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Does plant performance under stress explain divergent life history strategies? The effects of flooding and nutrient stress on two wetland sedges

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ABSTRACT

Novel hydrology and nutrient loads are common environmental filters in disturbed wetlands. These filters impact plant growth and influence the composition and productivity of wetland plant communities by selecting for flooding-tolerant species that can efficiently acquire and use nutrients. We performed a factorial greenhouse trial to elucidate how nutrients and flooding affect the growth and morphology of *Carex obnupta* and *Carex stipata*, two wetland sedges with divergent life history strategies. Individual seedlings of each species were exposed to a combination of flooding (deep or shallow) and fertilization (low or high) treatments. These treatment combinations create a stress gradient from high fertilization and shallow flooding (lowest stress) to low fertilization and deep flooding (highest stress). Plant biomass accumulation and allocation were measured after 77-days. Total biomass, leaf area, and shoot biomass were greatest in high fertilizer treatments, regardless of flooding treatment. There was an initial plant size effect in *C. obnupta*: small plants accumulated less biomass than large plants under high flooding. *C. stipata* had higher biomass and specific leaf areas than *C. obnupta* in all treatments. *C. obnupta* had higher net assimilation rates per unit biomass and leaf area than *C. stipata*. These stress responses illustrate that each species has a distinct life history strategy. *C. obnupta* is a stress tolerator that can persist across a range of flooding conditions. *C. stipata* is a ruderal species that outgrows flooding stress by allocating carbon to leaf growth. These species' niches likely diverge based on how each species grows and allocates biomass under environmental stress

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1. Introduction

Plant communities arise as products of biotic and abiotic environmental filters that shape plant species' growth and reproduction that drive interspecific competition (Keddy, 1992; Woodward and Diament, 1991). Environmental filters are any non-random process that shapes species assembly in a given environment (Díaz et al., 2007). Plant community composition shifts across environmental gradients based on species' functional capacities to acclimate to local environmental conditions (Cornwell and Ackerly, 2009; Wright and Westoby, 1999). As soil resources and/or environmental stress change across space or time, species that cannot acclimate to local conditions become less competitive and eventually fail to

successfully reproduce and maintain their place in a community (Díaz et al., 1998). This theory of community assembly dictates that a species' life history strategy must correspond to the local physical, chemical and biotic environments for it to persist (Keddy, 1992). The traits that comprise the life history strategies that govern community assembly may be most easily elucidated across strong environmental gradients, such as those found in wetland ecosystems.

Wetland vascular plants have evolved functional strategies to tolerate environmental stress and acquire the resources required to persist, grow, and reproduce under suboptimal conditions. Accordingly, wetland plant communities often assemble across hydrologic gradients (Keddy, 1992; Shaffer et al., 1999; van Eck et al., 2004), as the depth, duration and timing of flooding stress select for wetland plant species with morphological and physiological adaptations that match a given environment (Keddy, 2000; Kozłowski, 1984; Magee and Kentula, 2005). To not be "filtered" out of a community, wetland plant species must be able to not only tolerate flooding stress, but also effectively acquire environmental

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resources such as nutrients and light. While general plant strategies have been classified based on functional traits that allow individual plant species to thrive and compete in different environmental settings (Grime, 2001; Kahmen and Poschold, 2004) many species' life history strategies and their corresponding functional traits have not been assessed. Within this study we assess the abilities of two common wetland species, *Carex obnupta* and *Carex stipata*, to acclimate to persistent stress by quantifying how their functional traits—physiological and morphological characteristics that govern growth and reproduction—respond to the common wetland stressors and subsidies, flooding and nutrients.

