

RECOVERY OF STRUCTURE AND FUNCTION OF THE VEGETATION COMMUNITY
AFTER A DISTURBANCE AS AFFECTED BY INITIAL PLANTING RICHNESS IN
CREATED WETLANDS

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Abstract

RECOVERY OF STRUCTURE AND FUNCTION OF THE VEGETATION COMMUNITY AFTER A DISTURBANCE AS AFFECTED BY INITIAL PLANTING RICHNESS IN CREATED WETLANDS

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Creating and restoring wetlands is commonplace because many wetlands have been threatened or destroyed by urban expansion. Structural and functional aspects of created wetland ecosystems, however, remain poorly understood. One of the challenges facing created wetlands is ensuring that the ecosystem is able to recover after a disturbance, such as a mass herbivory eat-out or a large storm event. In this study, we use a controlled environment to examine how original planting diversity affects the ability of a created plant community to recover structurally (vegetation morphology and soil characteristics) and functionally (acquiring above ground biomass, potential denitrification and microbial respiration) after a disturbance. We used four macrophyte species, planted along a gradient of functional richness (FG 1 – FG 4) to assess community resilience based on morphometric measurements and biomass estimates. The vegetative results were compared to the two growing seasons prior to the disturbance. Soil biogeochemical

characteristics were examined both during the growing season and the non-growing season. After the disturbance (i.e., aboveground harvesting and extensive soil coring), total mesocosm percent vegetative cover increased as planting richness increased, indicating that higher richness positively impacts the recovery of the overall plant community. The facultative annual and the reed both produced fewer and shorter stems than prior to the disturbance. The sedge had a shorter maximum canopy height. The facultative annual and the sedge were unsuccessful in monoculture with 50% failure of monocultures for both species. All four species produced less biomass one year after the disturbance as compared to the two years prior. The obligate annual was dominant where it was planted, contributing positively to total mesocosm cover and aboveground biomass, and providing support for the growth of other species. No seasonal differences were observed with any of the soil characteristics measured, although unplanted mesocosms (FG 0) were consistently different than those of higher planting richness, with lower bulk density, soil moisture, and C:N. Potential denitrification increased with increasing planting richness. Microbial respiration was initially much higher in unplanted mesocosms, however over the 324-day soil incubation, all functional groups converged at a moderate respiration rate. Our results indicate the importance of having a species-specific planting regime when creating/restoring wetlands to ensure the development of functional resilience.

Introduction

Wetland vegetation is an important driver of ecosystem functioning through primary productivity, nutrient removal, and carbon cycling (Jordan et al. 2003; Moser et al. 2009; Koh et al. 2009; Dee & Ahn 2014). Plant diversity and richness play an important role in the development and stability of the wetland ecosystem functions (Grime 1998; Hooper et al. 2005; Bouchard et al. 2007). In addition to providing essential habitat for a wide range of species, a diverse vegetative community enhances the nutrient cycling (Moser et al. 2009; Levi et al. 2015), increases productivity and carbon storage potential (Cardinale et al. 2013; Williams and Ahn 2015; Means et al. 2016), and can inhibit the spread of invasive species (Byun et al. 2013; Ma'jekova et al. 2014; Beck et al. 2015). One of the challenges facing created or restored wetlands is ensuring that the ecosystem is able to recover after a disturbance, such as a mass herbivory eat-out or a large storm event (Wilson & Keddy 1986; Day et al. 2013; Feng et al. 2013; López-Mársico 2015). No studies could be identified that examine how the planting richness impacts the recovery of macrophyte community immediately after the disturbance.

The continued degradation of our environment through resource consumption and climate change is threatening many natural ecosystems, including wetlands, and is predicted to worsen over the coming decades (Day et al. 2013). Wetlands are protected

under section 404 of the Clean Water Act of 1972 (CWA 2002; USEPA and USACE 2012). Under this policy, the loss of any wetlands that are filled or removed for any reason must be compensated for through the restoration or creation of similar wetland area, striving for no-net-loss of structure or function. This often requires mitigation ratios well beyond 1:1 of restored area to destroyed area (USACE 2010; Brown and Lant 1999). Unfortunately, this has been largely ineffective with an estimated 50% of creation, restoration, and preservation sites failing to achieve the necessary functionality of those lost (Brown and Lant 1999; Dahl et al. 2011; Mitsch et al. 2012). By improving the policy by which we create and restore wetlands, we can preserve important ecosystem services (e.g., flood control, carbon storage, and the reduction of nitrates in our waterways).

To meet the US Army Corps of Engineers definition of a successful wetland, the soil must be inundated for a period of time sufficient to support hydrophytic vegetation (structure) and hydric soils (function) (Mitsch & Gosselink 2007). This requires frequent monitoring to ensure that all aspects of the wetland remain functioning for a minimum of five years (USACE 2010). Many considerations must go into the design of a created wetland including hydrology, geomorphology, land surface slope, soil quality, and vegetation. Successful development of the ecosystem is dependent upon the interactions among these factors. Wetland vegetation community structure can fluctuate over time depending on original planting, connectivity to surrounding waterways, invasive species, and disturbances from outside forces (Mitsch et al. 2012). Through the establishment of microtopography and subsequent increase in biodiversity (Moser et al. 2007; Lawrence

and Zedler 2011), plant communities can have lasting effects on the structure and function of a wetland including potential carbon storage and nutrient cycling (Grime 2001; Farrer and Goldberg 2009; Gutrich et al. 2009; Moser et al. 2009; Williams and Ahn 2015). Biodiversity loss significantly impacts ecosystem functioning (Cardinale et al. 2012; Isbell et al. 2013).

The complexity of macrophyte morphology enhances the uptake of nitrate from surrounding waterways (Levi et al. 2015). Richness of plant structural and functional traits contributes to the productivity (Tilman et al. 1997; Tilman et al. 2001; Hooper et al. 2005; Bouchard et al. 2007; Le Bagousse-Pinguet et al. 2012) and stability of vegetative communities (Loreau et al. 2002; Cardinale et al. 2013; Ma'jekova et al. 2014; Berendse et al. 2015) and to the overall resilience of the ecosystem (Carvalho et al. 2012). By increasing the morphological complexity, species richness, and diversity of plant traits in a system, more of the available niches can be occupied and exploited for increased macrophyte growth (Fox 2005; Lawrence and Zedler 2011). This also decreases the susceptibility of a community to invasive species such as *Typha spp.*, which can quickly overwhelm a wetland (Mitsch et al. 2012; Bernal and Mitsch 2013), causing a created wetland to fail. A planted community with a wide range of vegetative functional traits is more likely to successfully establish and maintain other wetland ecosystem services.

One of the most important ecosystem services provided by wetlands is their ability to remove nitrates from surrounding waterways, particularly in areas that receive excessive nutrients from fertilizers and agricultural activity. Nitrogen (N) can be a limiting nutrient in many ecosystems, however, human alterations to N cycling,

particularly in the widespread use of the Haber process to create fertilizers, have drastically increased inorganic nitrogen in many waterways (Davidson and Seitzinger 2006). This creates eutrophic environments in many coastal waterways, suppressing an ecologically and economically important ecosystem. Much of this excess N could be removed by the presence of wetlands, both coastal and inland. As with many soil properties, N cycling in young wetlands is slower than in more developed wetlands and natural wetlands (Wolf et al. 2011; Mitsch et al. 2012). Over decades of development, total N, total carbon (C), and soil moisture increase, and bulk density decreases, creating a more ideal environment for microbial functioning.

Nitrate in the system can be removed via plant uptake, denitrification, dissimilatory nitrate reduction (DNRA), or anaerobic ammonium oxidation (anammox). In wetland ecosystems, denitrification is the most prominent form of nitrate removal (Washbourne et al. 2011). During this process, denitrifying bacteria transform nitrate (NO_3^-) into nitrous oxide (N_2O) and nitrogen gas (N_2). The main driver of denitrification is available nitrogen in the soil (Groffman and Tiedje 1989; Morse et al. 2012). Denitrification enzyme activity (DEA) also requires an energy source (electron donor), which usually comes in the form of decomposing carbon-based matter, and therefore the process is not only limited by N, but also by available C (Paul 2007; Sutton-Grier et al. 2011). Created wetlands contain less biomass, soil C, and mineralizable C than their natural counterparts (Hossler and Bouchard 2010). The efflux of CO_2 from wetland soils depends on a suite of abiotic and biotic factors, including leaf litter and litter decomposition (Fang et al. 2015; Palta et al. 2012) and is the product of several

biogeochemical processes including microbial respiration, denitrification, and the reduction of nitrate, sulfate, and methane (Mitsch and Gosselink, 2007). CO₂ efflux can give insight to both the lability of carbon and the activity of the organisms as they cycle nutrients and can be used as a measure of whether the denitrification process is C or N limited (Robertson et al. 1999).

Denitrification is spatially and temporally heterogeneous (Davidson and Seitzinger 2006). Plants influence nitrogen cycling through ammonium and nitrate uptake and inputs from leaf litter. Higher rates of denitrification are often seen in the early spring before plant uptake of N is at its peak (Boyd 1978; Groffman and Tiedje 1991), and in the fall, where fallen leaf litter is more abundant and plant uptake of N has slowed (Hooker and Stark 2008; Morse et al. 2012). Individual plant species influence microbial functioning differently through various morphometric traits and nutrient removal rates (Eisenhauer et al., 2010). The rate of N cycling in wetlands can be heavily influenced by the root biomass (Moreau et al. 2015). Plant species evenness has also been shown to influence the soil ecosystem functioning (Palta et al. 2012). It has been found that plant diversity leads to higher N mineralization and higher denitrification rates (Zak et al. 2003) and may also lead to more consistent rates of denitrification over time (McGill et al. 2010). Planting diversity has been shown to influence denitrification potential and has implications for the availability of N in the system (Groffman and Tiedje 1989; DeMeester and Richter 2010; Schultz et al. 2011; Cantarel et al. 2015). However, in a nutrient-rich environment Mitsch et al. (2012), saw no relationship in the nitrate reduction as affected by plant diversity, which suggests that plant selection may be more

important in wetlands that are nitrogen limited. Plant community structure, litter accumulation, and the soil microbial community are each important factors in determining the potential removal of nitrates (Craft et al. 2003; Hooker and Stark 2008; Straahof et al. 2014; Fang et al. 2015). It is necessary to better understand the role that specific plant species play in NO_3^- removal and therefore enable the creation of wetlands to better address the problem of eutrophication in bays and estuaries.

Disturbances are common to every ecosystem and have been recognized for their part in ecosystem development for over a century (Cooper 1913). Natural disturbances come in many varieties (e.g., fire, herbivory, storm events, temperature changes, or drought) and can have a wide range of impacts on an ecosystem, from permanently altering the system or refreshing it for a new season of growth (Gunderson et al. 2010). While some disturbances, particularly man-made, can be devastating, others have been documented to have beneficial impacts for plant communities in certain wetlands. For example, increased sedimentation from hurricanes can stabilize coastal marshes against sea level rise and land subsidence (Baustian and Mendelssohn 2015). In addition, herbivory has been seen to increase native plant diversity and protect the community from an invasive grass species, creating a more stable ecosystem (Beck et al. 2015). The plant community can influence the impacts of disturbances. Certain plant species are more tolerant of stress and/or disturbances than others. Many species are capable of changing the way they allocate nutrients depending on the state of their environment (e.g., nutrient limitation). This can be observed in the plasticity of their morphology

(Grime 2001; Grasset et al. 2015). A plant community that is rich in plants of different life strategies should be more resistant to disturbance and quicker to recover.

In a created or restored wetland, it is important to understand the way in which the vegetation community responds to a disturbance so as to maintain wetland ecosystem services for many years. To understand the community response to disturbance, there are many assumptions to be made about the original state of the ecosystem, possible regime shifts, and the scale of the ecosystem (Gunderson et al. 2010). Ecosystem theory states that through succession and evolution, and despite disturbances, ecosystems move towards a condition of order and efficiency (Margalef 1968). The idea of ecosystem resilience has evolved with our understanding of ecosystem functioning and community response. Holling (1973), defined “resilience” as the parameter by which an ecosystem can absorb and accommodate unexpected events (e.g. disturbances), while maintaining existing relationships among community variables. Thus, a resilient community is one that is steadfastly resistant to change. Stability, on the other hand, is the ability of the ecosystem to return to equilibrium quickly and without fluctuation to the relationships existing prior to disturbance (Holling 1973). These rigid definitions make achieving resilience in created wetlands a challenge due to the changing nature of wetlands and their surroundings. As such, we use the extended definition of resilience from Walker et al. (2006), which states that a community can be considered resilient if the basic structure, function, and feedbacks are maintained in the face of disturbance. This allows for changes in species dominance and/or differentiation of niche fulfillment.

Likewise, it is necessary to understand how a disturbance to a created wetland could change the soil microbial functioning and subsequent nutrient cycling. The development of the microbial community and changes there in, either environmental or from an outside disturbance, can take anywhere from several weeks (Groffman and Tiedje 1991) to several years (Eisenhauer et al. 2010) to adapt to new environmental conditions. Disturbances can aerate wetland soils, increasing redox potential and reducing denitrification. If the soil were heavily oxidized during a disturbance, denitrification would be suppressed by the abundance of O₂, the preferential electron acceptor (Mitsch and Gosselink 2007). In addition, a disturbance in which much or all of the standing plant matter is removed greatly reduces the potentially available C necessary for DEA. A C-limited environment can lead to lower rates of denitrification because there is a lack of electron donor (Groffman and Tiedje 1991; Morse et al. 2012). Changes to the available C:N can impact the structure of the microbial community as well, either increasing or decreasing the presence of denitrifiers (Peralta et al. 2013; Nijburg et al. 1997).

Due to the multidisciplinary nature of wetland mitigation, the definition of resilience can be considered in a socio-ecological capacity as the ability of an ecosystem to maintain desired ecosystem services despite disturbances (Folke et al. 2002). For this study, we assumed that the original state, prior to the disturbance was stable and measure resilience based on whether or not structural and functional attributes important for ecological functions of wetlands (e.g., cover, and biomass accumulation) were restored after the disturbance. While there are many measurable components contributing to the resilience of ecosystems (Hodgson et al. 2015), quantifying resilience is difficult on a

limited spatial and temporal scale. Therefore, we can make assumptions on the disturbance threshold based on our observations of recovery, which can then be easily applied to the permitting process for creating and restoring wetlands (Standish et al. 2014). Using this information it is possible to develop practical active management plans that account for disturbances, such as weather events and herbivory, in created or restored wetlands.

This study took place using a set of 40 freshwater wetland mesocosms to investigate how planting diversity impacts the initial recovery of a wetland community after a disturbance. Here, I will expand on a two-year mesocosm study based on the functional diversity of wetland macrophytes. All mesocosms were planted in 2012 using four native freshwater emergent macrophytes, chosen based on their functional differences and their abundance in created wetlands in the Virginia Piedmont. At the end of the second year of growth all above ground biomass was harvested and the below ground biomass was cored for analysis on morphometric growth, biomass production, and nutrient cycling (Korol and Ahn 2015), drastically reducing autochthonous C inputs and causing a disturbance to the soil. The created disturbance mimics a mass herbivory event. This study will only examine the first year of recovery with the anticipated continuation of the project to assess the overall resilience of the mesocosm communities. Specific study questions to be addressed are as follows:

1. How does planting richness affect the overall vegetative community response one year after a severe disturbance?
2. How are individual species affected by the disturbance?

3. How does planting richness affect the soil characteristics and functioning one year after a severe disturbance?

Methods

Study setup

This study was conducted in the Ahn Wetland Mesocosm Compound that houses a set of 40 568 L Rubbermaid® tubs with a surface area of 1.15 m². The mesocosms in this study were built to mimic the large created wetlands in the Virginia Piedmont region (i.e., North Fork Wetlands Bank (created in 1999), Bull Run Mitigation Bank (created in 2002), and Loudon County Mitigation Bank (created in 2006)). Of the 40 mesocosms, six are unplanted and the remaining 34 are planted along a gradient of functional planting diversity (Boutin and Keddy 1993) using four common wetland species: *Eleocharis obtusa* (an obligate annual), *Juncus effusus* (an interstitial reed), *Mimulus ringens* (a facultative annual), and *Carex vulpinoidea* (an interstitial tussock). Functional group 1 (FG 1) consists of monocultures for each species, functional group 2 (FG 2) consists of two of the four species growing together, functional group 3 (FG 3) consists of three species, and functional group 4 (FG 4) consists of all four species. The tubs were originally vegetated in the spring of 2012 to begin a long-term study on the effects of plant functional diversity on the development of created wetland communities. At the end of the second (i.e., 2013) growing season, all above ground biomass was harvested and below ground biomass was heavily cored for a pair of companion studies (Korol and Ahn

2015; unpublished manuscript). This created a regime of disturbance to all wetland mesocosms. This study was conducted throughout the first growing season after the disturbance (i.e., 2014) to determine how planting richness impacts the ability of a planted community to recover after a disturbance.

Field Measurements

Bi-weekly morphometric measurements were taken between April 1 and November 18 of 2014 on all four species in the 34 planted mesocosms. Table 1 shows a complete list of morphological measurements, including cover analysis (percent vegetative cover and area (m²)) for all four species individually and for the total mesocosm cover. Maximum canopy height (CH) (cm) was determined for *C. vulpinoidea*, and stem count (SC) and average stem length (SL) (cm) were measured for *M. ringens* and *J. effusus*.

Table 1. Plant morphological characteristics measured for each species in the study. Measurements taken for each species.

Species	<i>J. effusus</i>	<i>M. ringens</i>	<i>C. vulpinoidea</i>	<i>E. obtusa</i>
%Cv	X	X	X	X
SL	X	X	-	-
CH	-	-	X	-
SC	X	X	-	-
AGB	X	X	X	X
RY	X	X	X	X
%RCv	X	X	X	X

%Cv (Standardized percent cover) is standardized to account for the number of originally planted individuals of each species; SL (Stem Length) measured in cm. CH (Canopy Height), measured in cm from the to maximum height of vegetation; SC (Stem Count), a count of all stems. AGB (Above Ground Biomass) is estimated based on species-specific morphometric measurements; RY (Relative Yield) based on biomass estimates comparing each species when grown in mixture to when grown in monoculture (Carvalho et al., 2012). %RCv (% Cover Restored) estimates the resilience of each species based on cover prior to the disturbance (based on Slocum and Mendelssohn, 2008).

Cover was determined using a grid comprised of 215 squares, each with an area of 51.4 cm². For each species, the total number of fully and partially occupied squares was counted. Using the counts of squares, percent cover and covered area were determined for each species and the total mesocosm, accounting for vegetative overlap of species. Cover for individual species was standardized over 1 m² (approximate surface area of each mesocosm) to account for differences in the original number of individuals planted in each mesocosm. This was accomplished dividing the percent of cover for each species by the total percent covered in the mesocosm. Using the complete set of morphometric measurements, an estimate for peak aboveground biomass (AGB) per species was determined using regression formulas adapted from Korol and Ahn (2015) (Table 2).

Table 2. Regression equations for AGB estimates based on morphological measurements (adapted from Korol and Ahn, 2015).

Species	Regression equation	R ²	p-value
Fac. Annual	$\text{Log}_{10}(\text{AGB}_M) = 0.007(\text{SL}) + 1.996(\text{Cv}) + 0.003(\text{SC}) + 1.167$	0.883	<0.001
Obl. Annual	$\text{Log}_{10}(\text{AGB}_E) = 0.480(\sqrt{\text{Cv}}) + 2.017$	0.603	<0.001
Sedge	$\sqrt{\text{AGB}_C} = 0.106(\text{CH}) + 6.651(\text{Cv}) - 7.353$	0.687	<0.001
Reed	$\text{Log}_{10}(\text{ABG}_J) = 0.012(\text{SL}) + 0.558(\text{Cv}) + 0.886$	0.571	<0.001

SC (# of stems); SL (stem length, cm); CH (maximum canopy height, cm), and Cv (cover, m²).

For a better understanding of species interactions and interspecific competition the estimated biomass of each species when grown in monoculture was compared to

those of the mixtures. This was accomplished using the following formula for Relative Yield (RY) (Carvalho et al. 2012):

$$RY_i = \frac{O_i}{(Y_i/x)}$$

in which O_i is the biomass of species (i) grown in mixture, Y_i is the biomass of species i when grown in monoculture, and x is the number of species in the mixture. For this study, Y_i was determined by averaging the two monocultures of each species. Because it is understood that a monoculture will produce more biomass than a species grown in a mixture, a $RY > 1$ indicates that species overyields when grown in mixture and that neighbors are beneficial for the productivity of that species. In comparing RY among species, this also gives insight as to which species are more dominant in a mixed environment.

We use percent cover data because this is often what is used to determine success of created wetlands (CWA 2002; USACE 2010). We estimated the percent recovery of the vegetation using data from the growing season after the disturbance for this study and the data collected from the previous two growing seasons prior to the disturbance (Korol & Ahn 2015). We accomplished this by adapting the equation for individual species percent recovery (%RCv) used by Slocum and Medelssohn (2008).

$$\%RCv = \frac{\%Cover_{2014}}{\%Cover_{2013}} \times 100$$

This gives us a numerical estimation of how successfully the mesocosms recovered. We also broke this down by species to determine which species were more successful than others in recovering after the disturbance.

Soil characteristics

Soil temperature was taken continuously during the growing season using iButtons (from June through October of 2014). Soils were analyzed for gravimetric soil moisture (GSM), total carbon (%C), total nitrogen (%N), and bulk density (BD) during the growing season and during the non-growing season of 2014 in order to observe the seasonal differences in the mesocosms. During both sampling seasons, three 20 mL soil cores from the top 10 cm were taken in three locations of each mesocosm for GSM, %C, and %N. BD was measured using small aluminum tins of known volume and weight. The samples for BD were weighed and dried at 105°C for two days then weighed again to get dry mass/volume. Samples were taken between July 27 and August 5, 2014 for the growing season measurements and on December 4, 2014 for the non-growing season measurements. Samples for GSM were weighed then dried in the oven at 105°C for two days and weighed again. Samples for C and N analysis were air dried for several weeks to avoid possible burning of organic matter then ground using a mortar and pestle. The ground samples were left to air dry for another two days to ensure all moisture was removed. The samples were then placed in vials. The samples were then run through the 2400 Series II CHN/O elemental analyzer (Perkin-Elmer, Waltham, Massachusetts) to determine %C and %N.

Potential denitrification

Potential denitrification enzyme activity (DEA) was analyzed using methodology adapted from Groffman et al. (1999). In the growing season, triplicate samples were analyzed for each mesocosm. During the non-growing season, one composite sample of

the three soil cores was sampled. All samples for potential DEA were kept in the refrigerator for no more than 24 hours before sampling. Samples were weighed to 25 g and placed in Erlenmeyer flasks. DEA media was created using 0.1 g chloramphenicol, 1.01 g KNO_3^- , 1.0 g dextrose, and 1.0 L deionized water. Each sample was mixed with 25.0 mL of media to create a slurry. The flasks were then stoppered and the slurry was bubbled with nitrogen. The headspace of each flask was flushed with nitrogen and vacuumed three times to create an anaerobic environment. Once flasks reached ambient temperature and pressure, 10 mL of scrubbed acetylene was added. The flasks were then placed on a shaker table at 125 rpm and the headspace was sampled after 45 minutes and 105 minutes. The gas samples were injected into monoject vials prior to being analyzed on a Shimadzu GC-8A gas chromatograph (Shimadzu Scientific Instruments, Columbia, MD). The DEA rate ($\mu\text{g N-N}_2\text{O/kg soil/h}$) is calculated as being equal to the concentration ($\mu\text{g N-N}_2\text{O}$) at 45 minutes subtracted from the concentration at 105 minutes, divided by the soil weight (kg) times the proportion of dry soil (1-soil moisture).

Carbon mineralization

Denitrification is often strongly linked with the abundance and quality of C in the soil and the production of CO_2 through microbial respiration (Craft et al. 2003; Straahof et al. 2014). Because we used laboratory methods to determine the potential denitrification, rather than *in situ* methods, we chose to also examine potential carbon mineralization. Measuring CO_2 efflux from the soil gives more insight into how active the microbial community is and the availability of soil C for DEA. To determine whether or not carbon is a limiting factor in potential DEA in our mesocosms, the labile and

refractory carbon pools were estimated via analysis of CO₂ respiration. Potential carbon mineralization was measured in the laboratory using a LiCor LI-8100 infrared gas analyzer. The LI-8100 was fit with a modified airtight jar lid to accommodate the laboratory samples. Soil samples from each of the 40 mesocosms were collected December 1, 2014 (for comparable analysis with the non-growing season DEA and soil characteristics measurements) and brought back to the US Geological Survey laboratory in Reston, VA for analysis. Field moist soils weighed and sieved using a 0.223 inch sieve to remove rocks and live roots. Samples were placed in airtight, 354.88-cm³ glass jars, then the headspace was flushed with nitrogen gas for three minutes to create an anaerobic environment. The jars were left at ambient temperature to incubate for a week and then sampled periodically over eleven months (December, 2014 – October, 2015). Measurements were taken on day 7, 14, 32, 57, 86, 121, 150, 203, 250, and 324. On days 179, 232, and 285 the jars were purged to release any built up pressure that may inhibit microbial activity. On each sampling date, the jars were individually analyzed three times in a row. Because the lid had to be changed prior to analysis, this included an initial three-minute flush with nitrogen to remove any oxygen. The three sampling periods followed the initial flush, including a five-minute deadband and two-minute sampling period. Between samples, the headspace was flushed. Flux rates were expected to lower towards 0 $\mu\text{mol CO}_2 \text{ kg}^{-1} \text{ s}^{-1}$ as the labile C in the sample is used up and the microbes begin to consume the refractory C (Robertson et al. 1999). The CO₂ flux rate ($\mu\text{mol C kg soil}^{-1} \text{ s}^{-1}$) was used to determine potential carbon mineralization in each mesocosm (mg C

kg soil⁻¹ day⁻¹). Microbial growth during the incubation period is assumed to be constant or insignificant (Robertson et al. 1999).

Data analysis

All data were tested for normality using a Shapiro-Wilkes test. Because the conditions of normality required for the analysis of variance (ANOVA) were not met, non-parametric analyses were applied using Kruskal-Wallis and Mann-Whitney U tests to determine differences between functional planting richness groups (FGs) for all variables (morphometric characteristics, soil characteristics, AGB, DEA, and CO₂ efflux, and labile C). Non-parametric analyses were also used to determine differences between individual species for the first year after the disturbance (2014). The vegetation data were also analyzed against the data from the two years prior to the disturbance (2012 and 2013) (Korol and Ahn 2015). In addition, Spearman rank correlations were run comparing the structural data (%C, %N, C:N, BD, GSM, total mesocosm %C_v and soil temperature) against the functional data (AGB, DEA and labile carbon mineralization rates). Significance is determined at $p = 0.05$. All statistical analyses were conducted using IBM SPSS Statistics version 21.0 (SPSS, 2012).

Results

Peak growth was determined after all measurements were taken. All species peaked at different times between August 5 and September 30 (Appendix I). 18 Aug 2014 was chosen for all peak growth analyses because it most closely encompasses the peak for each species as well being comparable to the timing of biomass harvest in 2013. Standardized cover (to assume 1 m² cover of individual species) made it possible to compare all planted mesocosms regardless of the number of original individuals.

Plant community recovery

Three of the four planted species (the reed, *J. effusus*, the facultative annual, *M. ringens* and the sedge, *C. vulpinoidea*) continued to send up shoots after the disturbance, as the 2013 growing season ended. Although these plants were able to allocate space to grow, not all were successfully re-established by the end of the 2014 growing season. The obligate annual exhibited ruderal behavior (the ability to thrive in disturbed land, often through intensive seeding (Hill et al. 2002) and became dominant (Korol and Ahn 2015). The obligate annual, *E. obtusa*, was the only species that recovered after the disturbance in all mesocosms in which it was planted. Of the other three species, many of the originally planted individuals failed to recover during this study after the disturbance compared to the growth achieved during the prior two growing seasons (2012-2013). The reed was the second most successful (100% of planted mesocosms recovered in FG 1 and

2, 88% in FG 3, and 63% in FG 4). The sedge experienced 100% recovery of planted mesocosms only in FG 2, and recovered 50% in FGs 1 and 4, and 78% in FG 3. The facultative annual showed the least successful recovery with 100% recovery in FG 2, but only 50% recovery in FG 3 and 4, and eventual failure in monocultures.

Table 3. Standardized percent cover recovery during this study (2014) of each plant species by functional planting richness.

	Fac. Annual	Reed	Obl. Annual	Sedge	Total MC %Cv
FG1	50.0 ± 25.0	100.0 ± 0.0a	65.2 ± 17.4	50.0 ± 25.0ab	43.49 ± 13.6a
FG2	17.9 ± 6.6	46.8 ± 18.7ab	96.5 ± 1.0	48.5 ± 8.2a	72.79 ± 12.2b
FG3	5.7 ± 2.3	35.2 ± 10.9ab	94.2 ± 2.2	12.6 ± 6.7ab	76.24 ± 6.4b
FG4	3.9 ± 2.0	9.6 ± 4.3c	95.2 ± 1.8	4.6 ± 3.1b	84.48 ± 1.0c

Significance ($p \leq 0.05$) is indicated by the letters a, b, and c. Where letters are absent, no significant differences were observed.

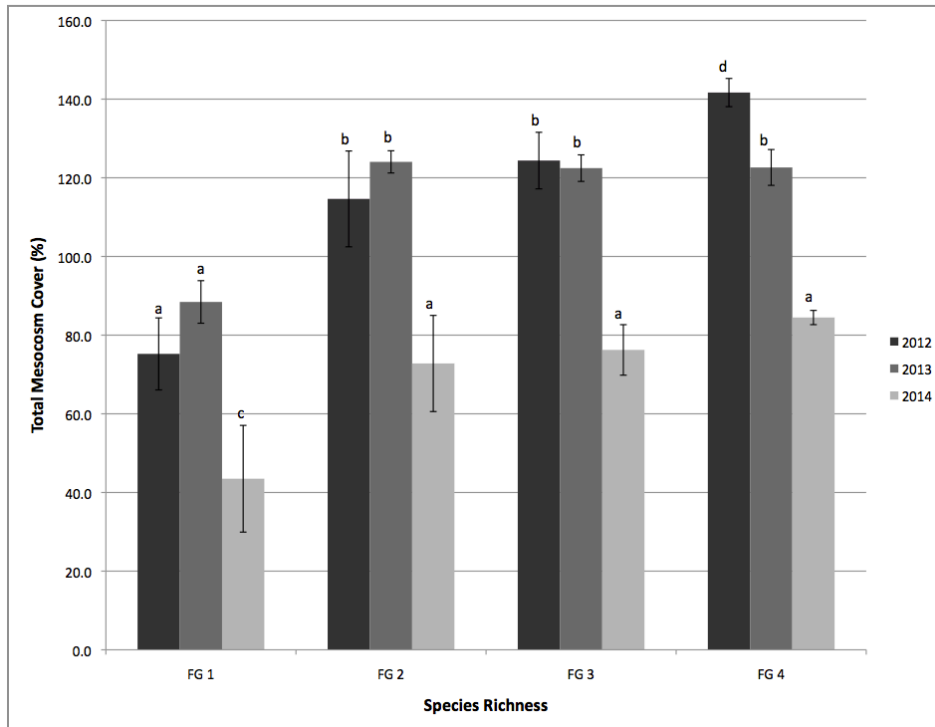


Figure 1. Yearly change in total mesocosm standardized percent vegetative cover. Significance ($p \leq 0.05$) is indicated by letters a, b, and c across both FG and year.

Total mesocosm cover in the first year after disturbance showed a significant increasing trend as planting richness increases (43.5% cover in FG 1 to 84.5% cover in FG 4) ($p = 0.028$) (Table 3). Total mesocosm cover in FG 1, however, is strongly influenced by failures in the monocultures of the sedge and the facultative annual. Between monocultures and mixtures, (FG 1 vs. FG 2, 3, and 4) the total percent coverage almost doubles (From 43.49% in FG 1 to 84.48% in FG 4; Table 3). Total mesocosm cover was also influenced by the reed, which significantly decreased cover as planting richness increased, as those in FGs 1 and 2 were significantly different from FG 4 ($p = 0.044$ and $p = 0.048$, respectively). The cover percentage of sedge decreased significantly between FG 1 (50% cover) and FG 4 (4.6% cover) ($p = 0.012$). In comparing our result of the plant community recovery with the two growing seasons prior to the disturbance, total mesocosm cover was significantly lower in FG 1 than in the mixtures for all years (Figure 1). Although the total cover in the 2014 growing season was significantly lower than the two years prior to the disturbance, the pattern of increased total cover as planting richness increases remains similar. In addition, looking at the error bars, we can see that there is more variability of individual recovery in the community of lower richness groups (e.g., FGs 1 and 2), which diminishes as the richness increases, suggesting a more stable recovery of the community as richness increases. It is notable though that in the 2013 growing season, when each plant community was fully established, there was little difference in the variability of total mesocosm percent coverage between the groups.

Species-specific resilience

We compared the ability of each species to restore vegetative cover after a disturbance using the previous growing season as the equilibrium (Figure 2). If a species achieved at least 100% recovered vegetative cover (%RCv) it can be considered resilient after just one growing season after the disturbance. Most resilient was the obligate annual, which was able to fully restore cover in all functional groups to levels observed in the year prior to the disturbance (104% RCv in FG 1, 190% RCv in FG 2, 301% RCv in FG 3, and 217% RCv in FG 4) (Figure 2). The reed reached 100% RCv only when grown in monoculture and the sedge reached 146% RCv in FG 2, although neither achieved cover similar to that of the growing seasons prior to the disturbance in any other functional group. The facultative annual, the least successful, was unable to recover to equilibrium in all richness groups, restoring no more than 59% RCv (in FG 2).

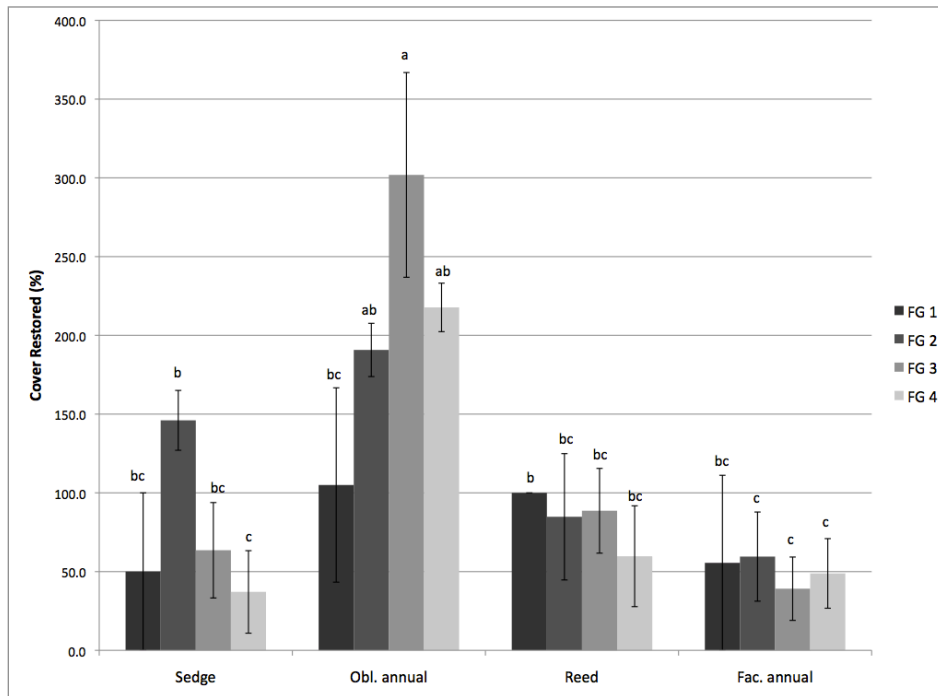


Figure 2. Percent cover restored 1 year after disturbance by individual species. Significance ($p \leq 0.05$) is indicated by letters a, b, and c.

Although vegetative cover is often used to make assumptions on the condition of wetland communities (NRC, 2001), species-specific morphometric measurements allow for a fuller understanding of the resilience of each species within the community (Diaz et al., 2004). We compared these measurements across the functional groups and across the three growing seasons (Figure 3). The facultative annual (Figure 3a) showed no significant difference in stem count (SC) between the first growing season (i.e., 2012) and the first year after the disturbance (i.e., 2014) (average SC, 10 and 7, respectively). The second growing season (i.e., 2013) (average SC, 31) had significantly more growth of stems ($p < 0.001$). Although the SC was the statistically same in the first growing season and the first year after the disturbance, the mean stem length (SL) was

significantly lower after the disturbance, and both were significantly lower than in the second growing season (2012: 47.7 cm, 2013: 98.5cm, 2014: 12.3 cm; $p = 0.008$). SC was also measured for the reed (Figure 3b) during the first growing season and the first year after the disturbance. Average SC for each functional group was significantly lower after the disturbance ($p < 0.001$). SL for the reed was statistically the same in the first growing season and the first year after the disturbance (overall average, 95.7 and 54.5 cm respectively) in all FGs, where as the second growing season produced significantly longer stems (overall average 118.7 cm). For the sedge (Figure 3c), between the first and second growing seasons, there was a significant increase in CH (51.9cm and 66.3cm respectively; $p = 0.034$). However, several individuals did not recover after the disturbance (2014 average CH = 29.3cm), leading to a significantly lower CH in the first year after the disturbance for all FGs except for FG 2 (CH = 58.7cm; $p = 0.034$). The obligate annual (Figure 3d) had significantly higher %Cv in the first year after the disturbance in FGs 2, 3, and 4 (96%, 94%, and 95% respectively) than compared to the two years prior to the disturbance ($p < 0.05$). In FG 1 showed no significant change in %Cv.

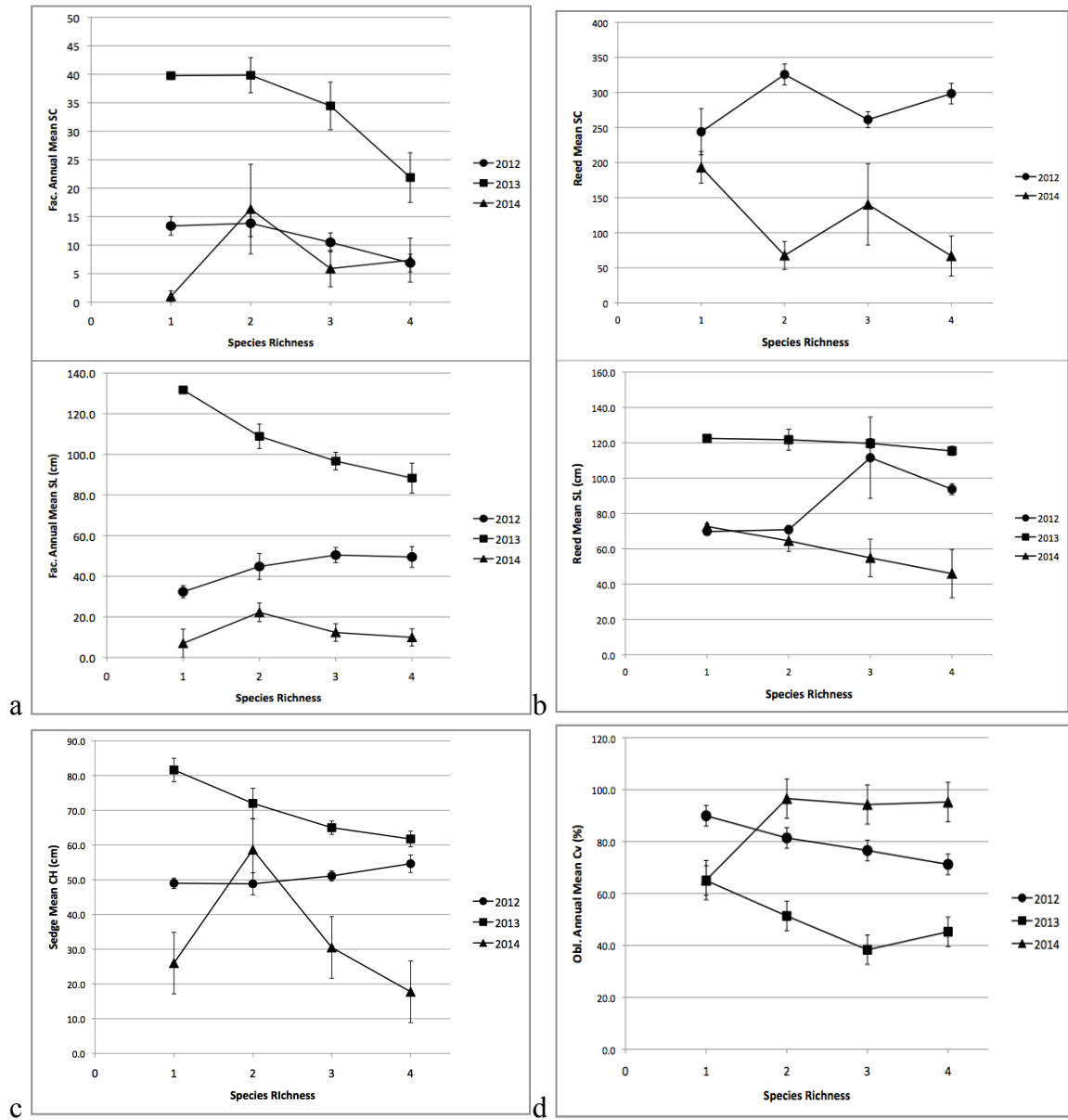


Figure 3. Yearly comparison of morphological measurements for individual species. a) Stem count (SC) and stem length (SL) for the facultative annual (*M. ringens*). b) Stem count (SC) and stem length (SL) for the reed (*J. effusus*). c) Canopy height (CH) for the sedge (*C. vulpinoidea*). d) Standardized percent cover (Cv) for the obligate annual (*E. obtusa*).

Table 4. Annual estimate of individual species AGB (g m^{-2}) based on morphological measurements.

Species	FG	2012	2013	2014
Fac. Annual				
	FG 1	$235.0 \pm 28.4\text{a}$	$1983.2 \pm 84.5\text{a}$	11.9 ± 11.9
	FG 2	$165.2 \pm 10.8\text{a}$	$576.8 \pm 209.4\text{ab}$	45.2 ± 10.1
	FG 3	$85.8 \pm 11.6\text{b}$	$286.1 \pm 54.6\text{b}$	21.9 ± 8.0
	FG 4	$85.8 \pm 11.6\text{b}$	$135.2 \pm 23.9\text{c}$	17.3 ± 7.5
	overall average	$98.1 \pm 13.4^*$	$425.1 \pm 116.7^*$	$22.5 \pm 4.8^*$
Reed				
	FG 1	$142.4 \pm 4.1\text{a}$	$1662.5 \pm 115.7\text{a}$	$174.4 \pm 15.9\text{a}$
	FG 2	$99.6 \pm 16.3\text{ab}$	$876.6 \pm 174.1\text{ab}$	$62.9 \pm 7.9\text{b}$
	FG 3	$88.3 \pm 13.1\text{ab}$	$462.7 \pm 81.0\text{b}$	$66.3 \pm 15.3\text{b}$
	FG 4	$74.3 \pm 6.9\text{b}$	$254.4 \pm 23.5\text{c}$	$50.4 \pm 14.8\text{b}$
	overall average	$89.7 \pm 7.3^*$	$552.5 \pm 96.2^*$	$69.9 \pm 10.9^*$
Obl. Annual				
	FG 1	$320.5 \pm 49.0\text{ab}$	$752.5 \pm 8.8\text{a}$	247.3 ± 63.1
	FG 2	$360.4 \pm 11.6\text{a}$	$463.8 \pm 45.6\text{ab}$	313.8 ± 4.6
	FG 3	$348.8 \pm 4.2\text{ab}$	$307.7 \pm 24.5\text{ab}$	300.2 ± 4.8
	FG 4	$341.2 \pm 2.8\text{b}$	$320.1 \pm 15.3\text{b}$	301.0 ± 4.4
	overall average	$345.0 \pm 4.5^*$	$373.9 \pm 31.0^*$	$297.5 \pm 6.0^*$
Sedge				
	FG 1	$218.1 \pm 13.5\text{a}$	$1031.4 \pm 206.7\text{a}$	109.0 ± 109.0
	FG 2	$144.2 \pm 37.2\text{ab}$	$348.3 \pm 113.1\text{ab}$	280.0 ± 38.5
	FG 3	$89.9 \pm 12.0\text{b}$	$167.1 \pm 56.3\text{b}$	137.4 ± 47.5
	FG 4	$69.7 \pm 9.1\text{b}$	$61.7 \pm 11.0\text{c}$	101.4 ± 66.7
	overall average	$101.6 \pm 11.9^*$	$232.1 \pm 65.6^*$	$141.1 \pm 33.5^*$

* Indicates overall significance among years at $p \leq 0.001$.

Significant difference ($p \leq 0.05$) between functional richness groups is indicated by letters a, b, and c. Where letters are absent, no significant differences were observed.

The average biomass recovered by individual species ranged from 5% (facultative annual) to 80% (obligate annual). The majority of recovered biomass could be attributed to the success of the obligate annual (Table 4). The reed shows a significant decrease based on planting richness with the AGB produced in FGs 2, 3, and 4 (62.9 g m^{-2} , 66.3 g m^{-2} , and 69.9 g m^{-2} respectively) significantly lower than that of FG 1 (174.4 g m^{-2} ; $p < 0.05$). The sedge produced higher AGB in FG 2 (280.0 g m^{-2}) with a slight decline as FG

increased (AGB = 137.4 g m⁻² in FG 3 and 101.4 g m⁻² in FG 4), however no significant difference was observed. The facultative annual showed a similar pattern of decline in FG 2, 3, and 4 (45.2 g m⁻², 21.9 g m⁻², and 17.3 g m⁻² respectively), although again this was not considered significant. The obligate annual shows a slight increase in AGB when grown in mixture (FG 2; 313.8 g m⁻², 3; 300.2 g m⁻², and 4; 301.0 g m⁻²) versus grown in monoculture (FG 1; 247.3 g m⁻²), however this increase was also not considered significant. Estimated biomass was significantly lower in the first year after the disturbance than the previous two growing seasons for all four planted species (Table 4; $p < 0.001$).

Relative yield (RY) was used to determine which species grew more successfully with neighbors (RY > 1) and which are better suited for monoculture growth (RY < 1; Table 5). The facultative annual showed a significant decrease between FG 2 (7.6) and FG 3 (5.5; $p < 0.05$). Although the reed under yielded only in FG 2 (RY=0.7), no significant change in RY was observed between FGs for reed or the sedge. The obligate annual showed an increase in RY as FG increased ($p < 0.001$).

Table 5. Relative yield (RY) of AGB growth in mixtures of multiple species as compared to monocultures.

	Sedge	Obl. Annual	Reed	Fac. Annual
FG 2	4.0 ± 1.6	2.5 ± 0.0a	0.7 ± 0.1	7.6 ± 1.7a
FG 3	3.8 ± 1.3	3.6 ± 0.1b	1.1 ± 0.3	5.5 ± 2.0b
FG 4	3.7 ± 2.4	4.9 ± 0.1c	1.2 ± 0.3	5.8 ± 2.5ab

Significance ($p \leq 0.05$) is indicated by letters a, b, and c. Where letters are absent, no significant differences were observed.

Soil characteristics

In comparing each variable between the growing and non-growing seasons, no significant differences were seen. Differences were seen across planting richness groups (Table 6), however no clear trends correlating to the planting richness gradient were observed. As expected, the unplanted mesocosms (FG 0) contained the lowest soil C during both the growing season and non-growing season (1.28%C and 1.37%C respectively). In the non-growing season, FG 1 (1.41%C) and the unplanted mesocosms had statistically similar C ($p = 0.950$). During the growing season, the highest C was found in FG 2 (1.52 %C), and was significantly higher than any other richness group ($p < 0.05$). During the non-growing season, the highest C was found in FG 3 (1.52%C), but no differences were observed between FGs 1, 2, 3, or 4. There was no significant difference in %N seen among the mesocosms, all of which had between 0.11%N and 0.12% N in both the growing season and non-growing season sampling. The C:N ratios observed also showed no pattern of increase as planting richness increased with the lower C:N seen in the unplanted, but the highest seen in FG 2 in the growing season (12.52) and in FG 3 in the non-growing season (13.19).

During the growing season, BD ranged from 0.96 g cm^{-3} (FG 4) to 1.07 g cm^{-3} (FG 2) with FG 2 being significantly higher than the others ($p < 0.001$). In the non-growing season, the unplanted mesocosms had significantly lower BD (0.75 g cm^{-3}), with no other differences observed among planting richness groups. Gravimetric soil moisture during the growing season was almost uniform across planting richness groups, with only FG 0 being significantly different (0.28; $p < 0.001$). In the non-growing season, FG 2

was the highest (0.32) and FG 0 (0.25) remained significantly lower than all other groups ($p < 0.05$).

Potential denitrification

During both sampling seasons, the unplanted mesocosms had significantly lower rates of potential denitrification than planted mesocosms ($p < 0.001$; Figure 4a). There was a significant decrease in DEA in FG 1 during the non-growing season, from an average of $83.53 \mu\text{g N-N}_2\text{O kg}^{-1}\text{h}^{-1}$ in August to $47.99 \mu\text{g N-N}_2\text{O kg}^{-1}\text{h}^{-1}$ in December (Figure 4). During the non-growing season, FG 0 ($24.94 \mu\text{g N-N}_2\text{O kg}^{-1}\text{h}^{-1}$) and FG 1 were not significantly different ($p = 0.081$). Removing the unplanted mesocosms from the statistical analysis reduced the illusion that there was an increase in DEA as planting richness increased (Figure 1b), which can be attributed to the very low rate in FG 1 during the December sampling. During the growing season, potential denitrification rates ranged from $20.12 \mu\text{g N-N}_2\text{O kg}^{-1}\text{h}^{-1}$ (FG 0) to $99.64 \mu\text{g N-N}_2\text{O kg}^{-1}\text{h}^{-1}$ (FG 3). In the non-growing season, rates ranged from $24.94 \mu\text{g N-N}_2\text{O kg}^{-1}\text{h}^{-1}$ (FG 0) to $108.05 \mu\text{g N-N}_2\text{O kg}^{-1}\text{h}^{-1}$ (FG 4). Overall, there was no significant difference between the growing season and non-growing season measurements. Both sets of DEA measurements were significantly positively correlated with total mesocosm cover ($p < 0.001$). The non-growing season measurements were significantly negatively correlated with temperature ($p < 0.001$) and the December C:N ($p < 0.05$; Table 7).

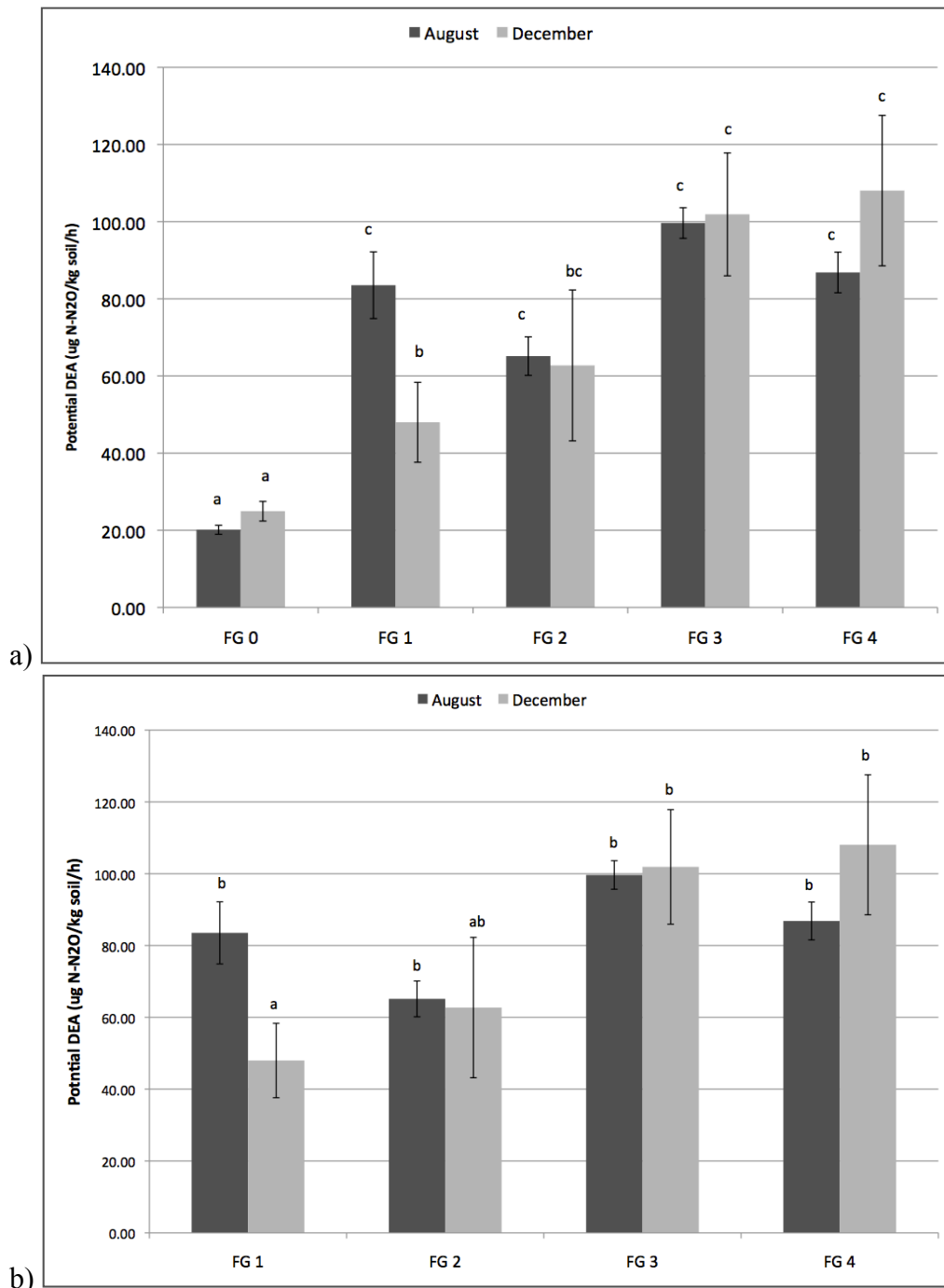


Figure 4. Seasonal denitrification potential ($\mu\text{mol N-N}_2\text{O kg soil}^{-1} \text{ hr}^{-1}$), examined including unplanted mesocosms (a) and excluding unplanted mesocosms (b). Statistically significant differences among FGs indicated by letters a through c ($p < 0.05$).

Table 6. Soil characteristics. Statistically significant differences among FGs indicated by letters a through c ($p < 0.05$). Where letters are absent, no significant difference was observed. No significant seasonal differences were observed.

	%C ¹	%C ²	%N ¹	%N ²	C:N ¹	C:N ²	BD ¹	BD ²	SM ¹	SM ²
FG 0	1.28 a	1.37 a	0.12	0.11	10.79 a	12.01 a	0.99 a	0.75 a	0.28 a	0.25 a
FG 1	1.42 b	1.41 ab	0.12	0.11	12.14 bc	12.63 b	1.02 ab	0.99 b	0.30 b	0.31 bc
FG 2	1.52 c	1.45 b	0.12	0.11	12.52 c	12.98 bc	1.07 b	1.03 b	0.30 b	0.32 c
FG 3	1.31 ab	1.50 b	0.11	0.11	11.71 b	13.19 c	0.98 a	0.97 b	0.30 b	0.30 b
FG 4	1.35 ab	1.46 b	0.11	0.11	11.82 b	12.81 bc	0.96 a	1.00 b	0.31 b	.31 bc

Soil Characteristics measured include % soil carbon (%C), % soil nitrogen (%N), ratio of carbon to nitrogen (C:N), bulk density (BD) (g/cm³), and gravimetric soil moisture (SM)(proportion water in soil)

¹Soils collected July 27, 2014.

²Soils collected December 4, 2014.

Table 7. Spearman rank correlations among soil characteristics, AGB, and total mesocosm percent cover (%Cv).

	LC	%C ²	%N ²	C:N ²	%C ¹	%N ¹	C:N ¹	DEA ²	DEA ¹	BD ²	BD ¹	SM ²	SM ¹	Temp	AGB
LC	1.000														
%C ²	-.074	1.000													
%N ²	.100	.781**	1.000												
C:N ²	-.215	.684**	.188	1.000											
%C ¹	.137	.034	-.026	.136	1.000										
%N ¹	.423**	.092	.234	.009	.788**	1.000									
C:N ¹	-.127	.015	-.222	.234	.800**	.307	1.000								
DEA ²	-.547**	.339*	.215	.324*	.020	-.001	.089	1.000							
DEA ¹	-.541**	.281	.084	.343*	-.198	-.293	-.056	.575**	1.000						
BD ²	-.398*	.303	.133	.301	.154	-.088	.307	.248	.532**	1.000					
BD ¹	.016	-.165	-.089	-.241	.283	.271	.101	-.111	.111	-.073	1.000				
SM ²	-.398*	.303	.133	.301	.154	-.088	.307	.248	.532**	1.000**	-.073	1.000			
SM ¹	-.208	.199	-.072	.450**	.235	.009	.369*	.355*	.328*	.423**	-.241	.423**	1.000		
T	.036	.040	-.077	.122	-.034	-.243	.164	-.439**	-.116	-.031	-.255	-.031	.158	1.000	
AGB	-.349*	-.059	-.298	.182	.049	-.371*	.402*	-.004	.270	.086	.250	.086	.096	.352*	1.000
%Cv	-.429**	.262	.112	.403**	.135	.100	.166	.738**	.570**	.531**	-.045	.531**	.403**	-.513**	-.152

FG (Functional group); LC (labile carbon measured December 11, 2014 - mg C kg soil⁻¹ day⁻¹); %C (percent soil carbon); %N (percent soil N); C:N (carbon to nitrogen ratio); DEA (denitrification enzyme activity - $\mu\text{mol N-N}_2\text{O kg soil}^{-1} \text{ hr}^{-1}$); BD (bulk density - g cm⁻³); SM (gravimetric soil moisture - proportion water in soil); T (Temperature - °C); AGB (aboveground biomass - g m⁻²); %Cv (total mesocosm percent cover)

¹Soils collected July 27, 2014.

²Soils collected December 4, 2014.

* Indicates significance at $p = 0.05$.

** Indicates significance at $p = 0.001$.

Carbon mineralization

The unplanted mesocosms showed the clearest transition from active C pool to intermediate C pool (Figure 5). The average CO₂ flux values were considerably high throughout the incubation, indicating that a transition from labile C consumption to refractory C consumption had not taken place in all samples (Paul et al. 2001).

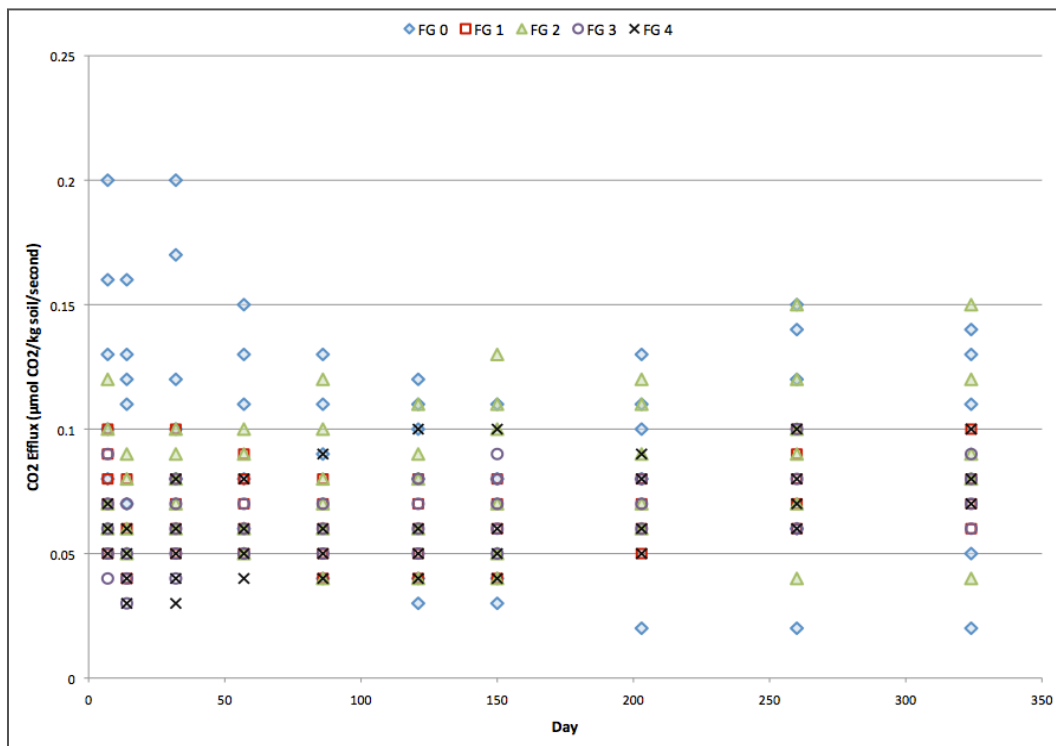


Figure 5. CO₂ flux (μmol CO₂ kg soil⁻¹ sec⁻¹) over the 324-day incubation (December, 2014 – October, 2015).

At the beginning of the analysis, the average of FG 0 was high relative to the other richness groups ($0.15 \mu\text{mol CO}_2 \text{ kg soil}^{-1} \text{ s}^{-1}$) and averaged $0.09 \mu\text{mol CO}_2 \text{ kg soil}^{-1} \text{ s}^{-1}$ by day 203. The planted mesocosms showed much less variation at each sampling date and very little variation throughout the incubation period (Figure 5). By the end of Feb (86 days after incubation), the CO_2 flux rate was statistically uniform across all FGs, fluctuating around $0.07 \mu\text{mol CO}_2 \text{ kg}^{-1} \text{ s}^{-1}$ across all functional groups for the remainder of the 324-day incubation.

The CO_2 flux was converted to C mineralization rates (Paul et al. 2001; Figure 6). FG 1 also showed no significant change throughout the incubation, with a mineralization rate between $18 \text{ mg C kg soil}^{-1} \text{ d}^{-1}$ and $24 \text{ mg C kg soil}^{-1} \text{ d}^{-1}$. FG 2 also showed no significant change throughout the incubation, with a mineralization rate between $20 \text{ mg C kg soil}^{-1} \text{ d}^{-1}$ and $30 \text{ mg C kg soil}^{-1} \text{ d}^{-1}$. FG 4 had the lowest mineralization rate at the beginning of the incubation ($12 \text{ mg C kg soil}^{-1} \text{ d}^{-1}$), and showed no statistical change throughout the incubation. After day 121, the average mineralization rate for all groups began to slowly increase, as did the variation in each planting richness group (Figure 6), however this change was only significant in FG 4 ($p = 0.003$; Appendix II). The labile C pool (derived from the December 11 CO_2 flux measurements) was significantly negatively correlated with DEA from both the growing season and non-growing season and with total mesocosm %Cv ($p < 0.001$; Table 7).

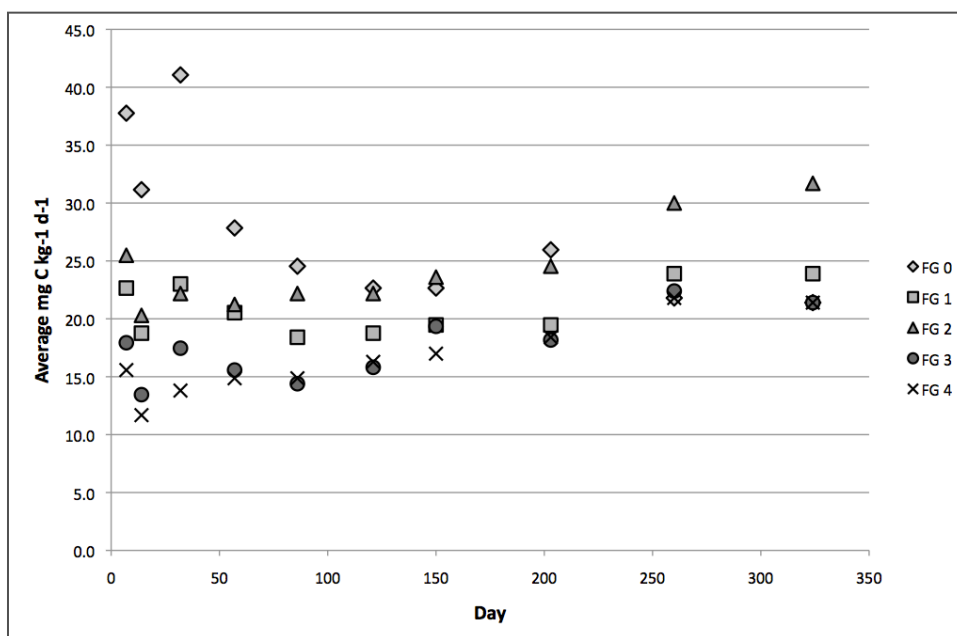


Figure 6. Potential carbon mineralization ($\text{mg C kg soil}^{-1} \text{ day}^{-1}$) over the 324-day incubation (December 11, 2014 – October 19, 2015) derived from CO_2 flux.

Discussion

Plant community recovery

This study aimed to determine the effects of the planting richness on the first year recovery of the vegetation community in created wetlands after a disturbance. Many studies have shown the positive relationship between planting diversity and productivity (Cardinale et al. 2013; Williams and Ahn 2015), stability (Loreau et al. 2002), and resilience (Carvalho et al. 2012). Using percent total vegetative cover, the most commonly used indicator of plant community development in evaluating created mitigation wetlands (NRC 2001), we hypothesized that communities with higher planting richness would recover more successfully after a disturbance. The total mesocosm percent cover, which was higher in mixtures, supported this hypothesis. We also found that in mixtures, variation was lower further indicating that higher planting richness increases recovery one year after a disturbance. This pattern was particularly evident when comparing monocultures to mixtures. The difference in success between monocultures and mixtures was exacerbated by the failure of several monocultures to recover after the disturbance. The majority of the total cover re-growth after the disturbance can be attributed to both the reed and the obligate annual, which both thrived in all functional groups.

While total mesocosm cover increased with the planting richness, none of the richness groups were able to recover to the prior equilibrium one year after the disturbance. Therefore, we may not say that the communities at this time are stable (Holling 1973). Although literature does support this hypothesis (Cardinale et al. 2011; Ma'jekova et al. 2014; Berendse et al. 2015) our study does not. The large standard error observed in the individual species' ability to recover (%RCv) was due to failure of individuals of certain species. This indicates that the community recovery is heavily dependent on the species-specific relationships. It is also likely that not all species were fully mature at the end of this study. Korol and Ahn (2015) observed the full growth of all four species was achieved in the second growing season, indicating that this is a temporal limitation of the study. Observation over a longer period of time is necessary to determine whether or not the individual species and overall communities will recover further.

Species-specific resilience

Responses of a community to a disturbance are dependent upon the species present and those that colonize after the disturbance (Speed et al. 2010). In an upland prairie study, the restoration of the community was influenced not only by individual plant traits, but also their neighbors (Roberts et al. 2010). Further exploring the impact of the disturbance on the planted community, we examined species-specific morphometric measurements. We were able to gain a more complete understanding of the vegetative dynamics in the ecosystem and determine which species are more resilient immediately after a disturbance. We hypothesized that morphometric growth of each species would be

similar to the first growing season, and suspected that the community would still be in an early stage of development. Many of the morphometric measurements for each species showed that growth was much less successful than prior to the disturbance. Only the obligate annual, a dominant ruderal (Grime 2001; Korol and Ahn 2015), was able to restore cover to the levels seen before the disturbance in all four functional groups. The obligate annual dominated each mesocosm in which it was planted. None of the other three species were able to thrive in FG 4, where *E. obtusa* was consistently present. This is the same dynamic present prior to the disturbance (Korol and Ahn 2015). The reed, *J. effusus*, produced large quantities of AGB when planted in monoculture relative to the mixtures. In addition, while stem counts showed no pattern in regards to planting richness, stem lengths were inversely related to planting richness. The low AGB production in higher mixtures and less successful morphometric growth of the reed could be attributed to over yielding of *E. obtusa*. Thus in isolated areas of a larger wetland, *J. effusus* is a good candidate for contributing cover and AGB, however growth may be limited by the presence of neighbors.

After the disturbance, the facultative annual, *M. ringens*, produced only a few short stems. The stems were shorter and weaker than those produced during the first growing season and were susceptible to storms and foraging by animals. In the first and second years of growth, the facultative annual produced large amounts of biomass when in monoculture, however, both post-disturbance monocultures eventually failed, suggesting this species is particularly prone to disturbance when grown alone. In addition, re-growth rates of *M. ringens* in mixtures were low, with 100% re-growth of

planted individuals only seen in FG 2. This suggests there may be nutrient or space limitation also acting on the facultative annual. Similarly, the sedge, *C. vulpinoidea*, failed in one of the monocultures and not all originally planted individuals successfully recovered. Again, we saw 100% recovery of originally planted individuals only in FG 2. This indicates that this species is also prone to disturbance when grown alone and may be hindered by the presence of multiple neighbors.

The species-specific observations lend evidence in support of creating a spatially explicit planting regime to promote a more resilient community. It is important to take into account the species that are planted with neighbors and which are isolated. *J. effusus*, for example, thrives alone, where as *M. ringens*, would likely be decimated by a severe disturbance. The obligate annual can quickly colonize after a disturbance. *E. obtusa* is fast growing and seeds multiple times throughout the growing season, making it able to spread before other species can be completely re-established. In a created system where rapid vegetation is desired, this is a good planting choice both for its ability to spread, thus preventing soil erosion, and to accumulate above ground biomass. However, nutrient availability as well as temporal and spatial species limitations should be considered when planting with *E. obtusa* with neighboring species. While other species also became established when grown in mixtures with the obligate annual, *E. obtusa* may hinder the growth of other species in the same community within the first growing season after a disturbance.

Although total mesocosm cover increased as planting richness increased, many of the morphological attributes showed signs of stress in FG 4 (short and thin shoots seen in

the facultative annual, shorter shoots seen in the reed, and a lower max canopy height in the sedge). Other studies have also found that vegetation performance after a disturbance is species specific (Speed et al. 2010; Pfiefer-Meister et al. 2012). Species are able to modify growth allocations and nutrient use as a response to stress from the disturbance or from shading from more resilient neighboring species (Grime 2001; Grasset et al. 2015). This can be seen in the morphology of shoots. Morphological plasticity, which caused the AGB to vary greatly within species, is dependent upon stress from the disturbance as well as from neighbors (Fargione and Tilman 2005; Thein et al. 2008; Lorentzen et al. 2008). These morphological responses to stress resulted in the more uniform distribution of AGB across both FG and species type.

Soil characteristics

Although no seasonal differences occurred, differences were observed in planted versus unplanted mesocosms for almost all soil characteristics measured. Nitrogen abundance, however, was one characteristic that was uniform across all richness groups. Freshwater wetlands act as a N sink, with more N cycling within the system than flowing out (Bowden 1987). It was expected that the nitrogen would vary with changing planting richness since it has been found that difference morphometric traits, plant evenness, and species diversity all influence the uptake and availability of N in the soil (Eisenhauer et al. 2010; Palta et al. 2012; Zak et al. 2013; McGill et al. 2010). The other soil characteristics did not follow any trends based on planting richness. This could be attributed to the fact that not all planted individuals grew back and the dominance of the obligate annual, after the disturbance or to the fact that the community has not yet

reached maturity (Means and Ahn 2016). FGs 2 and 3 had high levels of C and were the most successful in terms of morphometric growth for the sedge, obligate annual, and facultative annual. In the planted mesocosms, BD was around expected levels for a young wetland. In the unplanted mesocosms, however, it was significantly lower. As wetlands develop, the BD decreases and SM increases as the soil becomes more organic (Anderson et al. 2005; Ahn and Jones, 2013). Characteristics such as these are useful in determining the successful development of wetland functions such as denitrification (Ahn and Peralta 2012).

Potential denitrification

The most important factors affecting denitrification are the absence of oxygen, the presence of nitrate in the surface water, and temperature (Song et al. 2014). Plant species and their spatial distribution in a wetland have been shown to alter the availability of electron donors and acceptors (Sutton-Grier and Megonigal 2010), however the specific effects of the planting community on denitrification appear to be inconsistent. It has been found that higher plant diversity leads to higher N mineralization, which in turn leads to higher denitrification (Zak et al. 2003). However, it has also been found that DEA does not vary based on plant community structure, and higher diversity simply leads to more constant denitrification rates over time (Hopfensperger et al. 2009; McGill et al. 2010). The methods by which denitrification studies are performed and the inherent spatial and temporal variability within different wetlands make comparative analyses challenging (Davidson and Seitzinger 2006). The DEA rates seen in this study were similar to those found in the large created wetlands in the Virginia piedmont region in past studies. Most

recently, Ahn and Peralta (2012) found rates ranging from 41 $\mu\text{g N-N}_2\text{O kg}^{-1}\text{h}^{-1}$ in North Fork Mitigation Bank to 228 $\mu\text{g N-N}_2\text{O kg}^{-1}\text{h}^{-1}$ in Loudon County Mitigation Bank. These rates were found to be more dependent on soil condition, as determined by various soil characteristics (e.g., Total organic carbon, total nitrogen, BD, GSM and pH), rather than on age (Ahn and Peralta 2012; Dee and Ahn 2012). Our study saw no difference based on planting richness. The only group in which a difference in DEA was observed was FG 1 during the non-growing season analysis. In this richness group, however, two of the eight mesocosms did not recover at all after the disturbance and one failed after the end of the growing season, with only two withering stems. It is likely that the failure of these monocultures influenced the microbial community to more closely resemble that of the unplanted mesocosms. The strong positive correlation between DEA and cover during both sampling seasons suggests that the overall community success is more important than the individual species.

Seasonal variations in potential DEA show conflicting results. Some studies have found higher rates of denitrification taking place in the spring, before the plants begin to take up large amounts of N (Boyd 1978; Groffman and Tiedje 1991) or in the fall when there is an abundance of leaf litter, providing a C source for the microbial community (Hooker and Stark 2008; Morse et al. 2012). Conversely, Hopfensperger et al. (2009) found that denitrification was highest in June. In our study, no difference between samples taken in early August and those taken in December was found. The seasonal differences in denitrification rates are dependent on a suite of other variables including temperature, which in our study had a moderate negative correlation with the non-

growing season DEA. While ground temperatures were not measured in the mesocosms during the December sampling, the first freeze of the season took place almost a month prior to sampling. The week prior to sampling, however, was unseasonably warm with air temperatures reaching up to 79°F with temperature lows well above freezing (Vienna VA Weather Archive). This suggests that the microbial community may have been more active than is typical of winter, when activity is often low. To see a significant difference, it is probable that the temperature in the soil must drop to at least 5°C. Other factors driving seasonal differences in DEA include nitrate concentrations and C inputs (Cornwell et al. 1999; Richardson et al. 2004; Plata et al. 2012). A significant positive correlation between C:N and DEA was observed, however the relationship is weak to moderate at best. The mesocosms in this study are ombrotrophic and are not supplemented with NO_3^- the way many wetlands are through runoff and stream flow. Besides the topsoil used in the creation of the mesocosms, the only additional N was from ambient deposition. Although the analysis of DEA was performed in the lab, the low levels of N in the mesocosms may have shaped the microbial community (Nijburg et al. 1997; Groffman and Tiedje 1991; Richardson et al. 2004; Palta et al. 2012). Higher concentrations of nitrate have been shown to increase the presence of nitrate-reducing bacteria and higher potential nitrate-reducing activity in the rhizosphere. Where nitrate is readily available, denitrifying bacteria were abundant, however when nitrate is limited, the presence of C controlled the composition of the bacterial community and led to a dominance of DNRA in high C, low nitrate environment (Nijburg et al. 1997; Wallenstein et al. 2006).

Carbon mineralization

Potential denitrification as performed in this study is performed under ideal conditions and represents the highest potential rate of denitrification for the soil microbial community (Groffman et al. 1999). Therefore, this measure is more indicative of the microbial community structure than it is of the denitrification rate in the mesocosms. Because of this, it was useful to examine more about the C inputs that can be used as an energy source for microbes (Groffman et al. 1999). Throughout the incubation period, it was expected that the labile carbon would be consumed first, producing large quantities of CO₂ that would diminish as the microbes moved into the refractory carbon pool (Robertson et al. 1999). After 86 days of incubation, the rates of CO₂ efflux had yet to reduce to levels low enough to indicate that the microbial community was beginning to metabolize the refractory C pool. CO₂ production and C mineralization in this study indicated a steady intermediate C pool across the planting richness gradient. The most active C pool was seen early on in the unplanted mesocosms. Although there were no species planted, they did receive C inputs from external sources (e.g., leaf litter from surrounding trees) and the growth of algae. This suggests that these inputs are of higher quality and were more readily available for use by the microbial community than the litter from the planted species. The quality of C from leaf and root litter varies based on species (Faulwetter et al. 2009) as well as based on how recently it has senesced (Usselman et al. 2012).

The mesocosms of the highest planting richness (FG 4) showed an increase in microbial activity throughout the incubation. A possible explanation is that the high

planting richness increased the oxygenation of the soil/pore space and the three-minute flush of nitrogen was not enough to bring the soil condition back to fully anaerobic. The presence of oxygen in the soil would increase the CO₂ production greatly. Similar results were seen in a comparison of treatment wetlands comparing unplanted vs. planted areas (Faulwetter et al. 2009).

The high rates of CO₂ flux and the low rates of DEA in the unplanted mesocosms suggest that the microbial community may be using DNRA rather than denitrification (Nijburg et al. 1997; Washbourne et al. 2011). Under high C, low NO₃⁻ conditions, as seen in our mesocosms, DNRA may be favorable to denitrification. This is particularly true if oxygen is present since DNRA is not inhibited by O₂ the way that denitrification is. The disturbance and re-vegetation of the mesocosms could introduce oxygen to the soil, altering the microbial community. Other biogeochemical processes should be examined to determine the source of the CO₂ efflux and gain a better understanding of how the denitrification potential is affected.

Conclusions

The purpose of this study was to determine how the planting richness of a created wetland impacts the soil structure and function after a severe disturbance. In the studied disturbance, all above ground biomass was harvested for a companion study, limiting the leaf litter and dead plant matter entering the soil carbon pool after the end of the 2013 growing season. While several monocultures failed to recover any plants and the overall vegetation community was still not fully restored one year after the disturbance (2014), it is on the trajectory towards full recovery. The disturbance also involved coring the soil for below ground biomass (BGB) and for soil samples, resulting in an uneven soil surface and pockets of aerated soils. While not all of the BGB was removed for the companion study, much was removed, further limiting contributions to the soil C pool as well as reducing live root matter, which would otherwise oxidize the soil and support microbial activity.

This study demonstrates the importance of deciphering the many interconnections among wetland characteristics, both above ground and below ground. Overall, it was observed that the original planting richness has a positive impact on the re-establishment of a wetland plant community with regard to cover, while AGB was generally consistent across the planting richness gradient. It was also observed that the species that peaked

later in the season (i.e., the facultative annual and the sedge) were less likely to become fully established in all mesocosms in which they were originally planted. These species were thus unable to produce the high quantity of AGB that was observed in the obligate annual and the reed. Further study should address whether this was because of spatial, nutrient, or temporal limitation. Both the facultative annual and the sedge failed as monocultures, with 50% successfully re-growing for both species. For created wetlands in locations prone to disturbance, these two species are more likely to be devastated by a severe disturbance and should be planted with neighbors near by. While it is clear that certain species studied here are more immediately resilient than other, we will continue to monitor the morphological growth of the mesocosms for a fourth growing season to better understand the dynamics of species interaction in response to a disturbance.

After the disturbance, the soil characteristics and DEA were around levels that could be expected for a 3-year-old wetland indicating that the disturbance did not impact the structure or function of the soil microbial communities very dramatically. However, since the soil traits were only examined in the first year after the disturbance, it is possible that some effects are not evident without a comparison to the prior years. In addition, to gain a better understanding of the biogeochemistry and nutrient cycling a wider range of measurements may be needed, including BGB, and other microbial processes contributing to soil respiration (e.g., other nutrient transformations). Wetlands act as one of the largest C pools on the planet (Bridgman et al. 2006; Ahn et al. 2009) and one of the best ways to clean polluted waters (Brix and Shierup 1989). However, when they are destroyed, the created/restored wetlands that take their place require a time scale

of decades to centuries to recover the lost functionality (i.e. nutrient cycling) (Hossler and Bouchard, 2010; Mitsch et al. 2012). Created wetlands contained less biomass, less soil organic carbon, and less mineralizable C than natural wetlands. While the vegetation may be quick to develop, soil traits statistically similar to natural wetlands take decades to develop (Mitsch and Wilson 1996; Gutrich and Hitzhusen 2004; Mitsch et al. 2012).

Wetlands are a poorly understood, yet are an ecologically, economically, and culturally important ecosystem. Planners and policy makers, however, often overlook their importance for ecosystem services such as flood control, clean water, and carbon storage as well as their importance as habitat they provide for a wide range of species (*Millennium Ecosystem Assessment* 2005). The continued degradation of our environment through resource consumption and climate change is threatening many natural ecosystems, especially wetlands, and is predicted to worsen over the coming decades (Day et al. 2013). Increasing disturbances from climate change and urban expansion make successfully mitigating the loss of natural wetlands even more challenging. Disturbances can have a wide range of effects on vegetation and soil microbial communities, and may cause a shift in regime and loss of certain species. While many of these changes may be a natural part of the development of ecosystems, surpassing certain disturbance thresholds can be devastating for a created wetland and in some cases the ecosystem may not be able to recover without intensive human intervention (Standish et al. 2014). Although there was no defined disturbance threshold in this study, the failure of several planted individuals to recover after the disturbance suggesting that a critical threshold may have been surpassed and that it may be species-specific. This study

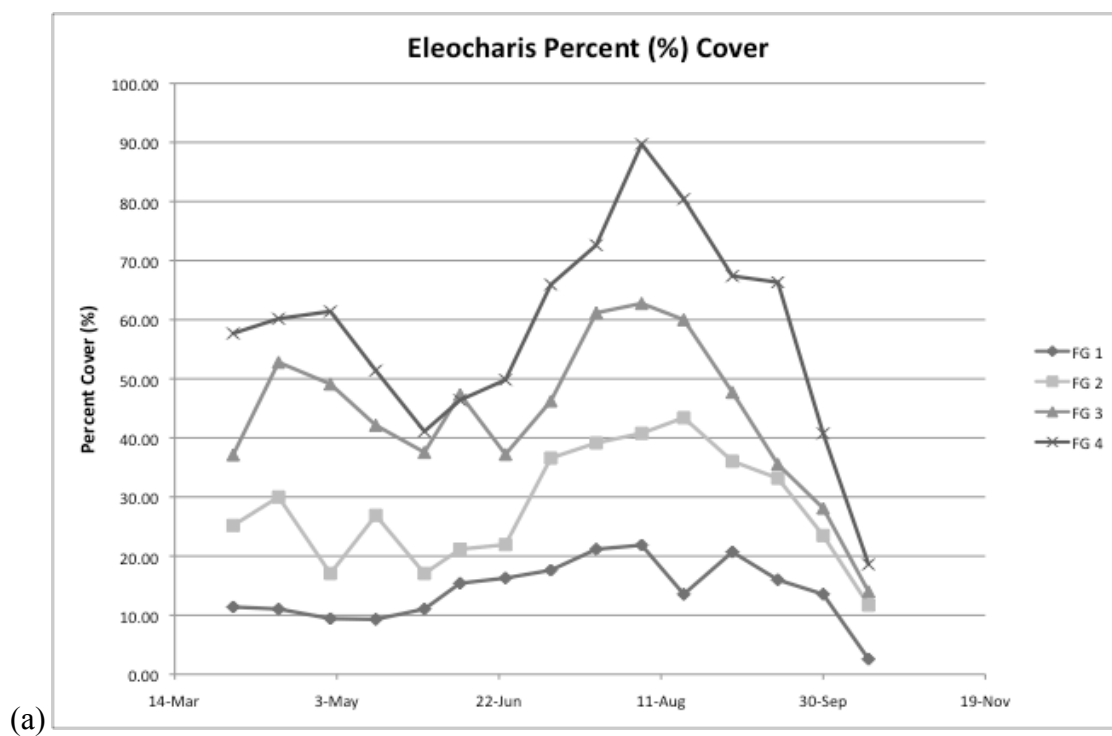
demonstrates how the immediate recovery of a wetland community can depend on the specific species used in planting and the spatial design of the wetland.

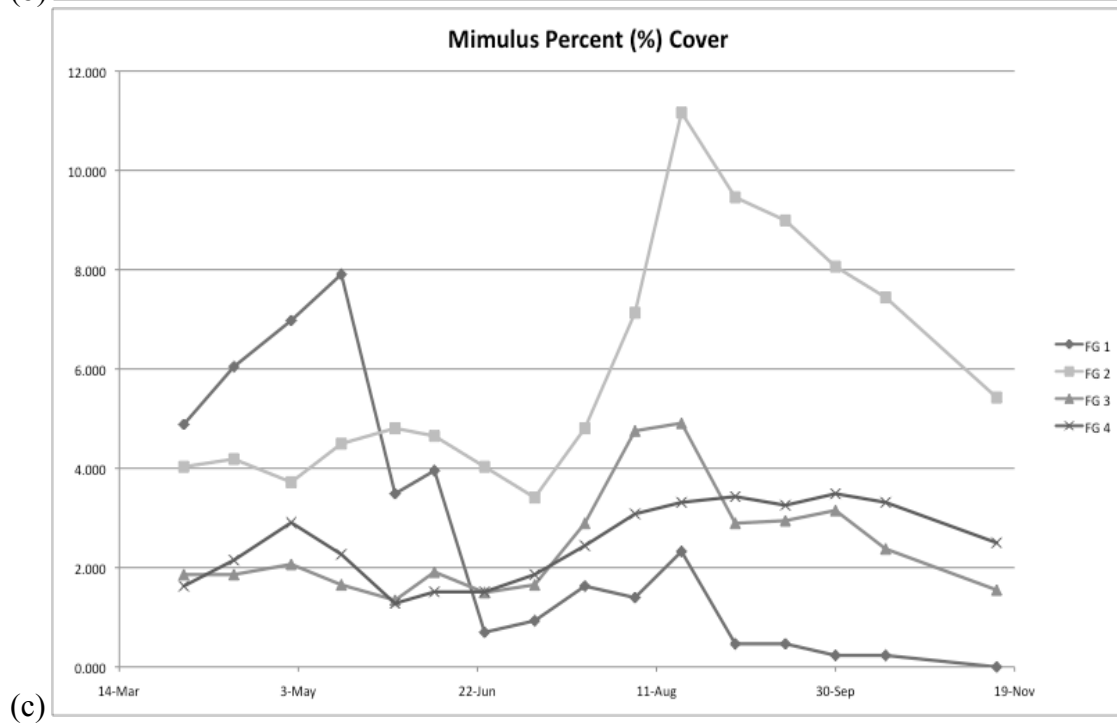
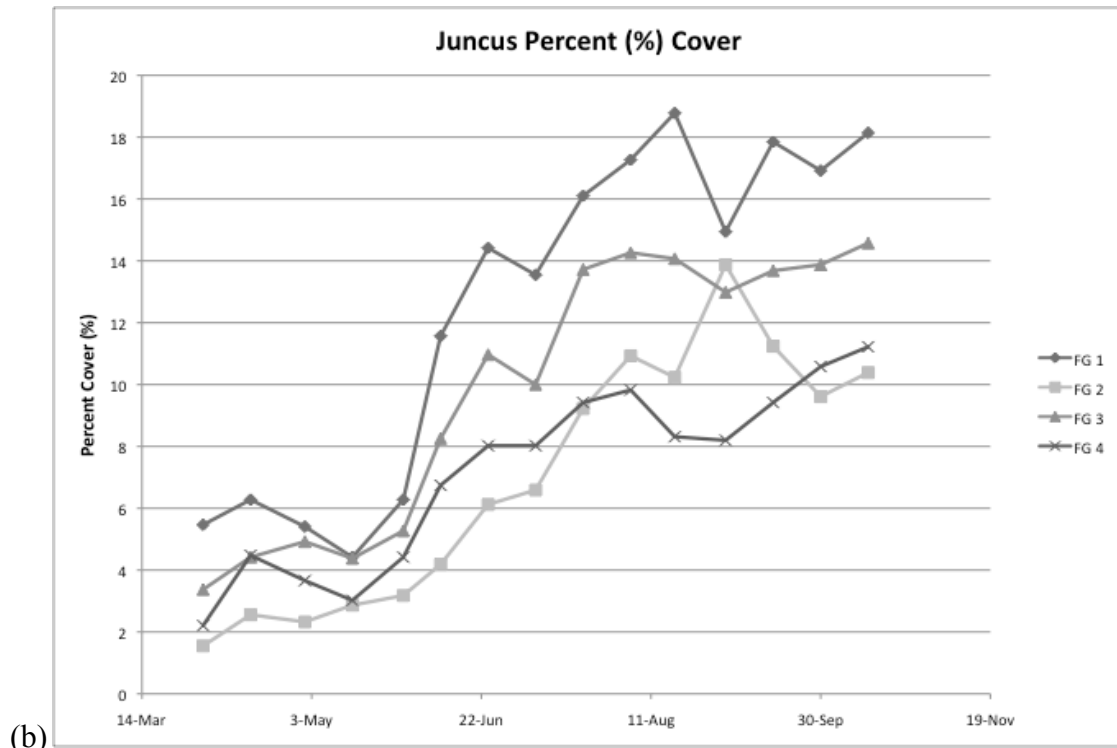
The initial planting of a mitigation wetland can determine the trajectory of the project for many years, however the requirements for planting are limited (NRC 2001). Percent cover and plant diversity are common metrics for determining success of a created wetland (CWA 2002; USACE 2010), however, current policy does not require a species-specific planting regime when creating or restoring a wetland. It is necessary to also consider the interactions of neighboring species during initial planting to prevent the failure of the mitigation project after the first major disturbance. For example, to maximize cover and AGB production, the facultative annual, *M. ringens*, should be planted with neighboring species and the reed, *J. effusus*, should be planted in monoculture. Without proper planning, accounting for species' neighbors and life strategies, a created or restored wetland is likely to fail after a severe disturbance. Likewise, many soil characteristics beyond inundation frequency are not considered. Created and restored wetlands have more homogeneous soil function than their natural counterparts (Bruland et al. 2006), however if properly accounted for, these functions can develop over time (Anderson et al. 2005). Occasionally, C-related characteristics (e.g., C storage and biomass production) of wetlands are used in the production of policy and permitting, however, N-related characteristics such as plant uptake, DEA, and DNRA are not (Moreau 2015).

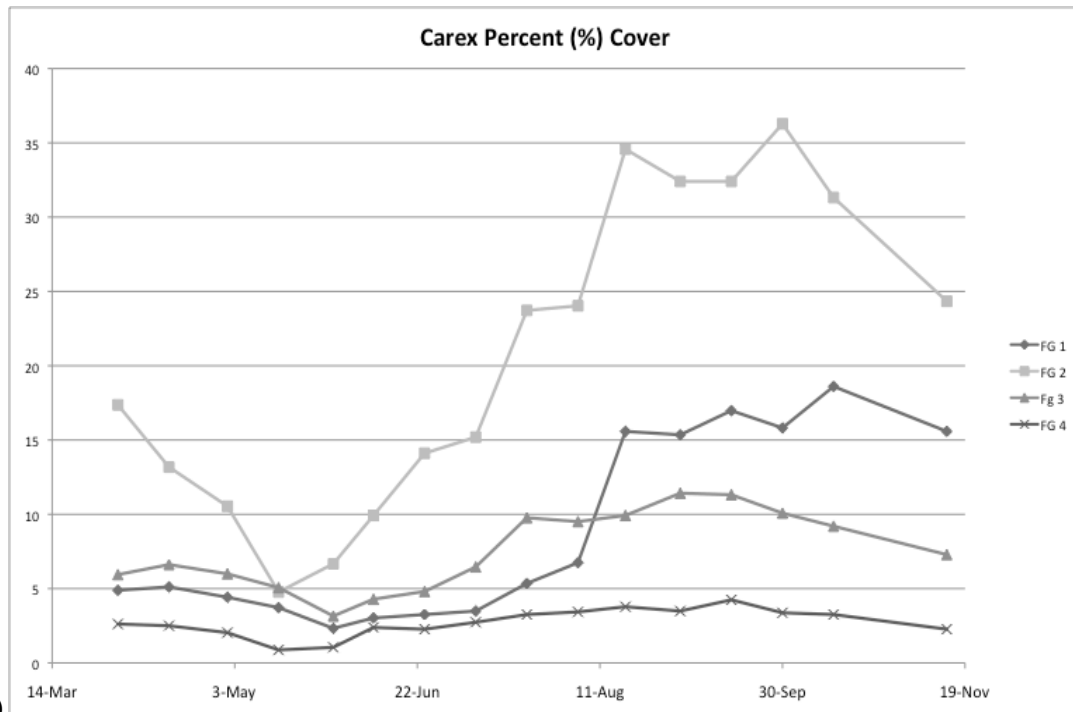
Each of the traits measured here are interconnected and exploring just one does not fully reflect the importance these connections in wetland functioning. Therefore, to

create successful mitigation wetlands that cycle nutrients and store carbon, it is essential that policy require biogeochemical standards for all projects, beyond the current standards for vegetative cover and hydric soils (USACE 2010). Often in the planning of mitigation wetlands, response to disturbances and community resilience may not be considered. Resilience is difficult to quantify as the definition means different things to different disciplines. It becomes more difficult when the pre-disturbance equilibrium we are trying to mimic reflects a centuries old natural wetland. However, as we will continue to convert natural lands to farm lands and urban centers, it is necessary to understand each factor contributing to the resilience and recovery of created/restored wetlands. It is our suggestion that current wetland mitigation policy be updated to include aspects of resilience and recovery. This is will increase the success and cost-effectiveness of mitigation projects, while ensuring continued ecosystem services provided by wetlands.

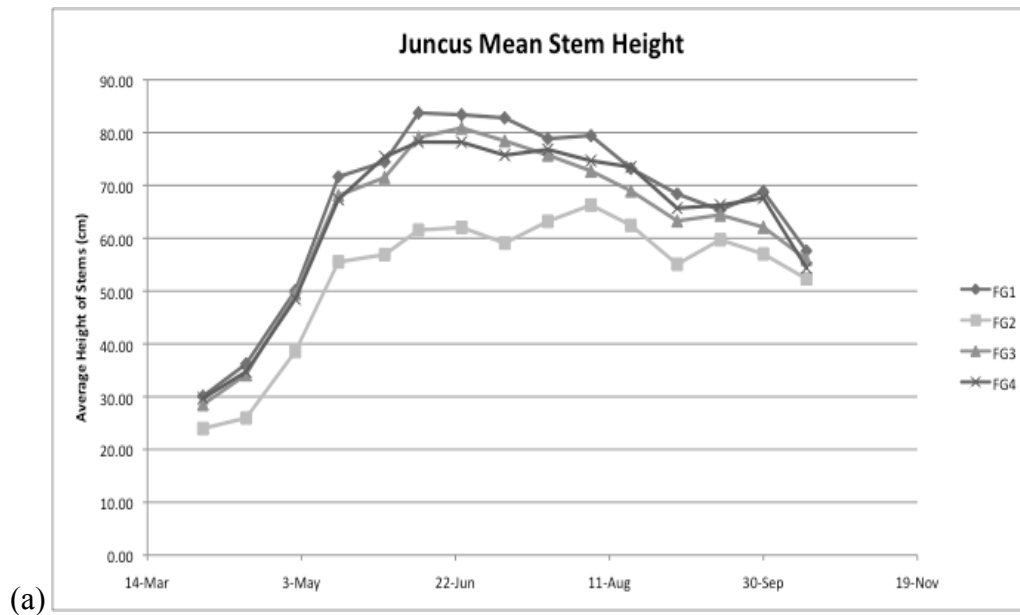
Appendix I



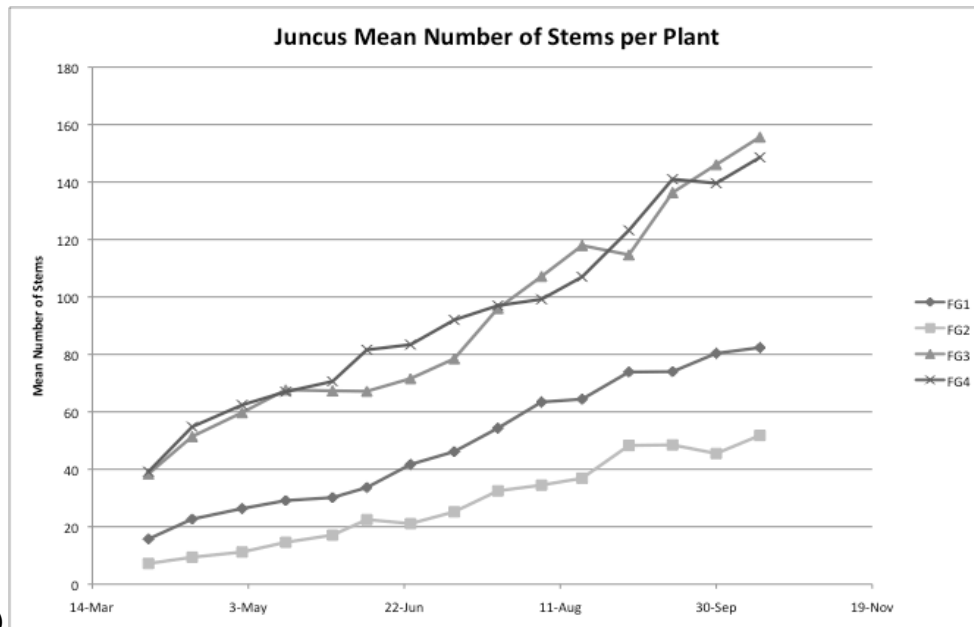




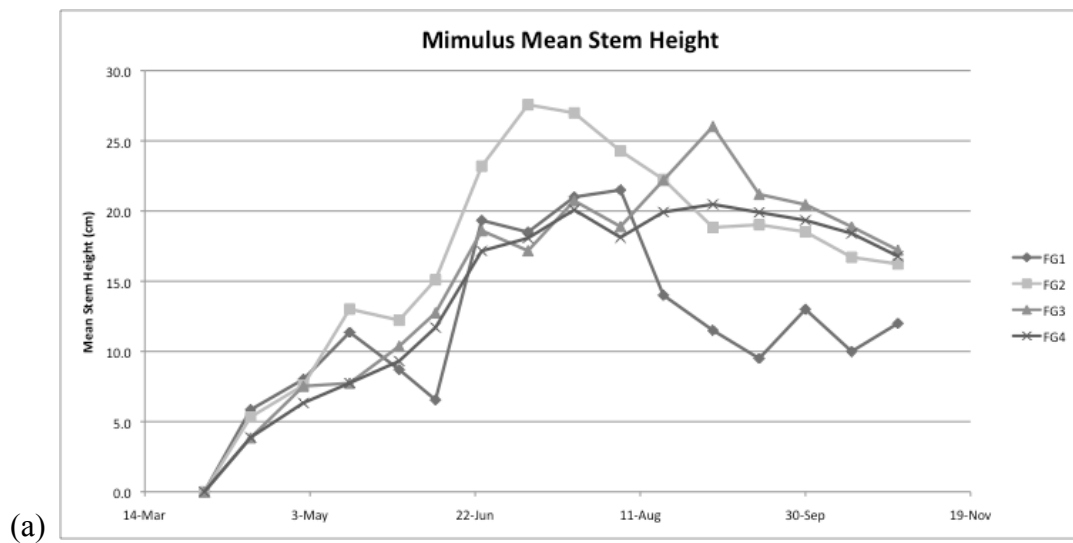
(d) Appendix I Figure 1. Trend in percent cover by functional group for (a) *Eleocharis obtusa* (b) *Juncus effusus* (c) *Mimulus ringens* and (d) *Carex vulpinoidea* over the growing season between April 1 and November 19, 2014.



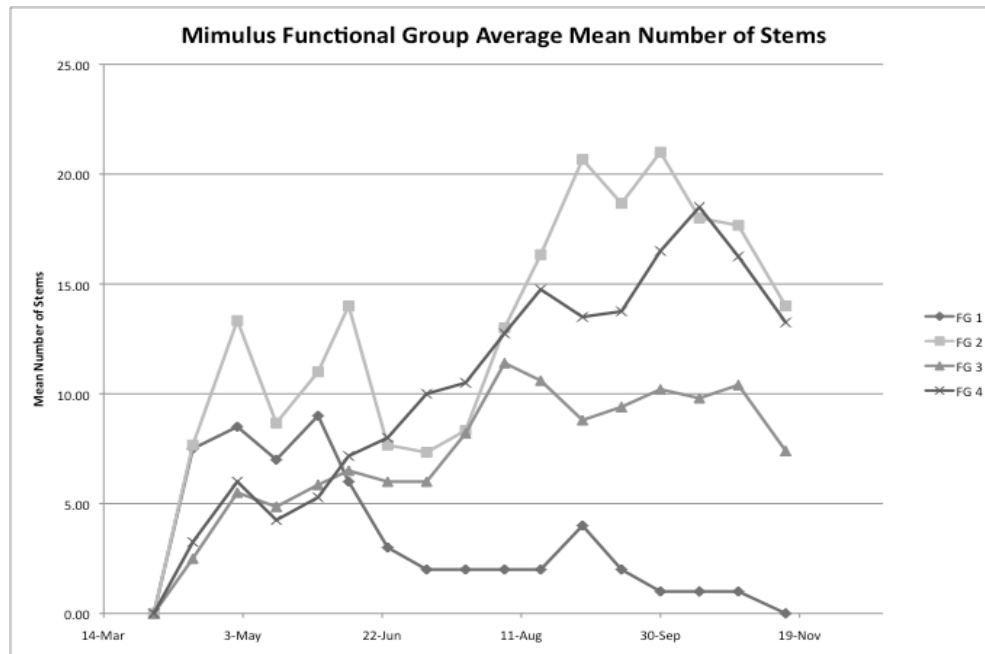
(a)



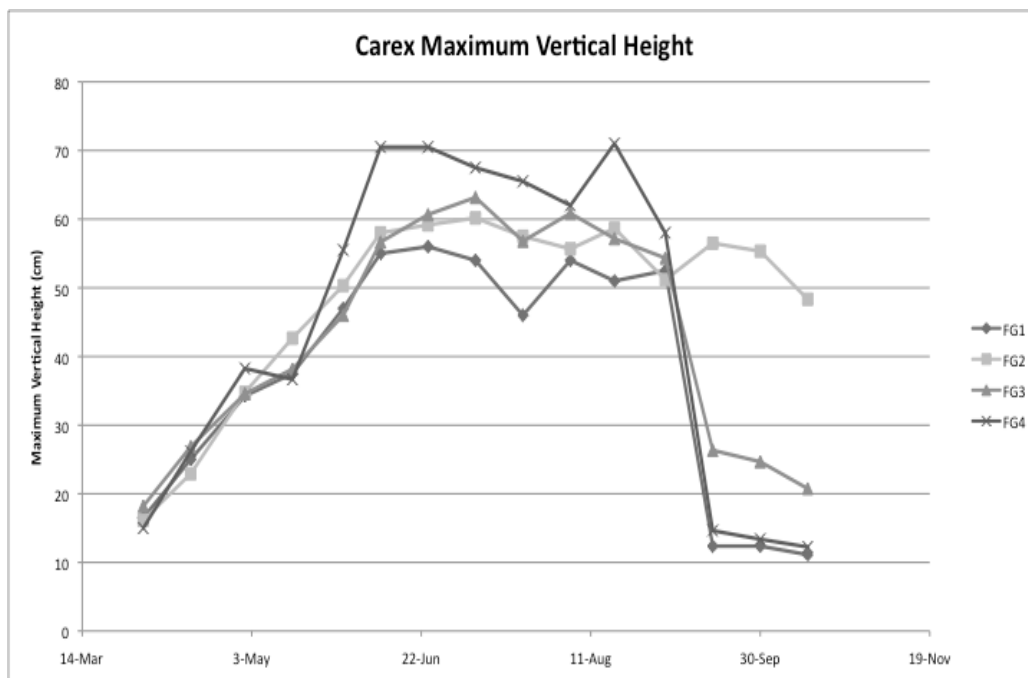
(b) Appendix I Figure 2. (a) Trend in mean stem height observed for each *Juncus effusus* plant in each functional group over the growing season between April 1 and October 28, 2014. (b) Trend showing the mean number of stems per *Juncus effusus* plant in each functional group over the growing season between April 1 and October 28, 2014.



(a)

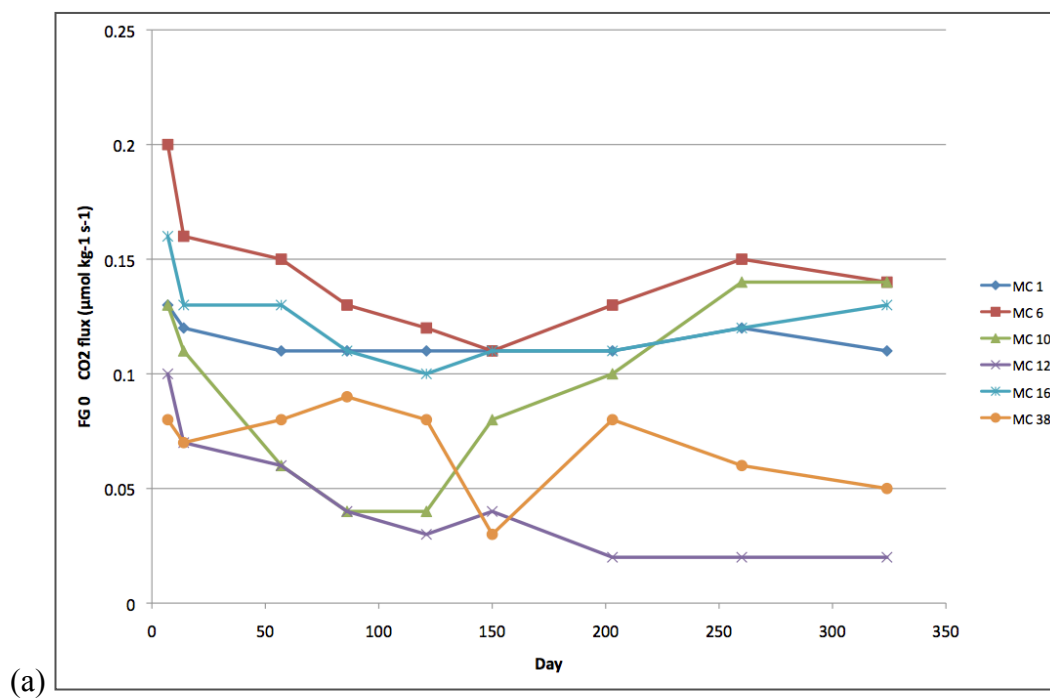


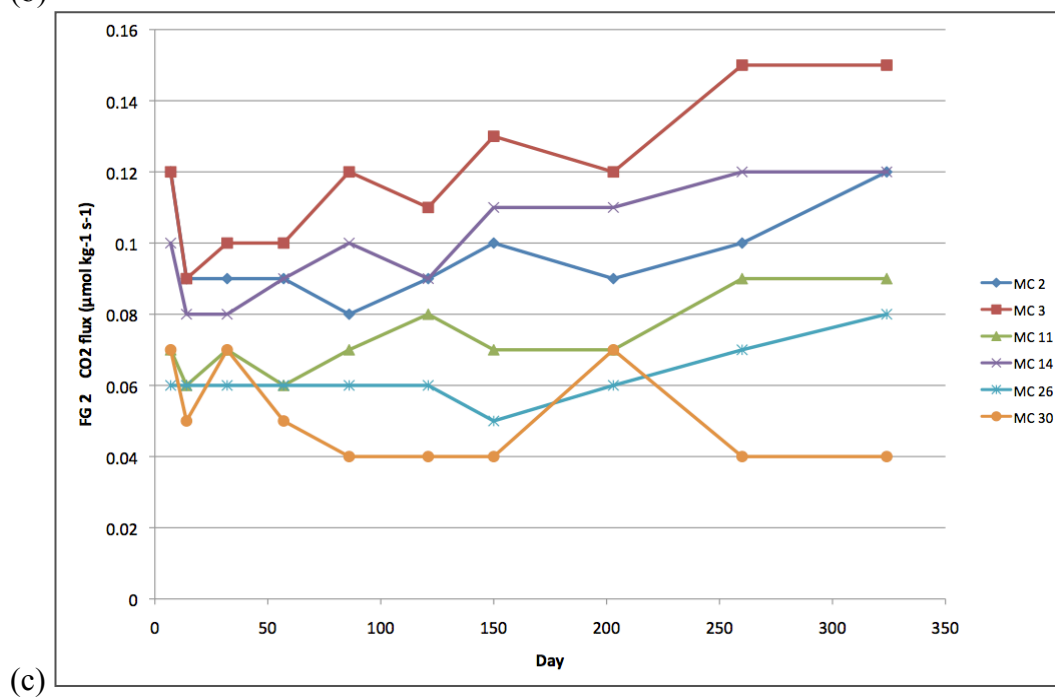
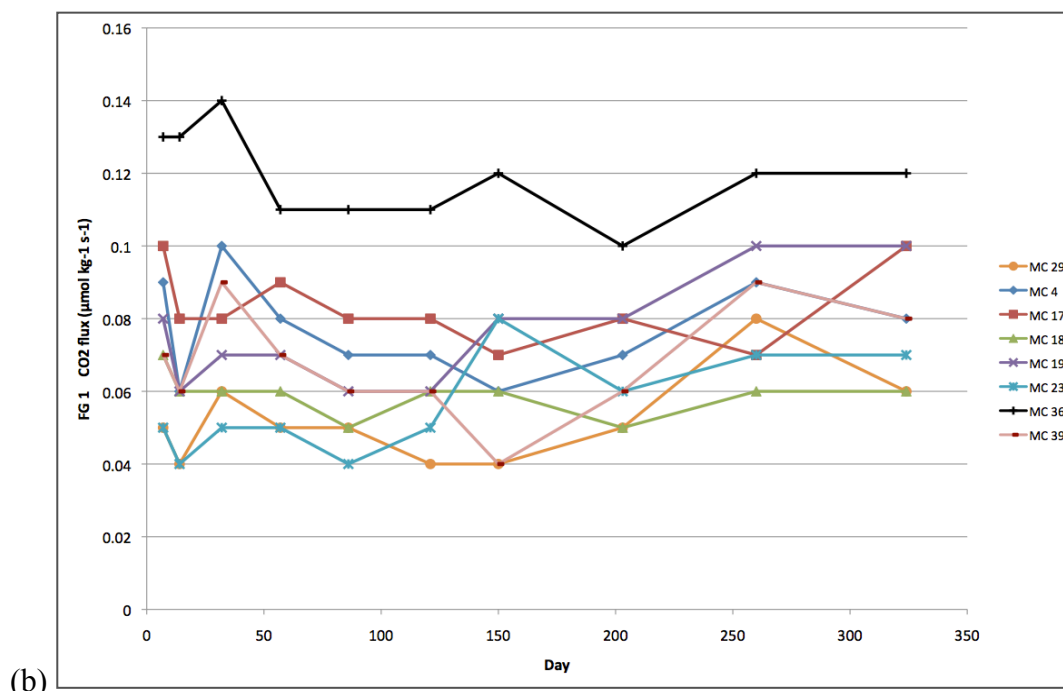
(b) Appendix I Figure 3. (a) Trend of mean stem height in *Mimulus ringens* for each functional group over the growing season between April 1 and October 28, 2014. (b) Trend of mean number of stems for each *Mimulus ringens* shoot in each functional group over the growing season between April 1 and October 28, 2014.

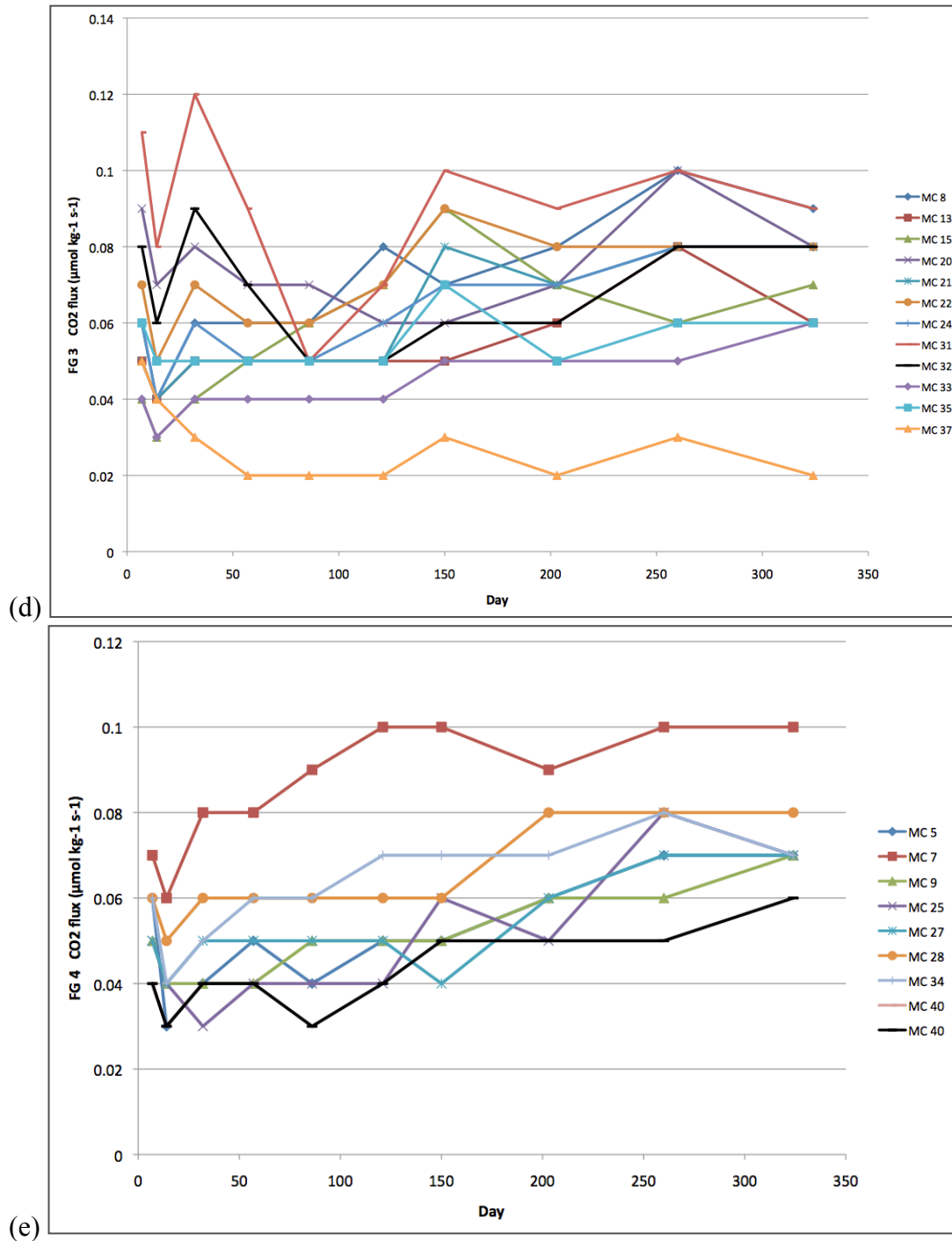


Appendix I Figure 4. Trend in the maximum height of *Carex vulpinoidea* by functional group over the growing season between April 1 and October 28, 2014.

Appendix II







Appendix II Figure I (a-e). CO₂ soil efflux over the 324-day incubation. (a) FG 0, no significant changes over time. (b) FG 1, no significant changes over time. (c) FG 2, no significant changes over time. (d) FG 3, significant decrease from day 7 to day 14 ($p = 0.039$); no change overall. (e) FG 4, significant decrease from day 7 to day 14 ($p = 0.021$), followed by a significant increase overall by day 260 ($p = 0.003$)

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Curriculum Vitae

I was born and raised in Arlington, Virginia. Growing up, my family often took trips to Savannah, Georgia, surrounded by the marshes. With two parents working for the Environmental Protection Agency, the policy involved in environmental science has always been part of my life. However, it wasn't until my AP Environmental Science class that I began to envision a career in the field. I attended the University of Georgia where I graduated in 2007 with a Bachelor of Science degree in Geography with a minor in Ecology. After taking two years off to work full time at a consulting firm, I decided it was time to further my education. It was my fond memories of the salt marshes in Savannah, coupled with the fun of Dr. Ahn's wetland class that encouraged my path in wetland ecology. Through my work at George Mason, I have grown considerably more attached to working in environmental science and I am excited to begin my search for a fulfilling career in environmental mitigation down in Atlanta, Georgia.