



## Research article

## Carbon storage potential by four macrophytes as affected by planting diversity in a created wetland

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## ABSTRACT

Wetland creation has become a commonplace method for mitigating the loss of natural wetlands. Often mitigation projects fail to restore ecosystem services of the impacted natural wetlands. One of the key ecosystem services of newly created wetlands is carbon accumulation/sequestration, but little is known about how planting diversity (PD) affects the ability of herbaceous wetland plants to store carbon in newly created wetlands. Most mitigation projects involve a planting regime, but PD, which may be critical in establishing biologically diverse and ecologically functioning wetlands, is seldom required. Using a set of 34 mesocosms (~1 m<sup>2</sup> each), we investigated the effects of planting diversity on carbon storage potential of four native wetland plant species that are commonly planted in created mitigation wetlands in Virginia – *Carex vulpinoidea*, *Eleocharis obtusa*, *Juncus effusus*, and *Mimulus ringens*. The plants were grown under the four distinctive PD treatments [i.e., monoculture (PD 1) through four different species mixture (PD 4)]. Plant biomass was harvested after two growing seasons and analyzed for tissue carbon content. Competition values (CV) were calculated to understand how the PD treatment affected the competitive ability of plants relative to their biomass production and thus carbon storage potentials. Aboveground biomass ranged from 988 g/m<sup>2</sup> – 1515 g/m<sup>2</sup>, being greatest in monocultures, but only when compared to the most diverse mixture ( $p = 0.021$ ). However, carbon storage potential estimates per mesocosm ranged between 344 g C/m<sup>2</sup> in the most diverse mesocosms (PD 4) to 610 g C/m<sup>2</sup> in monoculture ones with no significant difference ( $p = 0.089$ ). CV of *E. obtusa* and *C. vulpinoidea* showed a declining trend when grown in the most diverse mixtures but *J. effusus* and *M. ringens* displayed no difference across the PD gradient ( $p = 0.910$ ). In monocultures, both *M. ringens*, and *J. effusus* appeared to store carbon as biomass more effectively than the other species, suggesting that the choice of plant species may play an important role in facilitating the development of carbon accumulation/storage in created wetlands. Plant community diversity provides many ecosystem services (e.g., habitat and floristic quality) other than carbon storage function. Thus, a further study is needed that will focus on investigating how other design elements such as microtopography and hydrologic connectivity may interact with PD in terms of enhancing the carbon storage potential of newly created wetlands.

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## 1. Introduction

Wetland mitigation requires the development and establishment of plant communities as a priority (USACE, 2010; NRC, 2001; Spieles, 2005). Planting is an important part of wetland mitigation because vegetation development is the most commonly used metric for determining mitigation success and fulfillment of requirements under the Clean Water Act (CWA) section 404 (Clean

Water Act of 1972, 2002). However, vegetation establishment is most often achieved by intentional seeding or planting of wetland species along with natural recruitment of volunteer species from adjacent communities. Poor development of vegetation communities with lower species richness, lower total plant cover, and fewer native volunteer species, have previously been observed in many created mitigation wetlands compared to natural wetlands (Balcombe et al., 2005; Gutrich et al., 2009). Currently there is no consideration of planting diversity in created mitigation wetlands, nor is plant community diversity managed vigorously during post-construction monitoring. Lack of these considerations may have

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structural (e.g., biodiverse habitat development), as well as functional, consequences (e.g., lesser or no development of ecological functions) for the outcomes of wetland mitigation projects (Zedler and Callaway, 1999; Farrer and Goldberg, 2009; Williams and Ahn, 2015).

