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Research article

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

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A B S T R A C T

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^2 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^2 – 1515 g/m^2, 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^2 in the most diverse mesocosms (PD 4) to 610 g C/m^2 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.

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 (Vanelslander 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^2 by 0.64 m deep, which sat aboveground 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^2 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^2. 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^2 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^2, 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^2. 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