To understand how each species will respond to wetland stress-subsidy dynamics, it is important to understand how each stressor (flooding and low nutrition) and subsidy (high nutrition) influences plant performance. Flooding reduces the oxygen available to submerged plant tissues and inhibits aerobic root respiration and active metabolic processes including nutrient and water uptake (Laanbroek, 1990). Plant responses to flooding are determined by belowground tissue oxygen acquisition and the expansion of leaf area relative to overall growth (Evans and Poorter, 2001). For many plants, including *Carex* species, flooding triggers the formation of aerenchyma—porous tissues that allow oxygen to diffuse within plant tissues (Chen et al., 2002; Visser et al., 2000). Within *Carex* species, aerenchyma occurs predominantly by lysigenous processes where root tissues die and are replaced by new aerenchymous tissues (Visser et al., 2000). Proportional increases in root allocation to form aerenchyma may occur at the expense of plant growth because as relative photosynthetic tissue biomass decreases, so do photosynthetic carbon gain (Mielke et al., 2003).

Flooding not only changes plant allocation patterns, but also reduces leaf-level photosynthesis in many species (Kozlowski, 1984; Mielke et al., 2003). However, whole-plant photosynthesis may remain high due to high pre-stress specific leaf areas, the unit leaf area per unit leaf mass (SLA; (Shipley et al., 2005). High SLA allows fast-growing plants to maintain growth during flooded conditions, even when leaf-level photosynthesis is reduced (Bailey-Serres and Voesenek, 2008; Mommer et al., 2006). When an additional environmental stress, like low soil fertility, is paired with flooding, stressors may exacerbate flooding stress (Pezeshki, 2001; Pezeshki et al., 1999), selecting against species unable to build and maintain tissues under multiple stressors.

In contrast to flooding stress, nutrient enrichment is a common anthropogenic impact on wetlands (Verhoeven et al., 1993) and may increase plant nutrient uptake, photosynthesis and growth (DeLaune et al., 1998; McJannet et al., 1995). Wetland plant performance is enhanced by nutrient enrichment when plants are able to acclimate to the anoxic environment and essential nutrients do not occur exclusively as toxic, reduced compounds (Johnson and Leopold, 1994; Laanbroek, 1990; Merino et al., 2008; Rey Benayas and Scheiner, 1993). Fertile conditions are associated with high total productivity in wetlands at the expense of plant diversity as fast-growing, resource-efficient species outcompete stress-tolerant species (Bedford et al., 1999; Grime, 1977; Moore et al., 1989). This trade off between productivity and diversity in enriched environments may be explained by enhanced physiological performance (Laanbroek, 1990; McJannet et al., 1995) or biomass allocation strategies that allow plants to escape stress and continue to grow (Voesenek et al., 2004, 1992). In this way soil nutrition filters species that cannot use extra soil resources, favoring ruderal species that can quickly turn these resources into biomass.

Plants growing in flooded and nutrient-limited environments may allocate more biomass to roots, increasing root surface area to acquire nutrients (Morris and Ganf, 2001; Rubio et al., 1995) and oxygen. Anaerobic conditions created by flooding limit roots' ability to acquire nitrogen (N), specifically nitrate (NO_3^-),

decreasing tissue nitrogen content and photosynthesis (Willby et al., 2001). Aerenchyma allows oxygen to diffuse into the soil, providing an oxidized buffer where nitrate remains soluble for plant uptake, enabling continued aboveground growth and photosynthesis. Building aerenchymous roots that acquire oxygen allows for continued plant growth facilitates a “low oxygen escape syndrome” life strategy (Bailey-Serres and Voesenek, 2008; Colmer and Voesenek, 2009). Low oxygen escape syndrome can occur rapidly in enriched, flooded conditions where sufficient photosynthesis maintains rigid stem architecture, expedites aerenchyma formation and allows leaf growth to continue (Blom and Voesenek, 1996). This rapid leaf expansion strategy in response to prolonged flooding has been shown to enhance the survival and growth of wetland plants in numerous settings (Pierik et al., 2008; Voesenek et al., 2004). However, differences in low oxygen escape strategy have not been evaluated among species known to occupy different environmental niches (Merino et al., 2008; Morris and Ganf, 2001).