Wetlands have been studied as potential sources or sinks of carbon (Bridgham et al., 2006; Nahlik and Mitsch, 2010; Mitsch et al., 2012, 2013; Ahn and Jones, 2013; Bridgham et al., 2013; Neubauer, 2014). This research demonstrates the necessity of specifically designing created wetlands to store as much carbon as possible, particularly in the early stages of development. Newly created wetlands offer an opportunity for the development of active carbon sinks as plants grow, accumulate, and store carbon as biomass through photosynthesis. While the majority of wetland carbon storage takes place in soils (Bridgham et al., 2006; Lawrence and Zedler, 2013), vegetation plays an important role in the development of the soil carbon pool. *Typha* spp., for example, is known to produce and store significant amounts of carbon as biomass, yet they are undesirable species for mitigation projects due to their invasiveness and aggressive colonization (Mitsch et al., 2012; Bernal and Mitsch, 2013). Little, however, is known regarding the carbon storage capabilities of native plants commonly used in mitigation wetlands, or how their ability to store carbon may be affected by planting diversity. The information garnered could be a possible design element to incorporate into the construction of future mitigation wetlands.

The relationship between plant community diversity and productivity has recently been investigated, much of which were based on grassland ecosystems (Englehardt and Ritchie, 2001; Tilman et al., 2001; Hooper et al., 2005; Loreau et al., 2002). It has been found that more diverse species groups can lead communities to higher productivity by exploiting a greater number of niches and thus more fully extracting available nutrients (Cardinale et al., 2011). Alternatively, interspecific processes that directly or indirectly facilitate the growth of neighboring species, due to a release from intraspecific competition through niche differentiation or a release from multi-trophic competition, can promote greater productivity in more diverse mixtures (Vaneislander et al., 2009; de Kroon et al., 2012; Le Bagousse-Pinguet et al., 2012). There is currently a lack of research findings on planting diversity effects on biomass production and subsequent carbon storage potentials in created wetlands.

The object of the study was to investigate the biomass production and carbon storage potential of four species as affected by initial PD that can be incorporated as a potential design element in created/restored mitigation wetlands.

## 2. Methods

### 2.1. Wetland mesocosm set-up and planting

The experiment was conducted in 34 outdoor mesocosms (numbered from 1 through 34), 568 L Rubbermaid® tubs with a surface planting area of 1.15 m<sup>2</sup> by 0.64 m deep, which sat above-ground in Ahn Wetland Mesocosm Research Compound on George Mason University's Fairfax campus (Fig. 1). Mesocosms were bottom-filled with 20 cm layers of locally-quarried rock and sand, and topped with 30 cm of locally-produced garden-quality topsoil known to have been used in the creation of Virginia wetland mitigation wetlands. Water levels were determined by precipitation events but were periodically supplemented with dechlorinated tap water to maintain a minimum depth of 5 cm.

Four species of emergent freshwater macrophytes were chosen for this study – *Carex vulpinoidea* L. (an interstitial sedge), *Eleocharis obtusa* R. Br. (an obligate annual), *Juncus effusus* L. (an

interstitial reed), and *Mimulus ringens* L. (a facultative annual). All plants were grown in controlled outdoor mesocosms along a gradient of PD (i.e., PD1, PD2, PD3, and PD4) for two full growing seasons (2012–2013). The wetland plant species were selected with two criteria in mind – that they be commonly found, seeded, and/or planted in created mitigation wetlands in the piedmont region of Virginia, and that each could be classified as species belonging to either a ruderal or an interstitial functional group (Keddy et al., 1994). In early May 2012, the mesocosms were planted with plugs of between one and four different herbaceous wetland plant species in a linear alignment either monotypically or in combinations of two to four different plant species. A low experimental density level was chosen to reflect (as closely as possible within ~1 m<sup>2</sup> mesocosm) planting densities used in the creation of freshwater wetlands in the Virginia piedmont. Two monocultures per species, or eight mesocosms, comprised the replicates for the lowest planting diversity (PD1). The second level of planting diversity (PD2) consisted of six replicates representing all combinations of two species. Twelve mesocosms using an even-species representation for combinations of three species comprised the replicates of the third level of planting diversity (PD3). All species were present in the eight mesocosms representing the highest planting diversity (PD4). Volunteer plant species were weeded from mesocosms throughout the study to preserve the original planting diversity.

### 2.2. Plant tissue carbon analysis

At the end of the second growing season (mid-September of 2013), a cover analysis was performed for each of the 34 mesocosms using a grid consisting of 215 squares, each with an area of 51.4 cm<sup>2</sup>. All live aboveground biomass (i.e., not standing litter) was harvested and samples were dried at 48 °C (drying cabinet maximum temperature) until a constant mass was reached (i.e., <5 g difference). Dried plant matter including leaves, blades, and stems was then ground using a Wiley Mill. Aboveground carbon (AGC) was determined by dry combustion of ground plant biomass samples in a 2400 Series II CHN/O elemental analyzer (Perkin–Elmer, Waltham, Massachusetts).

### 2.3. Competition values (CV)

Total cover including overhang (see Ahn and Mitsch, 2002), was determined for each species in each mesocosm in the field prior to harvesting. To compare the AGB and AGC content of each species, it was necessary to adjust the cover and analyze each species over a uniform 1 m<sup>2</sup> area, the approximate surface area of each mesocosm used in this study. For monocultures with overhanging vegetation, we scaled down the total cover to 100%. For the mixtures, the cover for the individual species in each mesocosm was extrapolated to assume 100% cover of each species over 1 m<sup>2</sup>, accounting for differences in the original number of individuals planted in each mesocosm. The adjusted cover values were used to determine extrapolated aboveground biomass (AGB) for each species, which was then multiplied by the % C in plant tissue for each species per 1 m<sup>2</sup>. This data was then analyzed by both PD and species.

A competition value (CV) (Hong et al., 2014) was determined for each species grown in mesocosms of different PD. This value was used to compare each species when grown alone in monoculture to when grown with neighbors. The CV provided a means to determine the interactions taking place among the plant groups (Hong et al., 2014; Byun et al., 2013; Keddy et al., 1994; Twolan-Strutt and Keddy, 1996). We could then examine each species growth potential when grown with 1, 2, or 3 other neighbors (i.e., PD2, 3, and 4). In addition, we could compare biomass production using



**Fig. 1.** Wetland mesocosms for the study and planting diversity (PD) represented by four macrophytes native to, and commonly planted in, created/restored wetlands in Virginia (photo credit to Ahn).

specific species combinations. The CV was determined using the following formula:

$$CV = \frac{100(X_2 - X_1)}{X_2}$$

where  $X_2$  is the AGB of a species grown with neighbors and  $X_1$  is the AGB of a species grown in a monoculture. Because there are replicate monocultures for each species ( $X_1$ ), the CV was calculated using the average of the two. A CV value above zero indicates an increased AGB when grown in a mixture. As CV decreases, so does the species' ability to produce biomass in the presence of neighbors (Hong et al., 2014).

#### 2.4. Data analysis

The data were tested for normality using a Shapiro–Wilkes test. A non-normal distribution was found for all data and thus a non-parametric Kruskal–Wallis analysis was used followed by a series of Mann–Whitney tests to determine variance by species and functional group. Significance was determined at  $p = 0.05$ . All statistical analyses were conducted using IBM SPSS Statistics version 20 (SPSS, 2012).

### 3. Results

#### 3.1. Aboveground biomass and carbon content

Along the gradient of PD in mesocosms, the monocultures (PD1) produced more biomass than those with highest PD (i.e., PD4,  $p = 0.021$ ) (Table 1); however, there was no difference in AGB and C content values among those of PD1, PD2 and PD3. The monocultures produced an average AGB of  $1515 \pm 181$  g/m<sup>2</sup> after two growing seasons whereas the highest diversity mixture (PD4) produced an average AGB of  $988 \pm 62$  g/m<sup>2</sup>. Biomass production

**Table 1**

Aboveground biomass (AGB), percent carbon (% C), and aboveground carbon content (AGC) per mesocosm by planting diversity (PD) group (i.e., 1–4) (mean  $\pm$  standard error). Letters indicate significant differences between the PD groups.

| PD | AGB (g/m <sup>2</sup> )       | % C              | AGC (g C/m <sup>2</sup> )    |
|----|-------------------------------|------------------|------------------------------|
| 1  | 1515 $\pm$ 181.2 <sup>a</sup> | 44.31 $\pm$ 0.69 | 610 $\pm$ 92.9 <sup>a</sup>  |
| 2  | 1436 $\pm$ 214.8 <sup>a</sup> | 45.07 $\pm$ 0.61 | 513 $\pm$ 66.0 <sup>a</sup>  |
| 3  | 1147 $\pm$ 111.6 <sup>a</sup> | 44.08 $\pm$ 0.20 | 406 $\pm$ 40.8 <sup>a</sup>  |
| 4  | 988 $\pm$ 61.9 <sup>b</sup>   | 44.08 $\pm$ 0.19 | 344 $\pm$ 15.4 <sup>ab</sup> |

Scaled to 100% cover per mesocosm (~1 m<sup>2</sup>).

within species groups (i.e., PD2 and PD3) was more variable and was dependent upon the species present in each mixture. *M. ringens* produced the most AGB (an average of 2032 g/m<sup>2</sup>), irrespective of PD (Table 2). This was significantly more than the biomass produced by *J. effusus* (1536 g/m<sup>2</sup>), *E. obtusa* (895 g/m<sup>2</sup>) and *C. vulpinoidea* (846 g/m<sup>2</sup>) ( $p < 0.005$ ). The AGC data along the PD gradient showed the same trend, although there was no significant difference between PD1 ( $610.0 \pm 92.9$  g C/m<sup>2</sup>) and PD4 ( $344.1 \pm 15.4$  g C/m<sup>2</sup>) ( $p = 0.083$ ) (Table 2). *M. ringens* showed significantly higher AGC storage potential ( $940$  g C/m<sup>2</sup>) than all the other species, followed by *J. effusus* ( $703$  g C/m<sup>2</sup>). *C. vulpinoidea* and *E. obtusa* showed the least AGC storage potential, both averaging  $371$  g C/m<sup>2</sup> (Table 2). To gain a deeper understanding of the species-specific responses to increases in PD, the AGC storage potential data were broken down by species within each PD group (Fig. 2). Only *E. obtusa* showed a significant decrease in AGC storage potential as PD increased, both between PD1 ( $205$  g C/m<sup>2</sup>) and PD4 ( $125$  g C/m<sup>2</sup>) ( $p = 0.044$ ) and between PD2 ( $157$  g C/m<sup>2</sup>) and PD4 ( $p = 0.048$ ). All other species remained statistically constant across the PD gradient.

#### 3.2. Competition value

The average calculated CV values were below 0, indicating that a given species' ability to produce biomass declined in the presence of neighbors (Fig. 3). There was little difference along the gradient of PD, other than *E. obtusa*, which showed a further declining competitive ability when growing with three other neighbors compared to a single neighbor (Fig. 3). The CV for *E. obtusa* decreased significantly from PD2 to PD4 ( $p = 0.048$ ). Where the CV<sub>E</sub> was highest, the CV of all other species was negative. For example, in mesocosm 21 which was planted with *E. obtusa*, *M. ringens*, and *J. effusus*, CV<sub>E</sub> = 104.7; CV<sub>M</sub> = -811.0; and CV<sub>J</sub> = -74.7. *J. effusus* and *C. vulpinoidea* showed no significant change as PD increased,

**Table 2**

Aboveground biomass (AGB), percent tissue carbon (% C), and aboveground carbon content (AGC) per mesocosm by plant species (mean  $\pm$  standard error). Letters indicate significant differences between plant species.

| Species               | AGB (g/m <sup>2</sup> )     | % Tissue C.      | AGC (g C/m <sup>2</sup> )       |
|-----------------------|-----------------------------|------------------|---------------------------------|
| <i>M. ringens</i>     | 2032 $\pm$ 171 <sup>b</sup> | 46.18 $\pm$ 0.15 | 939.77 $\pm$ 80.10 <sup>b</sup> |
| <i>J. effusus</i>     | 1536 $\pm$ 100 <sup>a</sup> | 45.75 $\pm$ 0.38 | 702.60 $\pm$ 45.35 <sup>a</sup> |
| <i>E. obtusa</i>      | 895 $\pm$ 98 <sup>c</sup>   | 42.37 $\pm$ 0.11 | 371.04 $\pm$ 41.50 <sup>c</sup> |
| <i>C. vulpinoidea</i> | 846 $\pm$ 100 <sup>c</sup>  | 43.74 $\pm$ 0.23 | 370.59 $\pm$ 43.77 <sup>c</sup> |

Scaled to 100% cover per mesocosm (~1 m<sup>2</sup>).

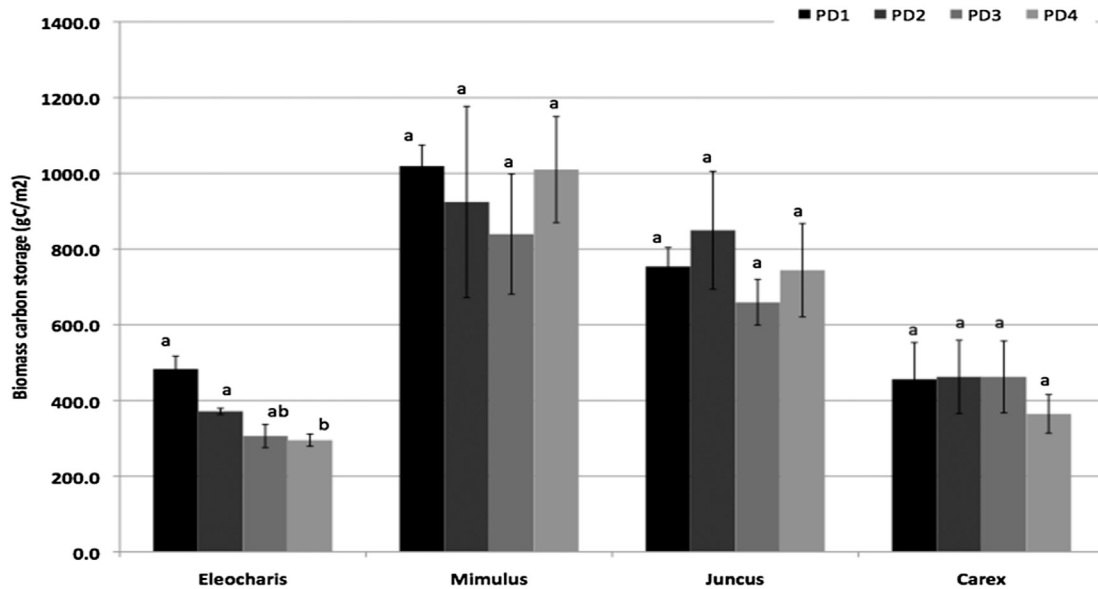


Fig. 2. Average aboveground carbon storage by each species along a planting diversity gradient (PD1 – PD4).

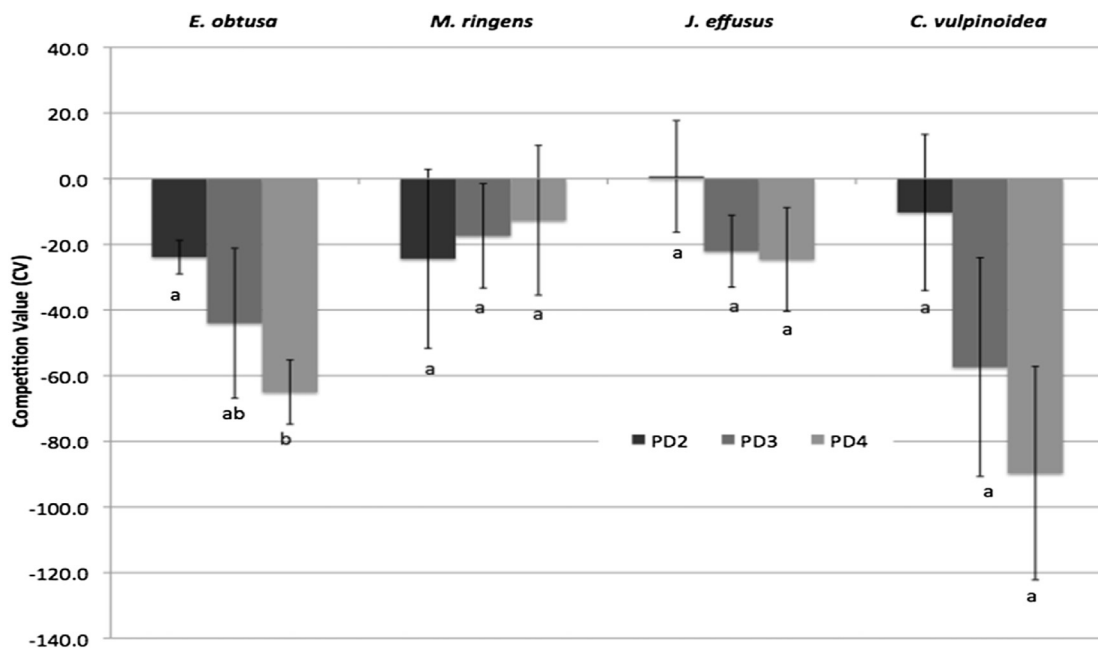


Fig. 3. Competition Values (CV) for each species along a planting diversity gradient (PD2 – PD4).

however the CV for *C. vulpinoidea* decreased with each additional neighbor by more than a factor of 5 (CV of  $-10.3$  in PD2;  $-57.4$  in PD3, and  $-89.7$  in PD4) although the difference was not statistically significant (Fig. 3). *M. ringens* and *Juncus* both showed very consistent and relatively high CVs (Fig. 3); both were significantly higher than *E. obtusa* ( $p = 0.021$  and  $0.021$ , respectively). The CV for *M. ringens* increased in its ability to store more biomass as the PD increased, from  $-24.4$  in PD2 to  $-12.7$  in PD4; however, this change was not significant ( $p = 0.910$ ). It was not able that CV values for *M. ringens* were higher when it was present in mesocosms grown with either *J. effusus* or *C. vulpinoidea*, or with both species. For example, in mesocosm 20 that contained *M. ringens*, *J. effusus* and *C. vulpinoidea* the  $CV_M = 36.0$  and mesocosm 30 where *M. ringens* grew with *C. vulpinoidea*,  $CV_M = 29.0$ . In mesocosm 3, *M. ringens*

was grown only with *E. obtusa*, the  $CV_M = -52.0$ . Mesocosms such as 20 and 30 show positive CV values for *M. ringens*, suggesting that *M. ringens* grows more successfully when planted alongside a tussock-forming species such as *J. effusus* or *C. vulpinoidea*.

## 4. Discussion

### 4.1. Aboveground biomass and planting diversity

The production of biomass is one of the most basic and encompassing ecosystem services provided by wetlands. This reflects the creation of habitat for rare species as well as supporting recreational birding and economic gain (Okruszko et al., 2011). Biomass can also be a reflection of the system's ability to remove



excessive nutrients such as nitrogen and phosphorous from the water. This can be seen in the presence of high-biomass accumulators such as *Typha*, which require large amounts of these nutrients and are often used in wetlands constructed specifically for nutrient removal (Tang et al., 2011). In our study, we observed *M. ringens* and *J. effusus* accumulated large amounts of biomass (Table 2) without the excessive colonization often observed with *Typha*, thus preserving plant community diversity. The decrease in biomass production at the highest PD (i.e., PD4), observed in this study (Table 1), is somewhat the opposite to the findings of other studies (Loreau, 2000; Tilman et al., 2001; Tilman et al., 1997; Shultz et al., 2012) where a positive relationship between plant diversity and productivity was found. One possible explanation for this change in biomass production may be that the taller species, *M. ringens* and *J. effusus*, shaded out the competing *E. obtusa* and *C. vulpinoidea* (Fig. 1). It was demonstrated in Ervin and Wetzel (2002) that shading produced by *J. effusus* limited richness and biomass of surrounding species. In addition, *Juncus effusus*, being a perennial species, exhibits year-round growth (Wetzel and Howe, 1999). This gives the species ample time to accumulate biomass, potentially limiting nutrients available for the other species between growing seasons. Because our mesocosms did not receive any additional nutrient input from fertilizer or local runoff, additional studies should be performed to determine if nutrient limitation impacted vegetation development.

Schultz et al. (2012) suggested that reeds might inhibit the growth of tussocks, explaining the limited AGB accumulation of *C. vulpinoidea* observed in our mesocosms. Lawrence and Zedler (2013) reported that the majority of biomass C in *Carex stricta* was allocated to the root system during the formation of tussocks. Based on AGB alone, *C. vulpinoidea* exhibited limited productivity in comparison to other species. The inclusion of belowground biomass would likely have presented a more complete view of overall biomass allocation in all planted species. *E. obtusa*, being a ruderal species, grows early and spreads quickly. This gives *E. obtusa* an advantage when initially populating a created wetland. Our study suggests, however, that after the first growing season this advantage no longer exists and the growth of *E. obtusa* is inhibited by the neighboring species, yielding limited biomass (unpublished data).

#### 4.2. Carbon storage potential of macrophytes

The amount of C stored in AGB is considered negligible relative to the C storage capacity of soils (Bridgham et al., 2006). However, this does not warrant the AGC in herbaceous wetland plants be ignored. It was clear from our study that the taller and sturdier species (i.e., *J. effusus* and *M. ringens*) were significant sinks of AGC; this has been documented (Tang et al., 2011; Gagnon et al., 2012; Hooker and Stark, 2008). However, a fast growing annual such as *E. obtusa*, that was observed to produce relatively high biomass in some mesocosms, particularly in monocultures, was also able to contribute to the AGC stock.

Shorter tussock-forming species such as *C. vulpinoidea* also contribute immensely to the C sequestration potential of wetlands; however, much of this contribution is stored as BGB, which is not quantified here. Lawrence and Zedler (2013) discovered that the composition of a *C. stricta* tussock could consist of up to 95% organic matter. Thus, although this study shows *C. vulpinoidea* as a low C accumulator in regard to AGC, the tussocks potentially trap a substantial amount of organic matter and sediment as water flows through the wetland (Lawrence and Zedler, 2013; Ervin, 2007). When paired with its ability to facilitate the growth of other species, particularly high C accumulator species such as *M. ringens*, *C. vulpinoidea* does have potential to increase the total C pool of a created wetland.

#### 4.3. Competition value (CV)

CV provides an insight for which species were driving the productivity in each mesocosms, revealing how the species-specific interactions either facilitate or prevent biomass production. Most of the calculated CV values in our study were negative, indicating that neighbors inhibited growth in all four species compared to when they were grown in monoculture. Despite this, *M. ringens* and *J. effusus* displayed the highest overall CV, ranking them as the most successful competitors as the PD increases (Fig. 2). We also found that *M. ringens* produced the most biomass when in a mesocosm accompanied by either *J. effusus* or *C. vulpinoidea* or when accompanied by both. This supports the idea that facilitation by tussock-forming species increases the potential for biomass production (Schmid and Harper, 1985; Hacker and Bertness, 1995; Ervin, 2007; Lawrence and Zedler, 2013). Planting *J. effusus*, *C. vulpinoidea*, and *M. ringens* together could ensure maximum biomass production and thus increase AGC storage potential in a created wetland based upon our finding.

In mesocosms where *E. obtusa* reached the highest CV, the neighboring species exhibited low CVs, indicating that they were not able to live up to their full potential; the lowest CVs for *J. effusus* and *M. ringens* were observed in mixtures with *E. obtusa*. This suggests a priority effect (Hong et al., 2014) by *E. obtusa*. Although  $CV_E$  was low overall, *E. obtusa* can produce large amounts of biomass under the right conditions (e.g., in low PD setting). This result supports a study by Byun et al. (2013), in which fast growing ruderal annuals, such as *E. obtusa*, often exhibit this behavior in AGB production. Mesocosms in which *E. obtusa* was able to out-perform neighbor species may be a residual effect of the first growing season when *E. obtusa* was the most productive species. The competitive ability of each of these four species must be carefully accounted for when proposing a planting regime for development of a created mitigation wetland. The competitive nature of a given species has been known to change over time (Byun et al., 2013; Zhang and Lamb, 2011), depending on its cover density and proximity to other plant species (Schmid and Harper, 1985). Therefore, the nature of each species and its interactions with the other species being planted together must be thoroughly examined over a longer time period, beyond a legally mandatory monitoring period (i.e., five years from construction) for created mitigation wetlands.

#### 4.4. Implications of study and recommendation

Policy makers have often been divided, choosing between conservation efforts or development. The no-net-loss wetland policy, established in 1988, sought to change this by suggesting that both goals are achievable. The new policy allocated hundreds of millions of dollars over the first few years to wetlands research, delineation, and mitigation (Deland, 1992). Currently, the most common solution for the no-net-loss is the creation of incentive-based wetland mitigation banks. Requirements for creating mitigation wetlands do not necessitate providing a specific planting scheme, although it is suggested that the vegetation should be the same as that being removed. The only requirement is that the created wetland be the same as those being destroyed, both by acreage and functionality (DOD and EPA, 2008). It is suggested that the species composition, diversity, and aerial coverage be kept consistent; however, this is not required (DOD and EPA, 2008). The vague language lends no guidance as to what species are most efficient in producing and supporting the desired wetland ecosystem services (Brown and Lant, 1999), especially when considering the challenge of carbon function of wetlands (i.e., wetlands as a potential carbon sink).

While carbon storage is not listed among the functions in the

federal mitigation bank guidelines (DOD/EPA, 2008), it must be acknowledged as an ecosystem service provided by wetlands. Devising incentives for wetland conservation and ecosystem services can often be challenging due to a lack of dollar-value for services such as biodiversity, wildlife habitat, and aesthetics. Carbon sequestration, however, is beginning to have a more tangible price. Carbon cap and trade programs were developed to help reduce atmospheric levels of carbon dioxide and to slow the impacts of climate change. Through such exchanges, a definitive price for C can be made and that value assigned to the C sequestration service provided by wetland mitigation banks (Hansen, 2009). For greenhouse gas emissions, such as CH<sub>4</sub> or CO<sub>2</sub>, the credits and payments for continuous emission offsets must take into account the resultant impacts from the credit sales (Cooley and Olander, 2012). By having a compensatory price for ecosystem services, mitigation parties can gain credits for their support of carbon-related ecosystem service.

Planting diversity should be required in planning mitigation bank wetlands and their monitoring and management so as to best facilitate the successful establishment of ecosystem functionality. The outcome of our study shows that ruderal species, and other fast growing annuals such as *E. obtusa*, can be good candidates for planting during early wetland development as they may help to minimize runoff and erosion. Over time, tussock-forming species such as *J. effusus* and *C. vulpinoidea* will induce microtopographic variation and spatial heterogeneity in the created habitat, which will lead to hydrologically diverse micro-sites that support a diverse plant community (Ervin, 2007; Moser et al., 2007; Wolf et al., 2011; Ahn and Dee, 2011). We also recommend that the perennial species *J. effusus* and *M. ringens* should be planted specifically for their ability to accumulate carbon as biomass, thus enhancing AGC storage potential in newly created wetlands. A further study is under way to include plant belowground biomass and soil carbon accumulation in relatively young created wetlands over time.

## 5. Conclusion

We investigated the effects of PD on carbon storage potential of four native wetland plant species that are commonly planted in created mitigation wetlands in Virginia. The carbon storage potential was not significantly affected by initial PD, but by the characteristics of each species. One limitation of this study is that it does not take into account belowground biomass, which may play a role in competition and total carbon storage potential of each mesocosm wetland. Concurrently, the soil C pool, which is the largest contributor to CO<sub>2</sub> sequestration, is left out of the study. In addition, the scale of species richness and planting density was limited in this study due to the space constraint of a mesocosm used (Ahn and Mitsch, 2002). These species may exhibit different patterns of growth and AGC accumulation when they are able to increase their cover and interact with more number of different species. A further study is needed over a longer period of time (i.e., longer than two growing seasons) to better track the progress of the development of plant community per initial planting regime.

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