RCFS	RIT	Tinker	Verona
Noninvasive Species											
Alismataceae	*Alisma subcordatum*				X				X		
	Sagittaria latifolia				X				X		
Araceae	*Peltandra virginica*							X			
Asteraceae	*Eutrochium maculatum*							X			
Cyperaceae	*Carex lupulina*	X						X			
	Carex vulpinoidea	X							X		
	Cyperus erythrorhizos	X			X						
	Elocharis congesta	X									
	Schoenoplectus tabernaemontani				X			X			
	Scirpus atrovirens								X		
	Scirpus cyperinus								X		
Juncaceae	*Juncus effusus*	X			X				X		
Pontenderiaceae	*Pontederia cordata*				X						
Scrophulariaceae	*Mimulus ringens*								X		
Verbenaceae	*Verbena hastata*	X									
Invasive Species											
Lythraceae	*Lythrum salicaria*	X			X			X	X		
Poaceae	*Phalaris arundinacea*	X			X			X	X		
	Phragmites australis	X			X				X		
Typhaceae	*Typha angustifolia*	X		X	X	X	X		X	X	X
	Typha latifolia		X		X	X			X	X	
	Typha x glauca							X			