C. stipata and *C. obnupta* occupy distinct niches in wetland environments and we anticipate that their functional responses to stress will explain these differences. While both species occur in wetlands, the types of wetlands they're capable of occupying (potential niche) may differ based on their ability to acclimate to stress and subsidy. We ask the question, does each species' performance under environmental stress explain its potential niche? To define how each species responds to flooding stress and nutrient subsidy, we addressed intraspecific and interspecific hypotheses on plant biomass accumulation and allocation. Intraspecific hypotheses compare individual species' performance across the range of treatments:

Environmental stress from high flooding and low nutrients will shape plant allocation and biomass accumulation. We anticipate that flooded plants or nutrient-stressed plants will accumulate less total biomass, have lower leaf areas and specific leaf area, and allocate a greater proportion of total biomass to roots than in unflooded and well fertilized plants.

Specifically, we expect that plants experiencing shallow flooding and high fertilization will accumulate more total biomass than plants experiencing deep flooding and high fertilization or deep flooding and low fertilization and accumulate high leaf area, and show high specific leaf area, leaf mass ratio and leaf area ratio. We also anticipate that high flooding may be overcome by high fertilization that allows plants to outgrow environmental stress.

The interspecific hypotheses address differences in the growth and allocation of the two species based on their life-history strategies:

Deciduous *C. stipata* will exhibit low oxygen escape syndrome-like properties when flooded, rapidly growing leaves and producing more total biomass than *C. obnupta*, with higher specific leaf area and leaf area ratios. The evergreen *C. obnupta* will be more flood-tolerant than *C. stipata*, growing slowly and thriving under high nutrient conditions required to produce long-lived, evergreen leaves with high leaf mass ratios and low specific leaf areas. Specifically, we anticipate that *C. stipata* will have higher leaf area ratios and specific leaf areas and a lower leaf mass ratio than *C. obnupta*. We anticipate that *C. stipata* will photosynthesize less per unit leaf area and per unit total biomass, but have greater total leaf areas than *C. obnupta*.

2. Materials and methods

2.1. Plant species and experimental rationale

To evaluate the stress responses of two wetland sedges under a combination of flooding and fertilization treatments, we established a greenhouse experiment in June 2009 at the University of

Washington Center for Urban Horticulture (Seattle, WA, USA). *C. obnupta* (slough sedge) and *C. stipata* (awlfruit sedge) were selected as experimental species for their regional abundance in wetlands of the U.S. Pacific Northwest as well as the global distribution of the genus *Carex* (Cyperaceae). *C. obnupta* is an evergreen, rhizomatous species that occurs from British Columbia, Canada, to northern California, USA in wetlands with variable hydroperiods including coastal marshes, hydric forests, riparian zones and tidal channels (Wilson, 2008). It occurs commonly in hydric, organic soils that experience their deepest flooding in winter (Minore, 1969), and spreads rapidly by rhizome and seed, forming dense monocultures in many habitats. *C. stipata* is a deciduous, tussock-forming species that inhabits wetland edges and seasonally wet habitats across North America, Europe and eastern Asia (Wilson, 2008). *C. stipata* is common in meadows and wet prairies but rarely occurs in deep, open water (Magee and Kentula, 2005), and does not grow well under prolonged flooding (Ewing, 1996). *C. stipata* seeds have been shown to exhibit reduced dormancy (Hough-Snee and Cooper, 2011; Kettenring and Galatowitsch, 2007) and are dispersed by buoyant perigynia.

2.2. Plant materials and allocation to experimental treatments

In May of 2009, one hundred 6-month old bare-root seedlings of each species were acquired from a nursery (Fourth Corner Native Plants, Bellingham, WA, USA). Initial wet biomass was measured for each seedling before individual seedlings were planted into 3.8 l plastic pots containing ~3.7 l of seed-sterilized sand. We selected sand substrate to allow better nutrient control (Fig. S1) and ensure heterogeneous capillary action in the soil column. Seedlings were randomized and placed onto greenhouse benches where they were watered and monitored for mortality for three weeks. Both species experienced transplant stress and mortality, shedding leaves immediately following transplant. Of the seedlings that survived the hardening off period, 60 individuals of each species were randomly selected for inclusion in the experiment. The 60 plants of each species were rank-ordered 1–60 by initial wet weight and plants were stratified into four groups based on weight ($N=60$, $n=15$, $k=4$). For example, plants with weight ranks of one, two, three, and four were allocated to four different groups, a, b, c and d. Ranked plants were then continuously allocated to the four different groups to prevent the random clumping of plants into size clusters (all plants large or all plants small) that might confound treatment effects. Following stratification, each group of plants (a, b, c, and d) was randomly assigned to a fully-crossed experimental treatment combination of flooding and fertilization.

2.3. Experimental design and treatments

A fully crossed, factorial experiment was undertaken in which individuals of both species were exposed to combinations of two flooding levels and two fertilization levels. The shallow flooding treatment consisted of each plant being placed into an individual five-liter plastic bucket and filled to a depth of 5 cm (~600 ml) inundating the bottom third of the rooting zone while maintaining aerated conditions elsewhere in the root zone. The deep flooding treatment partially submerged plants in individual buckets that were filled to 4 cm above the substrate level (~5000 ml). The low fertilization treatment consisted of a modified, one-eighth strength Hoagland's solution while the high fertilization treatment consisted of a modified, half-strength Hoagland's solution (Table S1; (Kinmonth-Schultz and Kim, 2011)). These fertilization levels provided nutrient concentrations that were potentially limiting (low) or in excess of the minimum amounts required for plant growth (high). Combinations of the nutrient and flooding treatments are referred to with two letter codes. For example, high

nutrients, deep flooding = HD; low nutrients, shallow flooding = LS, etc (Table S2).

Consistent growing conditions were maintained during the experiment by discarding and replacing treatment solutions on a weekly basis. To compensate for evapotranspirational water loss between treatment replacements, solution levels were refilled daily with deionized water to the assigned flooding depth. Plants were evenly spaced on greenhouse benches to prevent canopy effects and re-randomized across benches weekly. Greenhouse temperatures ranged from 17 to 29 °C and the light period consisted of artificial light levels set at 14 h per day at $\approx 1200 \mu\text{mol m}^{-2} \text{s}^{-1}$ around midday throughout the duration of the experiment. Ambient natural light within the greenhouse was as high as $1700 \mu\text{mol m}^{-2} \text{s}^{-1}$.

2.4. Photosynthesis, plant biomass and allocation

Seven seedlings of each species from each treatment combination (28 plants per species) were harvested on day 31 of the trial (results not presented here). The remaining 64 plants, 32 of each species, were harvested on day 77 and are referred to as the second harvest (h_2 ; Table S2). Prior to destructive harvest, four individuals of each species and treatment combination were measured for net carbon assimilation across a range of ambient CO_2 levels (Hough-Snee, 2010). We graphically present photosynthesis per unit leaf area and per unit leaf mass from the $400 \mu\text{mol mol}^{-1} \text{CO}_2$ level to aid interpretation of plant allocation and morphological data. At each harvest leaf area and whole plant wet biomass were measured and each plant was separated into two sections: aboveground (stems and shoots), and belowground (roots and rhizomes). The biomass from each plant was dried to a constant weight at 80 °C for 72 h. Dried biomass was measured for aboveground and belowground portions. Additionally, leaf area ratio (LAR), leaf mass ratio (LMR) and specific leaf area (SLA) were calculated for each plant to assess how the treatments were affecting the proportional allocation to the photosynthetic surfaces. LMR is defined as the proportion of leaf biomass to total plant biomass while SLA is the ratio of leaf area to leaf biomass. LAR is the product of SLA and LMR (Causton and Venus, 1981). With the exception of leaf area and wet weight, all biomass results presented herein use dry biomass values. Herein, we discuss analyses and results only from the second harvest (h_2).

2.5. Statistical analyses

At the time of stratification and treatment allocation, one-way ANOVA was used to test the null hypothesis that initial plant size did not differ between the four treatment combinations for each species. For total biomass, leaf area, root:shoot ratio, aboveground biomass, belowground biomass, LAR, LMR and SLA, a two-way factorial analysis of covariance (ANCOVA) was used to test for differences in treatment group means using plant initial wet weight as a covariate. Tests were performed independently for each species. Tukey pairwise comparisons were made within each species including the initial wet weight as a covariate. Combined fertilization and flooding effects on photosynthesis were compared *only between species* at each treatment combination using *T*-tests with Bonferroni-corrected *p*-values. For interspecific hypotheses, we tested the difference in means between each species at each treatment combination using pairwise *T*-tests with Bonferroni-adjusted *p*-values for each species at a given treatment combination. Interspecific hypotheses were tested independently from intraspecific hypotheses because our re-randomization scheme within the greenhouse prevented block or gradient effects within the greenhouse from compromising independence between experimental units. Test results were considered significant at

an alpha level of $p < 0.05$. Prior to analyses, data were assessed for normality and homogeneous variance using Shapiro–Wilk and Bartlett's tests and log transformed as necessary. All data are presented untransformed. Statistical analyses were performed in R version 2.9.1 (R Development Core Team, 2011).

3. Results

Initial wet weights ranged from 5.5 g to 80.6 g with a mean of 29.48 g for *C. stipata*, and from 3.8 g to 61.8 g with a mean of 22.32 g for *C. obnupta*. Mean plant wet weights did not significantly differ between treatment groups for either species prior to experimental treatments began ($p = 0.24$ for *C. stipata*; $p = 0.94$ for *C. obnupta*).

Harvest results for *C. obnupta* showed the highest total biomass, leaf areas, aboveground biomass, and belowground biomass in the least stressful highly fertilized, shallow flooding, (HS) treatment (Fig. 1; Table S3). For *C. obnupta* the lowest total biomass, leaf areas, aboveground biomass, and belowground biomass were all measured in the low fertilization, shallow flooding, (LS) treatment group (Fig. 1). Differences in *C. obnupta* total biomass, leaf area, above- and belowground biomass, could be attributed to fertilization and initial wet weight effects (Table 1). Flooding was also a significant factor in determining *C. obnupta* belowground biomass growth. Total biomass, leaf areas, and aboveground biomass of *C. obnupta* did not differ between deep and shallow flooding treatments within a given fertilizer treatment (Fig. 1). *C. obnupta* belowground biomass was highest in the HS treatment (Fig. 1). Flooding and fertilization, significantly affected *C. obnupta* root:shoot ratio (R:S). Plants grown with low nutrients or shallow flooding had proportionally greater R:S than their counterparts in the high nutrients or deep flooding treatments. Differences in *C. obnupta* leaf morphology (LAR, SLA, and LMR) were significantly influenced by fertilization. LMR was also significantly affected by the flooding treatment. Pairwise comparisons revealed that there were not significant LAR differences within nutrient treatments, SLA only differed between HS and LD treatments. Leaf mass ratio only differed between HD and LS (Fig. 2).

Differences in *C. stipata* total biomass and leaf areas could be primarily attributed to flooding (Table 1, Fig. 1). Unlike *C. obnupta*, initial *C. stipata* seedling wet weights did not affect final total biomass or leaf areas. While the most biomass accumulated in *C. obnupta* in the HS treatment, for *C. stipata* the greatest total biomass, leaf areas, aboveground biomass, and belowground biomass were in the HD group (Fig. 1). Similar to *C. obnupta*, the least total aboveground and belowground biomass and lowest leaf areas were measured for *C. stipata* in the LS treatment combination (Fig. 1). Although the general responses to fertilization were similar between species, total biomass was greater in deciduous *C. stipata* (Fig. 1) than evergreen *C. obnupta*. The R:S ratio for *C. stipata* followed the same pattern as *C. obnupta*, with the greatest R:S occurring in the LS group, and the lowest R:S ratio in the HD treatment. Root:shoot ratios were attributable to both flooding and fertilization. Differences in *C. stipata* LAR were primarily attributable to fertilization, with no significant differences within flooding treatments within either fertilizer group (Fig. 2). Flooding–fertilization interaction terms were only significant for *C. stipata* (Figs. 1–2). Specifically, wet weight, leaf area, aboveground biomass, total biomass and root-to shoot-ratio were influenced by flooding–fertilization interactions. For *C. stipata* SLA, there were no significant differences between any treatment combinations. *C. stipata* LMR was affected by both flooding and fertilization, with significant differences between flooding treatments within the high nutrient treatment, and between high and low nutrients (Fig. 2). Net carbon assimilation did not differ between treatments within *C. obnupta*. Within *C. stipata* the HS treatment had significantly higher

photosynthesis per unit mass than the other treatment combinations (Fig. 3).

Between the two species, there were numerous differences in allocation, morphology and photosynthesis at each treatment combination (Figs. 1–3). Total biomass, leaf area, and R:S ratio were higher in *C. stipata* than *C. obnupta* at the same treatment levels (Fig. 1). Within high fertilization treatments at both flooding levels, *C. stipata* produced more above and belowground biomass than *C. obnupta* (Fig. 1). Between *C. stipata* and *C. obnupta* LAR, SLA and LMR differed at each treatment combination (Fig. 2). Leaf area ratio and SLA were higher in *C. stipata*, showing that it accumulated the most leaf area per unit total biomass and per unit leaf biomass. Leaf mass ratio was highest in *C. obnupta* showing that it preferentially allocated biomass to thick leaves.

4. Discussion

4.1. Plant biomass and allocation

Previous research has demonstrated that *Carices* (*C. limosa*, *C. sempervirens*, *C. rostrata* and *C. davalliana*) proportionally allocate biomass to roots when submerged or partially flooded (Visser et al., 2000). Our results suggest that this flooding response can be mitigated under eutrophic conditions in fast-growing *Carex* species. For instance, in high fertility treatments individuals of both species proportionally allocated less biomass to roots than unfertilized individuals of the same flooding treatment. Both species' allocation responses to flooding and nutrients show that *Carex* responses to flooding may be driven by available soil resources. Nutrients generally exerted a stronger effect on total biomass and leaf area than flooding in *C. obnupta* and *C. stipata* (Table 1; Figs. 1–3), indicating that nutritional subsidy is more important than flooding stress in shaping plant morphology and allocation.

We observed rapid fertilization-driven leaf area expansion in both species regardless of flooding level. Both species effectively utilize soil resources to overcome flooding stress, but *C. stipata* builds thin and short-lived leaves very quickly (Figs. 2–3). The allocation costs of building new leaves is higher for evergreen species than deciduous species (Aerts, 1995; Wright et al., 2004). Accordingly, initial plant size had a more significant effect on *C. obnupta* total, and above- and belowground biomass, than on the same metrics in deciduous *C. stipata*. Photosynthesis was also lower per unit leaf mass for *C. obnupta* than for *C. stipata* (Fig. 3). *C. stipata* showed lower photosynthesis per unit leaf area, indicating that it has a less efficient canopy, and perhaps lower construction costs per unit leaf mass than *C. obnupta* under all treatments. This observation corresponds to the high leaf mass ratios and thick leaves of *C. obnupta*. Increased size at planting has been shown to correspond to greater growth, final plant height, and survival in *Carex nebrascensis*, *C. rostrata*, and *C. lanuginosa* in riparian meadows (Steed and DeWald, 2003), and may be a product of total canopy area. Size effects may influence how rapidly individual plants can overcome resource limitations and also how plants compete with one another across environmental gradients. