



# Planting richness affects the recovery of vegetation and soil processes in constructed wetlands following disturbance



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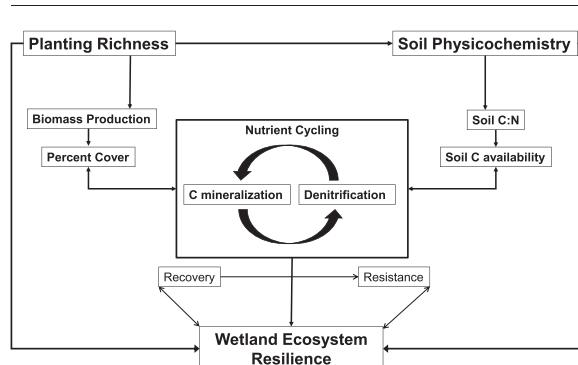
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## HIGHLIGHTS

- The ability of constructed wetlands to recover after a disturbance is poorly understood.
- The study examined first-year responses of vegetation and soil biogeochemistry to disturbance using 40 wetland mesocosms.
- Denitrification potential and CO<sub>2</sub> efflux are linked to the performance of vegetation community recovery.
- Planting richness positively affects plant cover and soil function recovery after a disturbance.

## GRAPHICAL ABSTRACT



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## ABSTRACT

The resilience of constructed wetland ecosystems to severe disturbance, such as a mass herbivory eat-out or soil disturbance, remains poorly understood. In this study, we use a controlled mesocosm experiment to examine how original planting diversity affects the ability of constructed freshwater wetlands to recover structurally and functionally after a disturbance (i.e., aboveground harvesting and soil coring). We assessed if the planting richness of macrophyte species influences recovery of constructed wetlands one year after a disturbance. Mesocosms were planted in richness groups with various combinations of either 1, 2, 3, or 4 species (RG 1–4) to create a gradient of richness. Structural wetland traits measured include morphological regrowth of macrophytes, soil bulk density, soil moisture, soil %C, and soil %N. Functional wetland traits measured include above ground biomass production, soil potential denitrification, and soil potential microbial respiration. Total mesocosm cover increased along the gradient of plant richness (43.5% in RG 1 to 84.5% in RG 4) in the growing season after the disturbance, although not all planted individuals recovered. This was largely attributed to the dominance of the obligate annual species. The morphology of each species was affected negatively by the disturbance, producing shorter, and fewer stems than in the years prior to the disturbance, suggesting that the communities had not fully recovered one year after the disturbance. Soil characteristics were almost uniform across the planting richness gradient, but for a few exceptions (%C, C:N, and non-growing season soil moisture were higher slightly in RG 2). Denitrification potential (DEA) increased with increasing planting richness and was influenced by the abundance and quality of soil C. Increased open space in unplanted mesocosms and mesocosms with lower species richness increased labile C, leading to higher C mineralization rates.

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## 1. Introduction

The effect of plant richness on wetland resilience to disturbance is poorly understood. 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 has been found to contribute 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 niches that are available 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; Byun et al., 2013), causing a constructed wetland to fail. A planted community with a wide range of vegetative functional traits is more likely to establish and maintain other wetland ecosystem services successfully. A diverse vegetative community in wetlands may enhance nutrient cycling (Moser et al., 2009; Beck et al., 2015; Levi et al., 2015), and increase productivity and carbon storage potential (Cardinale et al., 2013; Dee and Ahn, 2014; Means et al., 2016).

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). In constructed wetlands, disturbance can take the form of harvesting plant matter, which has been used as a means of permanently removing nitrogen and phosphorus from the ecosystem (Vymazal, 2007). 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). Disturbances can also impact the structure of soil microbial communities, for example by aerating the soil and increasing available O<sub>2</sub>. 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. In addition, a disturbance in which much or all of the standing plant matter is removed greatly reduces the potentially available C necessary for denitrification. One of the challenges facing constructed or restored wetlands is ensuring that the ecosystem is able to recover and maintain vegetative richness after a disturbance, such as a mass herbivory eat-out or a large storm event (Wilson and Keddy, 1986; Day et al., 2013; López-Mársico et al., 2015). There are, however, few data describing the effects of such disturbances on wetland vegetation communities and subsequent effects on soil biogeochemical functions.

One of the most important functions of wetlands is their ability to remove excess nitrate from waterways, thereby preventing eutrophication downstream. In wetlands, denitrification is the most prominent form of nitrate (NO<sub>3</sub><sup>-</sup>) removal (Washbourne et al., 2011). The main driver of denitrification is available N in the soil (Groffman and Tiedje, 1989; Morse et al., 2012). Denitrification enzyme activity (DEA) also requires an energy source, usually in the form of decomposing carbon-based matter, making the process not only limited by available N, but also by available C (Paul, 2007; Sutton-Grier et al., 2011). Over decades of constructed wetland development, total nitrogen (N), total carbon (C), and soil moisture increase, and bulk density decreases, creating a more ideal environment for microbial functioning (Zak et al., 2003; Paul, 2007; Straathof et al., 2014). Individual plant species influence microbial functioning differently through various morphometric traits and nutrient removal rates (Eisenhauer et al., 2010). Litter accumulation

and the soil microbial community are also important factors in determining the potential removal of nitrates (Craft et al., 2003; Hooker and Stark, 2008; Straathof et al., 2014; Fang et al., 2015). 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 (Hooker and Stark, 2008; Morse et al., 2012). It is necessary to better understand the role that specific plant species play in NO<sub>3</sub><sup>-</sup> removal.

The efflux of carbon dioxide (CO<sub>2</sub>) from wetland soil is by product of many metabolic processes, including denitrification (Mitsch and Gosselink, 2007) and depends on a suite of abiotic and biotic factors, including leaf litter and litter decomposition (Fang et al., 2015; Palta et al., 2012). Denitrification is often strongly linked with the abundance and quality of C in the soil and the production of CO<sub>2</sub> through microbial respiration (Craft et al., 2003; Straathof et al., 2014). Constructed wetlands contain less biomass, soil C, and mineralizable C than their natural counterparts (Hossler and Bouchard, 2010). CO<sub>2</sub> efflux can give insight to the lability (quality) and quantity of carbon as well as 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).

We experimentally tested the effect of planting richness of wetland macrophytes on the resilience of plant and microbial functions following a disturbance. Our study was conducted using a set of 40 freshwater wetland mesocosms that had previously been used in a companion study (Korol and Ahn, 2016) that investigated how planting diversity impacts structural and functional development of plant community and soil nitrogen processes. At the end of the companion study all mesocosms were severely disturbed for sampling by cutting the entire aboveground biomass of the plant community and removing multiple soil cores, drastically reducing autochthonous C inputs. We used the disturbed state of mesocosms as an opportunity to examine how the plant communities and soil biogeochemical processes recover immediately (one year) after the disturbance. Specific study questions are as follows:

1. How does planting richness in constructed mesocosm wetlands affect the recovery of individual plant species, and the plant community as a whole, in the first growing season following the disturbance?
2. How does soil physicochemistry respond following a disturbance?
3. Is there a relationship between plant recovery (as measured in percent cover) and denitrification functional recovery in these wetlands and how does carbon availability influence the relationship?

## 2. Methods

### 2.1. Study set up

This study was conducted in the Ahn Wetland Mesocosm Compound that houses a set of 40, 568 L Rubbermaid® tubs each with a surface area of 1.15 m<sup>2</sup>. The tubs were rain-fed, supplemented by dechlorinated tap water during the driest summer months to maintain 5 cm of standing water at all times. The mesocosms in this study were built to mimic the large constructed wetlands in the Virginia Piedmont region (Dee and Ahn, 2014). Of the 40 mesocosms (Fig. S1), six were unplanted and the remaining 34 were planted along a gradient of planting richness (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). Richness group 1 (RG 1) consisted of eight monocultures, two for each species. Richness group 2 (RG 2) consisted of six mesocosms, each containing two of the four species growing together. Richness group 3 (RG 3) consisted of 12 mesocosms, each containing all possible combinations of three species. Richness group 4 (RG 4) consisted of eight mesocosms, each containing all four species. The tubs were originally vegetated in the spring of 2012 (year 1) to begin a long-term study on the effects of planting richness on the development of constructed wetland communities. All tubs were weeded as necessary to maintain the planting treatments throughout the study.

## 2.2. The disturbance

At the end of the 2013 growing season (year 2), all above ground biomass (AGB) was harvested at the soil surface and below ground biomass (BGB) was cored (3–4 cores per mesocosms) to a depth of 30 cm at the base of each original planting location using 7.62 cm steel duct pipes (Korol and Ahn, 2016). This created a regime of disturbance to all wetland mesocosms. Measurements were taken throughout the 2014 growing season (year 3) after the 2013 disturbance to determine how the initial planting richness impacts the ability of a planted community to recover after disturbance.

## 2.3. Field measurements

Percent cover was measured because it is the most frequently used to determine success of constructed wetlands (CWA, 2002; USACE, 2010). Cover was determined using a grid comprised of 215 squares, each with an area of 51.4 cm<sup>2</sup>. 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 relativized over 1 m<sup>2</sup> (approximate surface area of each mesocosm) and to account for differences in the original number of individuals for each species planted in each mesocosm (for example, when first planted, some mesocosms in RG 3 had two *M. ringens* individuals planted and other had just one individual to have “four” individuals of plants in all mesocosms involved in the study). This was accomplished by dividing the percent cover for each species by the total percent cover in the mesocosm. Although vegetative cover is often used to evaluate the developmental status of wetland plant communities (NRC, 2001), additional species-specific morphometric measurements allow for a fuller understanding of the ability of each species to recover within the community (Diaz et al., 2004). Specific measurements differed based the unique morphology of each species, for example *M. ringens* produces tall, dispersed shoots, whereas *E. obtusa* produces many short shoots that blanket the ground (Table 1). 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. 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*. The morphology data was compared to the two growing seasons prior to the disturbance to better gauge the recovery the recovery. SC was not measured for *J. effusus* in year 2 because it was not a strong predictor for AGB and therefore not useful for the original study by Korol and Ahn (2016). This measurement was re-introduced in year 3 to better compare morphology of the reed after the disturbance.

**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
%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 soil surface 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; %RCv (%Recovery of Cover) estimates the resilience of each species based on cover prior to the disturbance (based on Slocum and Mendelssohn, 2008).

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 (2016). Equations were derived using morphometric measurements and AGB as measured after the harvest in year 2 (Table 2).

We estimated the regrowth of each species after the disturbance (percent recovery of the vegetation) using data from year 2 (Korol and Ahn, 2016) and year 3 (this study). We calculated this by adapting the equation for individual species percent recovery (%RCv) used by Slocum and Mendelssohn (2008).

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

## 2.4. Soil physicochemistry

Soil temperature was taken at the center of each mesocosm (10 cm deep) continuously during the growing season (between June and October of 2014) using iButtons (iButtonLink, Whitewater, Wisconsin). 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 30 mL soil cores (2 cm diameter) from the top 10 cm were taken in three locations of each mesocosm for GSM, %C, and %N. 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. Three soil sub-samples from each mesocosm were composited and a subsample for GSM was weighed, dried in the oven at 105 °C for 2 days, and weighed again. Samples for C and N analysis were air dried for several weeks to avoid possible burning of organic matter and volatilization of N then ground using a mortar and pestle. The ground samples were left to air dry for another 2 days to ensure all moisture was removed. The samples were then analyzed using a 2400 Series II CHN/O elemental analyzer (Perkin-Elmer, Waltham, Massachusetts) to determine %C and %N. BD was measured using intact cores taken with a small aluminum tin of known volume (i.e., 173.5 cm<sup>3</sup>) and weight for each mesocosms within the top 10 cm of soil. The samples for BD were dried at 105 °C for 2 days then weighed again to estimate g dry mass/volume.

## 2.5. Denitrification potential

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 >24 h before analysis. Samples were weighed to 25 g-ww and placed in Erlenmeyer flasks. DEA media was created using 0.1 g chloramphenicol, 1.01 g KNO<sub>3</sub>, 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 gas. 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 min and 105 min. 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 (μg N-N<sub>2</sub>O/kg soil/h) is calculated as the concentration (μg N-N<sub>2</sub>O) at 45 min subtracted from the concentration at 105 min, divided by the soil weight (kg) times the proportion of dry soil (1-soil moisture).

**Table 2**  
Regression equations for AGB estimates based on morphological measurements (adapted from Korol and Ahn, 2016).

Species	Regression equation	R <sup>2</sup>	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, m<sup>2</sup>).

2.6. Soil respiration potential

To determine if carbon is a limiting factor in potential DEA in our mesocosms, the labile and refractory carbon pools were estimated via analysis of CO<sub>2</sub> respiration to estimate soil respiration potential. 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 (Craft et al., 2003). 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 were sieved using a 0.223 in. sieve to remove rocks and live roots. Samples approximately 120.0 g-ww were placed in airtight, 359-cm<sup>3</sup> glass jars, then the headspace was flushed with nitrogen gas for 3 min to create an anaerobic environment (Bridgham et al., 1998). We chose anaerobic incubations because the mesocosm soils were constantly inundated. The jars were left at ambient benchtop temperature to incubate over ten 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 sample analysis, this included an initial three-minute flush with nitrogen to remove any oxygen. Three measurements followed the initial flush, including a five-minute deadband and two-minute sampling period. The headspace was flushed between each triplicate measurement and after the incubation lid was replaced after the third measurement. The CO<sub>2</sub> flux rate (μmol CO<sub>2</sub> s<sup>-1</sup>) was used to calculate potential carbon mineralization in each mesocosm at each sample time (mg-C kg-soil<sup>-1</sup> day<sup>-1</sup>) (Paul et al., 2001). The Labile C pool (LC) is characterized by the rate of carbon mineralization during the first week of incubation (Day 7 mineralization). Increases in microbial biomass during the incubation period is assumed to be constant or insignificant (Robertson et al., 1999).

2.7. Data analysis

All data were tested for normality using a Shapiro-Wilk test. Log and square root transformations were attempted on all data not meeting normality requirements, however no better results were achieved. 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 planting richness groups (RGs) for all variables (morphometric characteristics, soil characteristics, AGB, DEA, and CO<sub>2</sub> efflux, and labile C) as well as being compared across the growing seasons (year 1, year 2, and year 3). Non-parametric analyses were also used to determine differences between individual species for year 3 (2014). In addition, Spearman rank correlations compared the structural data (%C, %N, C:N, BD, GSM, soil temperature (T), total mesocosm cover, and species-specific morphological traits) with the functional data (AGB, DEA and labile C). Significance is determined at p = 0.05. All statistical analyses were conducted using IBM SPSS Statistics version 23.0 (IBM Corp, 2012).

3. Results

3.1. Plant community regrowth

Total mesocosm cover in year 3, the year after disturbance, showed a significant increasing trend with planting richness (43.5% cover in RG 1 to 84.5% cover in RG 4) (p = 0.028) (Table 3). The total percent coverage almost doubled in species mixtures compared to monoculture (RG 2, 3, and 4 vs. RG 1). Total mesocosm cover in RG 1 was influenced by failures (i.e. 0% cover) in the monocultures of the sedge and the facultative annual.

Although the total cover in year 3 was significantly lower than the two years prior to the disturbance, the pattern of increased total cover as planting richness increases remains similar to before disturbance. Total mesocosm cover was significantly lower in RG 1 than in the mixtures for all years, both before and after the disturbance (Fig. 1). There was a 50.8% relative decrease in total mesocosm percent cover in year 3 compared to year 2 (%Cv) in RG 1, 41.3% in RG 2, 37.8% in RG3, and just 31.1% decrease in RG 4 (Fig. 1). In addition, we can see that there is more variability of individual recovery in the community of lower richness groups (e.g., RGs 1 and 2), which diminishes as the richness increases. This suggests a more stable recovery of the community as planting richness increases. It is notable though that in year 2, when each plant community was fully established, there was little difference in the variability of total mesocosm percent coverage within the planting treatments.

3.2. Regrowth of individual species

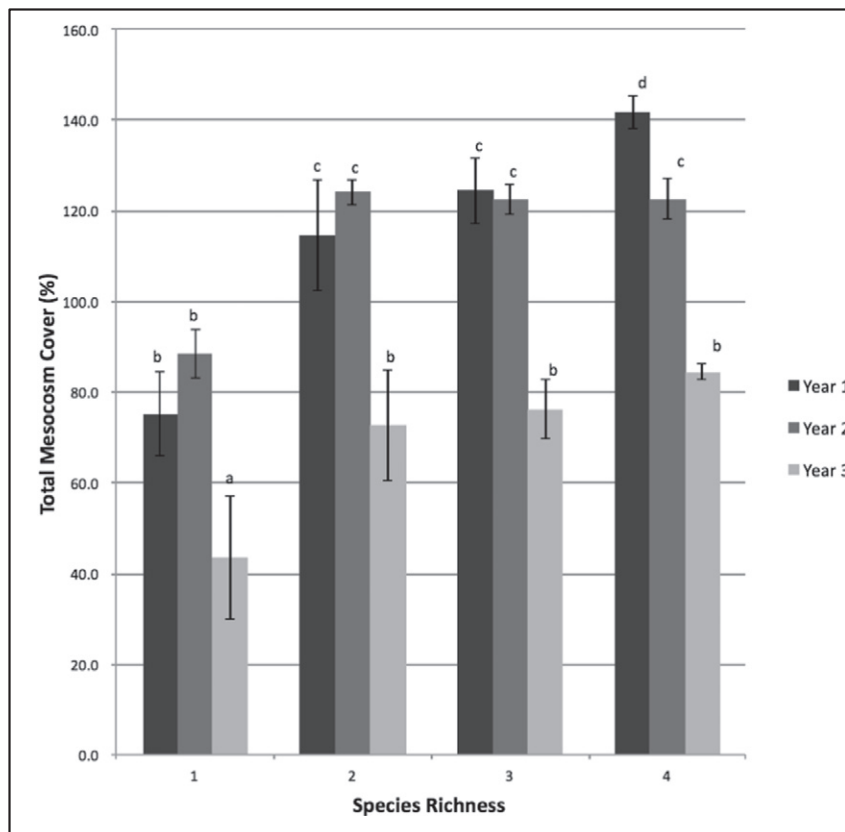
In year 3 the growth of each species peaked at different times between August 5 and September 30. However, we chose a single date, August 18, 2014, to represent peak growth of all species because it most closely encompasses the peak for each species and is comparable to the timing of aboveground biomass harvest in year 2. The obligate annual, *E. obtusa*, became dominant in all mesocosms in which it was planted (Table 3). After the disturbance, the obligate annual was the only species that recovered beyond what was seen in the year 2 growing season across all mesocosms (i.e. >100%RCv). Many of the originally planted individuals of the other three species failed to recover after the disturbance.

The reed, *J. effusus*, was the second most successful in terms of regrowth, although the extent of recovery was inversely related to the number of inter-specific neighbors (Table 3). The reed had significantly less cover as planting richness increased, with monocultures having

**Table 3**  
Relativized percent cover (%Cv) at peak growing season during year 3 (2014) of each plant species by planting richness.

	Fac. annual	Reed	Obl. annual	Sedge	Total MC
RG 1	50.0 ± 25.0	100.0 ± 0.0a	65.2 ± 17.4	50.0 ± 25.0ab	43.5 ± 13.6a
RG 2	17.9 ± 6.6	46.8 ± 18.7b	96.5 ± 1.0	48.5 ± 8.2a	72.8 ± 12.2b
RG 3	5.7 ± 2.3	35.2 ± 10.9b	94.2 ± 2.2	12.6 ± 6.7b	76.2 ± 6.4b
RG 4	3.9 ± 2.0	9.6 ± 4.3c	95.2 ± 1.8	4.6 ± 3.1b	84.5 ± 1.0c

Total MC represents the total vegetative cover over the mesocosm. Significance as determined by Mann-Whitney tests (p ≤ 0.05) is indicated by the letters a, b, and c. Where letters are absent, no significant differences were observed.



**Fig. 1.** Yearly change in total mesocosm standardized percent vegetative cover as compared across both RG and year. Significance as determined by Mann-Whitney tests ( $p \leq 0.05$ ) is indicated by letters a, b, c, and d. Error bars represent  $\pm 1$  standard error.

greater %Cv than RGs 2 and 3 ( $p = 0.044$ ) and RG 4 ( $p = 0.048$ ). Relativized cover of the sedge decreased as planting richness increased (Table 3). The cover percentage of sedge decreased significantly between RG 2 (49% cover) and RG 4 (5% cover) ( $p = 0.012$ ), in addition, one of the two monocultures failed to recover after the disturbance, with 0% cover throughout the 2014 growing season. The facultative annual, *M. ringens*, was the least successful species during the 2014 growing season, with relativized cover between 4% and 18% in RGs 2 through 4 (Table 3) and the eventual failure of both monocultures, one of which only produced two small shoots, which decomposed soon after peak growth measurements were taken.

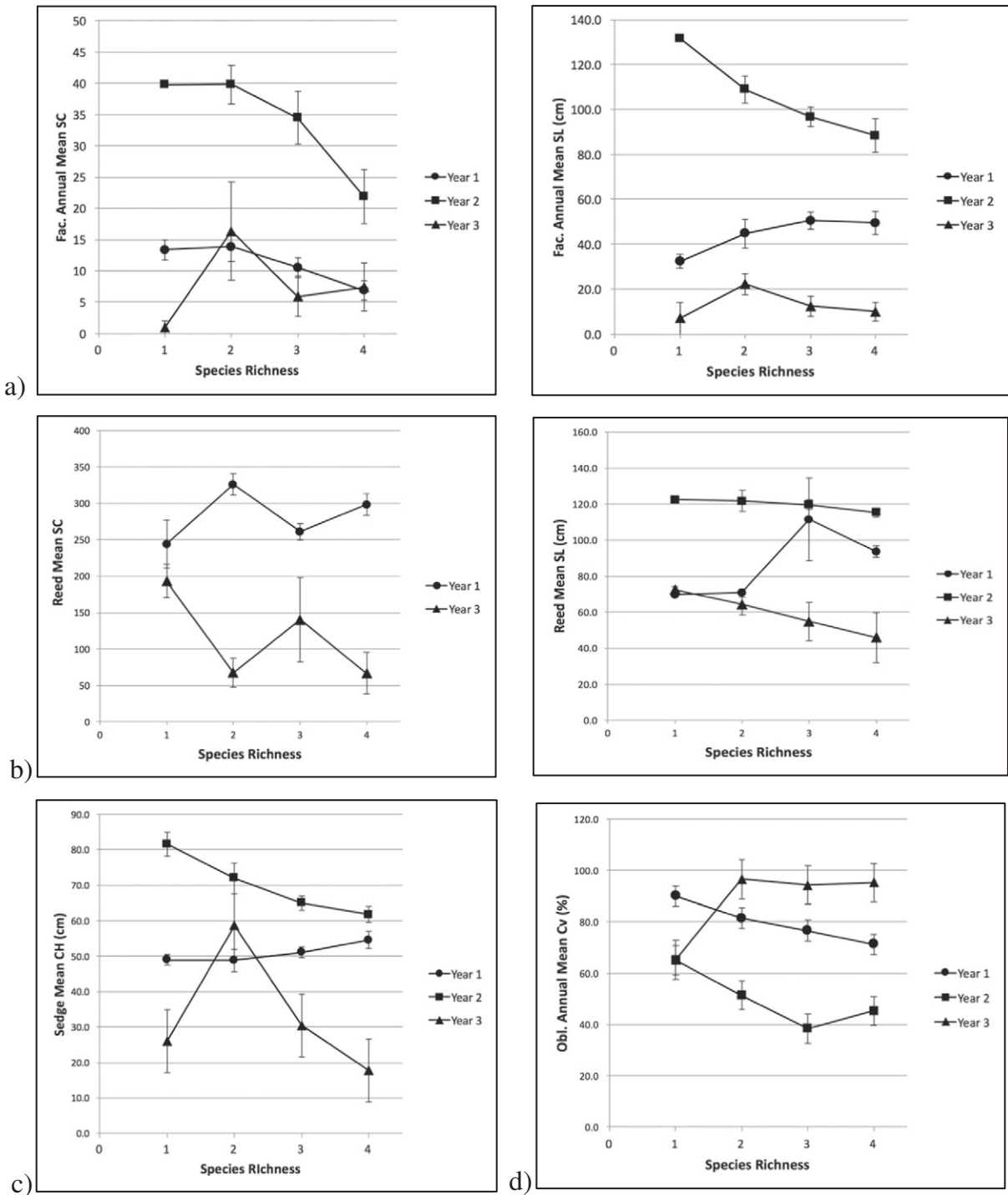
To further investigate the post-disturbance recovery of plant communities we used Kruskal-Wallis followed by Mann-Whitney tests to compare morphometric measurements for each species across the planting group treatments among the three growing seasons (Fig. 2). The facultative annual (Fig. 2a) had significantly more stems in year 2 than in either year 1 or year 3 ( $p < 0.001$ ). Although the SC was statistically the same in year 1 and year 3, the mean stem length (SL) of all richness groups was significantly lower in year 3 than in either of the previous two years (2012: 47.7 cm, 2013: 98.5 cm, 2014: 12.3 cm;  $p = 0.008$ ). For the reed, SC was only measured during year 1 and in year 3 (Fig. 2b). Average SC in each planting richness group was significantly lower in year 3 ( $p < 0.001$ ; Fig. 2b). SL for the reed, which was measured during all three growing season, was statistically the same in year 1 and year 3 (overall average, 95.7 and 54.5 cm respectively) in all RGs, whereas year 2 produced significantly longer stems (overall average 118.7 cm). The sedge (Fig. 2c) increased significantly in canopy height (CH) between year 1 and year 2 (51.9 cm and 66.3 cm respectively;  $p = 0.034$ ). After the disturbance, however, one monoculture and several individuals did not recover leading to a significantly lower overall CH in year 3 (overall average CH = 29.3 cm) for all RGs. Only in RG 2 was CH significantly higher than in year 1 (CH = 58.7 cm;  $p = 0.034$ ).

The obligate annual (Fig. 2d) had significantly higher relativized percent cover (%Cv) in year 3 for RGs 2, 3, and 4 (96%, 94%, and 95% respectively) than compared to the two years prior to the disturbance ( $p < 0.05$ ).

The morphometric estimates of overall average biomass recovery by individual species ranged from 5% (facultative annual) to 80% (obligate annual). The majority of recovered biomass overall was attributed to the success of the obligate annual, which was consistently high across all planting richness groups (Table 4). The reed shows a significant biomass decrease based on planting richness with the year 3 AGB produced in RGs 2, 3, and 4 ( $62.9 \text{ g m}^{-2}$ ,  $66.3 \text{ g m}^{-2}$ , and  $69.9 \text{ g m}^{-2}$  respectively) significantly lower than that of RG 1 ( $174.4 \text{ g m}^{-2}$ ;  $p < 0.05$ ). The sedge produced the most AGB in RG 2 ( $280.0 \text{ g m}^{-2}$ ). Estimated biomass was significantly lower in year 3 than in the previous growing season (i.e., the year 2) for all four species (Table 4;  $p < 0.001$ ).

### 3.3. Soil physicochemistry

No significant differences were observed in soil physicochemistry between the growing and non-growing seasons, however some differences were found between planting richness treatments although with no clear trends across the planting richness gradient (Table 5). As expected, the unplanted mesocosms contained the lowest soil %C during both the growing season and dormant (i.e., non-growing season) (1.3% C and 1.4% C, respectively). In the non-growing season, monocultures and unplanted mesocosms had statistically similar %C ( $p = 0.950$ ). During the growing season, the highest C was found in richness group 2 (1.5% C), particularly in combinations including the facultative annual. This was significantly higher than any other richness group ( $p < 0.05$ ). During the non-growing season, no differences were observed between planted mesocosms. There was no significant difference in %N seen among the mesocosms, all of which had between 0.11% and 0.12% N in both the growing season and non-growing seasons. In addition, we



**Fig. 2.** 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) Relativized percent cover (Cv) for the obligate annual (*E. obtusa*). Error bars represent  $\pm 1$  standard error.

compared %C, %N, and C:N from the year 3 growing season to the measurements taken during year 2, prior to the disturbance (Korol et al., 2016), and found no significant differences.

During the growing season, BD ranged from  $0.96 \text{ g cm}^{-3}$  in RG 4 to  $1.07 \text{ g cm}^{-3}$  in RG 2 with RG 2 being significantly higher than the other treatments ( $p < 0.001$ ). In the non-growing season, the unplanted mesocosms (RG 0) had significantly lower BD ( $0.75 \text{ g cm}^{-3}$ ), with no other differences observed among planting richness treatments.

Gravimetric soil moisture during the growing season was almost uniform (0.30–0.31) across planting richness treatments, with only RG 0 being significantly different (0.28;  $p < 0.001$ ). In the non-growing season, RG 2 was the wettest (0.32) and RG 0 (0.25) remained significantly drier than all other groups ( $p < 0.05$ ).

Soil %C and %N were significantly correlated with each other during both sampling seasons ( $r_s = 0.788, p < 0.01$  and  $r = 0.781, p < 0.01$  respectively, Table 6). Total mesocosm cover (%Cv) was significantly

**Table 4**  
Annual estimate of individual species AGB (g m<sup>-2</sup>) based on morphological measurements.

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

Significant differences between planting richness groups as determined by Mann-Whitney test ( $p \leq 0.05$ ) are indicated by letters a, b, and c. Where letters are absent, no significant differences were observed.

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

correlated with many of the soil functions and characteristics ( $p < 0.01$ ; Table 6), including DEA ( $r_s = 0.570$  during the growing season and  $r_s = 0.738$  during the non-growing season), LC ( $r_s = -0.429$ ), and GSM ( $r_s = 0.403$  during the growing season and  $r_s = 0.531$  during the non-growing season). Maximum height for the sedge was most closely correlated with soil characteristics GSM ( $r_s = -0.454$ ,  $p < 0.05$ ), BD ( $r_s = 0.502$ ,  $p < 0.05$ ), and T ( $r_s = -0.452$ ,  $p < 0.05$ ) during the growing season. It was also significantly correlated with the C:N during the non-growing season ( $r_s = -0.507$ ,  $p < 0.05$ ). The morphological growth characteristics, %Cv, number of stems, and stem height were also correlated with BD ( $r_s = 0.512$ ,  $p < 0.05$ ;  $r_s = 0.477$ ,  $p < 0.05$ ; and  $r_s = 0.615$ ,  $p < 0.01$ , respectively).

### 3.4. Denitrification and carbon mineralization potential

During both the growing season and non-growing season of year 3, the unplanted mesocosms had significantly lower rates of potential denitrification than planted mesocosms ( $p < 0.001$ ; Fig. 3). During the year

**Table 5**  
Soil characteristics. Statistically significant differences among planting richness groups as determined by Mann-Whitney test are 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 <sup>1</sup>	%C <sup>2</sup>	%N <sup>1</sup>	%N <sup>2</sup>	C:N <sup>1</sup>	C:N <sup>2</sup>	BD <sup>1</sup>	BD <sup>2</sup>	SM <sup>1</sup>	SM <sup>2</sup>
RG 0	1.28a	1.37a	0.12	0.11	10.79a	12.01a	0.99a	0.75a	0.28a	0.25a
RG 1	1.42b	1.41ab	0.12	0.11	12.14bc	12.63b	1.02ab	0.99b	0.30b	0.31bc
RG 2	1.52c	1.45b	0.12	0.11	12.52c	12.98bc	1.07b	1.03b	0.30b	0.32c
RG 3	1.31ab	1.50b	0.11	0.11	11.71b	13.19c	0.98a	0.97b	0.30b	0.30b
RG 4	1.35ab	1.46b	0.11	0.11	11.82b	12.81bc	0.96a	1.00b	0.31b	0.31bc

Soil Characteristics measured include % soil carbon (%C), % soil nitrogen (%N), ratio of carbon to nitrogen (C:N), bulk density (BD) (g/cm<sup>3</sup>), and gravimetric soil moisture (SM) (proportion water in soil).

<sup>1</sup> Soils collected July 27, 2014.

<sup>2</sup> Soils collected December 4, 2014.

3 growing season, average potential denitrification rates ranged from 20.1 (RG 0) to 99.6  $\mu\text{g N-N}_2\text{O kg}^{-1} \text{h}^{-1}$  (RG 3), with RG 3 having significantly greater DEA rates than RG 2. In the year 3 non-growing season, average rates ranged from 24.9 (RG 0) to 108.1  $\mu\text{g N-N}_2\text{O kg}^{-1} \text{h}^{-1}$  (RG 4). During the year 3 non-growing season, RG 0, RG 1, and RG 2 were not significantly different ( $p = 0.081$ ). However, DEA in RG 3 and RG 4 were significantly greater than in RG2 and RG1 (Fig. 3). There was a significant decrease in DEA in RG 1 between the growing and non-growing seasons, from an average of 83.5  $\mu\text{g N-N}_2\text{O kg}^{-1} \text{h}^{-1}$  (August 2014) to 48.0  $\mu\text{g N-N}_2\text{O kg}^{-1} \text{h}^{-1}$  (December 2014) (Fig. 3).

DEA measurements were also compared to those taken in the year 2 growing season prior to the disturbance (July 2013). Rates of DEA significantly decreased from before (year 2) compared to after the disturbance (year 3) in richness groups 1 and 2. However, DEA did not change in mesocosms with the highest richness groups (RG 3 and RG 4) or in unplanted mesocosms following disturbance.

The unplanted mesocosms showed the clearest transition from an active, labile C pool (high rate of C respiration potential at start of incubation) to intermediate C pool during anaerobic incubation (Fig. 4). In RG 0 the potential carbon mineralization rate was high during the first three measurements (days 7 to 32) and then decreased to a lower rate for the remainder of the incubation. Monocultures showed no significant change throughout the incubation, with a mineralization rate between 18 mg C kg soil<sup>-1</sup> day<sup>-1</sup> and 24 mg C kg soil<sup>-1</sup> day<sup>-1</sup>. Richness group 2 also showed no significant change throughout the incubation, with a mineralization rate between 20 mg C kg soil<sup>-1</sup> day<sup>-1</sup> and 30 mg C kg soil<sup>-1</sup> day<sup>-1</sup>. Richness groups 3 and 4 had somewhat lower C mineralization potential than richness groups 1 and 2, especially earlier in the incubation. At the beginning of the incubation, RG 4 had the lowest mineralization rate (12 mg C kg soil<sup>-1</sup> day<sup>-1</sup>). After day 121, the average mineralization rate for all groups began to slowly increase and level out (Fig. 4). This increase was only significant between day 14 and day 250 in richness group 4, which had the least variation within the group during each sampling date (SE between 0.9 and 2.0). The variation with richness groups 0, 1, 2, and 3 was large throughout the incubation leading to no differences among the 4 groups over the course of the incubation.

In addition to being positively correlated with cover, both seasons of DEA measurements were significantly negatively correlated with soil labile C (LC) ( $r = -0.541$ ;  $p < 0.01$  during the growing season and  $r = -0.547$  during the non-growing season;  $p < 0.01$ , Table 5). Also, both sets of DEA measurements were positively correlated with soil C:N ( $r = 0.324$ ;  $p < 0.05$ ). The non-growing season DEA measurements were significantly negatively correlated with average May–October soil temperature ( $r = -0.439$ ;  $p < 0.01$ ) and positively with December soil %C ( $r = 0.339$ ,  $p < 0.05$ ). The LC pool (Day 7C mineralization potential) was significantly negatively with GSM during the non-growing season ( $r = -0.398$ ;  $p < 0.05$ ), and with total mesocosm %Cv ( $r = -0.429$ ;  $p < 0.01$ ; Table 4). C:N during both growing seasons was also significantly correlated with GSM ( $r = 0.369$ ;  $p < 0.05$  during the growing season and  $r = 0.450$ ;  $p < 0.01$  during the non-growing season).

Table 6

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

	Cv	AGB	%C <sup>a</sup>	%N <sup>a</sup>	C:N <sup>a</sup>	%C <sup>b</sup>	%N <sup>b</sup>	C:N <sup>b</sup>	DEA <sup>a</sup>	DEA <sup>b</sup>	LC
Cv	1										
AGB	-0.152	1									
%C <sup>a</sup>	0.135	0.049	1								
%N <sup>a</sup>	0.1	-0.371*	0.788**	1							
C:N <sup>a</sup>	0.166	0.402*	0.800**	0.307	1						
%C <sup>b</sup>	0.262	-0.059	0.034	0.092	0.015	1					
%N <sup>b</sup>	0.112	-0.298	-0.026	0.234	-0.222	0.781**	1				
C:N <sup>b</sup>	0.403**	0.182	0.136	0.009	0.234	0.684**	0.188	1			
DEA <sup>a</sup>	0.570**	0.27	-0.198	-0.293	-0.056	0.281	0.084	0.343*	1		
DEA <sup>b</sup>	0.738**	-0.004	0.02	-0.001	0.089	0.339*	0.215	0.324*	0.575**	1	
LC	-0.429**	-0.349*	0.137	0.423**	-0.127	-0.074	0.1	-0.215	-0.541**	-0.547**	1
T	-0.513**	0.352*	-0.034	-0.243	0.164	0.04	-0.077	0.122	-0.116	-0.439**	0.036
GSM <sup>a</sup>	0.403**	0.096	0.235	0.009	0.369*	0.199	-0.072	0.450**	0.328*	0.355*	-0.208
Gumbo	0.531**	0.086	0.154	-0.088	0.307	0.303	0.133	0.301	0.532**	0.248	-0.398*
BD <sup>a</sup>	-0.045	0.25	0.283	0.271	0.101	-0.165	-0.089	-0.241	0.111	-0.111	0.016
BD <sup>b</sup>	0.073	-0.142	0.057	0.085	0.04	-0.009	0.038	-0.018	-0.029	-0.021	0.289
%Cv <sub>M</sub>	0.211	-0.044	0.366	0.203	0.42	-0.008	0.001	-0.164	0.209	0.054	-0.082
#St <sub>M</sub>	0.238	-0.07	0.351	0.186	0.4	-0.012	0.01	-0.21	0.243	0.078	-0.073
St Ht <sub>M</sub>	0.169	-0.002	0.214	0.067	0.322	0.151	0.061	0.047	0.219	-0.035	-0.063
%Cv <sub>J</sub>	-0.109	0.109	0.023	0.049	0.03	-0.012	0.048	-0.047	-0.178	-0.406	0.312
#St <sub>J</sub>	-0.257	0.257	-0.055	-0.037	-0.01	0.055	0.139	-0.139	-0.198	-0.291	0.212
St Ht <sub>J</sub>	0.014	-0.014	0.145	0.179	0.084	-0.146	0.039	-0.329	-0.237	0.205	-0.26
%Cv <sub>E</sub>	0.633**	-0.633**	0.421	0.409	0.311	0.173	0.134	0.284	-0.337	0.097	0.31
%Cv <sub>C</sub>	0.011	-0.011	0.194	0.2	0.013	-0.264	-0.148	-0.362	-0.26	-0.404	0.37
Max Ht <sub>C</sub>	0.035	-0.035	0.106	0.098	-0.018	-0.333	-0.208	-0.507*	-0.12	-0.282	0.2

Cv (total mesocosm cover (m<sup>2</sup>); AGB (Above ground biomass); %C (percent soil carbon); %N (percent soil N); C:N (carbon to nitrogen ratio); DEA (denitrification enzyme activity – μmol N-N<sub>2</sub>O kg soil<sup>-1</sup> h<sup>-1</sup>); LC (labile carbon measured day 7 of incubation – mg C kg soil<sup>-1</sup> day<sup>-1</sup>); T (Temperature - °C); BD (bulk density – g cm<sup>-3</sup>); GSM (gravimetric soil moisture – proportion water in soil); %Cv<sub>x</sub> (percent cover for individual species); #St<sub>x</sub> (number of stems); St Ht<sub>x</sub> (stem height); Max Ht<sub>C</sub> (Max height of *Carex*).

<sup>a</sup> Soils collected July 27, 2014.

<sup>b</sup> Soils collected December 4, 2014.

\* Indicates significance at  $p = 0.05$ .

\*\* Indicates significance at  $p = 0.01$ .

## 4. Discussion

### 4.1. Vegetative recovery

Many studies have shown the positive relationship between planting diversity and plant community productivity (Cardinale et al., 2013; Williams and Ahn, 2015), stability (Loreau et al., 2002), and resilience (Carvalho et al., 2012). We hypothesized that communities with higher planting richness would recover more successfully after a disturbance, using percent total vegetative cover as a metric. Cover is the most commonly used indicator of plant community development in evaluating constructed mitigation wetlands (NRC, 2001). The total mesocosm percent cover after disturbance, which was higher in planting mixtures with more species, supported this hypothesis. We also found less variability in mixtures, further indicating that higher planting richness increases the likelihood of recovery one year after a disturbance. This pattern was particularly evident when comparing monocultures to mixtures due to the high variability in the success of monocultures. 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 planting richness groups.

While total mesocosm cover increased with the planting richness in year 3 after disturbance, none of the richness groups were able to reach the percent cover observed in year 2 (prior to the disturbance). Mesocosms with the highest richness (RG 4) had the strongest recovery following disturbance, with the greatest percent recovery of plant cover (84%) compared to richness groups 2 and 3, each of which were greater than monocultures. Thus, planting diversity improved the regrowth of plant cover after the disturbance. However, recovery was not yet complete and we cannot say that the communities at this time are stable (Holling, 1973). The failure of individuals of certain species indicates that the community recovery is heavily dependent on the species-

specific relationships. It is likely that not all species were fully mature at the end of this study. The morphology of *M. ringens* in year 3, for example, was quite similar to that of year 1, suggesting that the species requires a second growing season to become fully established within the community. Korol and Ahn (2016) observed the full growth of all four species achieved at the end of the year 2 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.

In an upland prairie study, the restoration of the community was influenced not only by individual plant traits, but also their neighbors (Robert 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. Many of the morphometric measurements for each species showed that growth was much less successful after the disturbance (year 3). Only the obligate annual, *E. obtusa*, a dominant ruderal (Grime, 2001), was able to restore cover to the levels seen before the disturbance in all four planting richness groups, the same observed in Korol and Ahn (2016). None of the other three species were able to thrive in mesocosms with the highest richness, where *E. obtusa* was consistently present.

It is important to take into account which species are planted with neighbors and which are isolated. 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 constructed wetland where rapid vegetation colonization 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 *E. obtusa* with neighboring species so as to avoid a potential monoculture. While other species also became established when



Table 6 (continued)

T	GSM <sup>a</sup>	Gumbo	BD <sup>a</sup>	BD <sup>b</sup>	%Cv <sub>M</sub>	#St <sub>M</sub>	St Ht <sub>M</sub>	%Cv <sub>J</sub>	#St <sub>J</sub>	St Ht <sub>J</sub>	%Cv <sub>E</sub>	%Cv <sub>C</sub>
1												
0.158	1											
-0.031	0.423**	1										
-0.255	-0.241	-0.073	1									
-0.155	0.104	0.023	-0.071	1								
-0.108	-0.204	0.138	0.246	0.15	1							
-0.03	-0.105	0.194	0.242	0.135	0.969**	1						
-0.044	-0.202	0.282	0.23	0.205	0.769**	0.815**	1					
-0.127	-0.216	-0.122	0.512*	0.271	0.089	0.128	0.348	1				
-0.149	-0.329	-0.21	0.477*	0.183	0.222	0.307	0.42	0.887**	1			
-0.741**	-0.418	-0.217	0.615**	-0.036	0.472	0.429	0.363	0.392	0.506*	1		
-0.312	0.101	0.03	-0.204	-0.19	0.038	-0.094	-0.282	0	-0.313	-0.205	1	
-0.359	-0.367	0.061	0.528*	0.217	0.624*	0.527*	0.539*	0.349	0.286	0.507	0.081	1
-0.452*	-0.454*	0.104	0.502*	0.122	0.535*	0.485	0.497	0.253	0.262	0.517*	0.008	0.922**

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. The reed, *J. effusus*, produced large quantities of AGB when planted in monoculture relative to the mixtures, although it was not able to produce as many tall stems as prior to the disturbance.

The stems produced by *M. ringens* in year 3 were shorter and weaker and were potentially susceptible to storms and foraging by animals. Both post-disturbance monocultures eventually failed, suggesting this

species is particularly prone to disturbance when grown alone. In addition, *M. ringens* did best in mixtures with only one other species (RG 2) indicating that one other species provides support for growth, but increasing the richness too much could cause nutrient or space limitations. The failure of the sedge in one monoculture also indicates that this species is prone to disturbance when grown alone. However, as with the facultative annual, *C. vulpinoidea* thrives with just one other species but may be hindered by the presence of multiple neighbors.

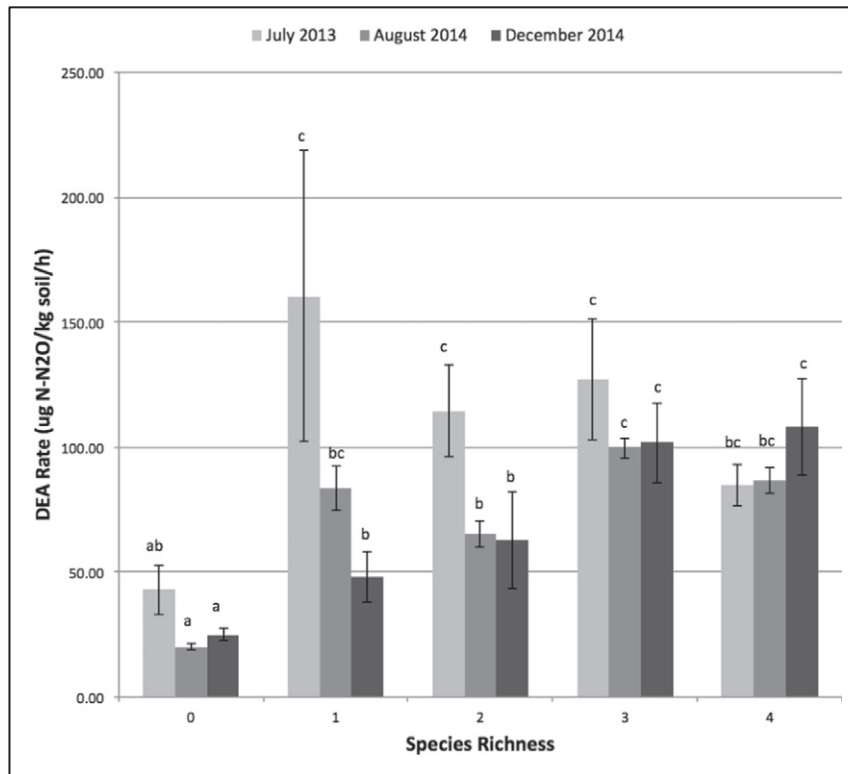


Fig. 3. Seasonal denitrification potential ( $\mu\text{mol N-N}_2\text{O kg soil}^{-1} \text{h}^{-1}$ ). Statistically significant differences among planting richness groups as determined by Mann-Whitney tests are indicated by letters a through c ( $p < 0.05$ ). Differences were measured across both RG and year. Error bars represent  $\pm 1$  standard error.

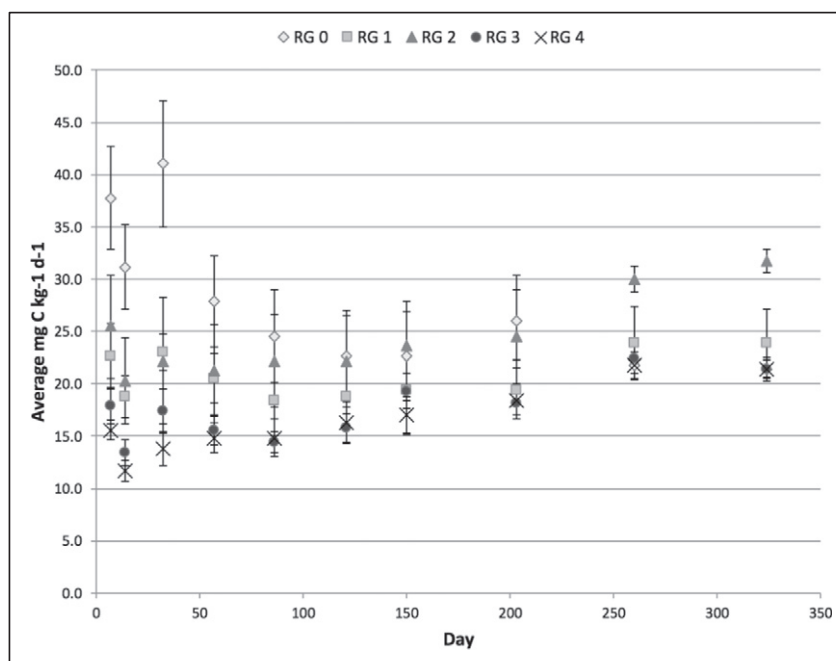


Fig. 4. Potential carbon mineralization ( $\text{mg C kg soil}^{-1} \text{ day}^{-1}$ ) over the 324-day incubation (December 11, 2014 – October 19, 2015) derived from  $\text{CO}_2$  flux. Error bars represent  $\pm 1$  standard error.

Other studies have also found that vegetation performance after a disturbance is species specific (Speed et al., 2010; Pfeifer-Meister et al., 2012). Although total mesocosm cover increased as planting richness increased, many of the morphological attributes showed signs of stress in highest planting richness scheme; short and thin shoots were seen in the facultative annual, shorter shoots seen in the reed, and a lower max canopy height in the sedge. 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 planting richness and species type.

#### 4.2. Soil physicochemistry

No seasonal differences were observed in planted versus unplanted mesocosms for almost all soil attributes measured. Nitrogen abundance was one characteristic that was uniform across all richness groups. Freshwater wetlands typically act as an N sink, with more N cycling and uptake within the system than flowing out (Bowden, 1987). It was expected that the soil total nitrogen would vary with changing planting richness since it has been found that different morphometric traits, plant evenness, and species diversity all influence the uptake and availability of N in the soil (Eisenhauer et al., 2010; Zak et al., 2013; McGill et al., 2010; Korol et al., 2016), however we did not see this in the soil N content. The other soil characteristics did not follow any monotonic trends based on planting richness, with few significant differences apparent in our study. RGs 2 and 3 had slightly higher levels of soil 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. BD was the most influential soil characteristic in terms of morphological recovery, as it dictates the ability for plant roots to grow. Lower bulk densities tend to allow for better root growth,

although the type of soil can also play a role (Tracy et al., 2013). In this study, BD was positively correlated with the recovery of both the sedge and the reed. As wetlands develop, the BD decreases and SM increases as the soil becomes more organic (Anderson et al., 2005; Ahn and Jones, 2013). Because the sedge is positively correlated with BD and negatively correlated with SM, it is likely that in more mature wetlands, the re-growth of *C. vulpinoidea* after a disturbance would be hindered.

#### 4.3. Denitrification and carbon mineralization potential

The most important factors affecting denitrification are the absence of oxygen, the presence of nitrate in the surface water, and temperature (Groffman et al., 1999; Song et al., 2014). 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  $\text{O}_2$ . In addition, changes to the vegetation community can alter the available C and uptake of N after a disturbance. Plant species and their spatial distribution in a wetland have been shown to alter the availability of C, another integral contributor to the denitrification rate (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 observed that DEA does not vary based on plant community structure, but rather higher diversity simply leads to more constant denitrification rates over time (Hopfensperger et al., 2009; McGill et al., 2010). Our study found that in response to a disturbance, planting richness and plant recovery influences DEA, with higher richness groups (RG 3 and RG 4) having higher rates of DEA in the non-growing season after disturbance compared to those of lower richness. Two of the eight monocultures, however, did not recover at all after the disturbance and one failed after the end of the growing season. The strong positive correlation between denitrification potential and plant cover during both sampling seasons suggests that the overall community success, and therefore increased macrophyte C input into the soil, is more important than the individual species for biogeochemical processes to continue.

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 past studies of the large constructed wetlands in the Virginia piedmont region. 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. Similarly, studies on seasonal variations in potential DEA show conflicting results. The seasonal differences in denitrification rates are dependent on a suite of variables including timing of plant growth, abundance of leaf litter, concentration of C, and temperature to regulate microbial activity, (Boyd, 1978; Groffman and Tiedje, 1991; Hooker and Stark, 2008; Morse et al., 2012; Hopfensperger et al., 2009; Cornwell et al., 1999; Richardson et al., 2004; Palta et al., 2012). Temperature, however, had a moderate negative correlation with the non-growing season DEA. In our study, no difference between samples taken in early August and those taken in December was found. The first freeze of the season took place almost a month prior to sampling, however the week prior to sampling was unseasonably warm with air temperatures reaching up to  $26.1^\circ\text{C}$  and lows well above freezing (Vienna Virginia Weather Archive, 2015). 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, temperature in the soil must drop to at least  $5^\circ\text{C}$  (Cornwell et al., 1999).

Changes to the available C:N can impact the structure of the microbial community as well, either increasing or decreasing the presence of denitrifying bacteria (Peralta et al., 2013; Nijburg et al., 1997). A significant positive correlation between C:N and DEA was observed, however the relationship is weak to moderate at best. Other than the topsoil used in the creation of the mesocosms, the only additional N was from ambient deposition. The mesocosms in this study are ombrotrophic and, unlike large-scale wetlands, are not supplemented with  $\text{NO}_3^-$  except through atmospheric inputs and negligible amounts in tap water. Although the DEA technique removes N limitation during the assay, 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). The C:N in our mesocosms reflects both the low availability of N as well as the reduced C from litter input due to harvesting AGB in year 2.

Denitrification potential 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). Measuring  $\text{CO}_2$  production from the soil gives insight into the activity of the microbial community and the availability of soil C for DEA. Potential C mineralization indicated a steady intermediate C pool across the planting richness gradient.  $\text{CO}_2$  flux values from the planted mesocosm soils in all richness groups never reduced to low enough levels to indicate a complete transition from labile C consumption to refractory C consumption (Paul et al., 2001). The highest C mineralization rates were seen early in the incubations of the unplanted mesocosm soils and decreased over time, suggesting consumption of a labile C pool. This suggests that C inputs to unplanted mesocosms were more readily available to support respiration of the microbial community than the litter from the planted species (Uselman et al., 2012). The high rates of  $\text{CO}_2$  flux and the low rates of DEA in the unplanted mesocosms and some replicates of richness groups 1 and 2 were likely influenced by the abundance of algae produced in unplanted mesocosms (McAndrew et al., 2016) and mesocosms with little vegetative cover. Although it was not specifically measured, increased algal growth was observed in mesocosms with little to no vegetative cover throughout the year 3 growing season. Algae are a form of labile C that microbes preferentially consume also leading

to greater rates of N uptake and incorporation into microbial biomass (Norrman et al., 1995; Fouilland et al., 2013). The high microbial metabolic rates from algal C inputs would produce  $\text{CO}_2$  while also reducing the available N for DEA.

Another possibility is that the microbial community may be using dissimilatory nitrate reduction to ammonium (DNRA) rather than denitrification (Nijburg et al., 1997; Washbourne et al., 2011). Where nitrate is limited, the presence of C, particularly highly labile C, controls the composition of the bacterial community and leads to a dominance of DNRA and lower denitrification rates (Nijburg et al., 1997; Wallenstein et al., 2006). Thus, the labile C and low  $\text{NO}_3^-$  conditions, especially in mesocosms that were unplanted or planted with low richness and did not recover after disturbance, may have led to DNRA favored over denitrification (Burgin and Hamilton, 2007). The disturbance to the soil and re-vegetation of the mesocosms also could have introduced oxygen to the soil, altering the microbial community. The possible presence of oxygen from disturbance increases the likelihood of DNRA because it is not inhibited by  $\text{O}_2$  in the way that denitrification is.

## 5. Conclusion

We measured how a planting richness influenced the recovery of the macrophyte community and soil biogeochemistry after a disturbance in constructed wetlands. Higher planting richness resulted in greater plant cover growth and higher denitrification potential. The responses of individual species varied with *Eleocharis obtusa* and *Juncus effuses* being more successful in their recovery than the others. Although the overall vegetation community was still not fully recovered only one year after the disturbance, it may be approaching pre-disturbance levels and may likely continue towards full recovery in the future. Potential denitrification after the disturbance was greater in higher richness groups (RGs 3 and 4) compared to RGs 1 and 2 (or RG 0), following the trend in total plant cover following disturbance. Denitrification rates in species poor treatments were likely lower in part due in part to greater algal biomass leading to enhanced microbial respiration of labile soil C and associated nitrate uptake, although this requires further quantitative study. The outcomes of the study suggest that constructed wetlands planted with more species are likely to have greater functioning and stability after a disturbance. A diverse and specific planting regime is recommended for consideration while planning the creation or restoration of wetlands in order to provide insurance against severe disturbances they may face. To address the limitation on the spatial scale and number of species inherent with the use of mesocosms, additional studies in large-scale wetlands that include more species rich treatments are recommended to gain a more in-depth understanding of structural and functional resilience of wetlands.

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.scitotenv.2016.11.134>.

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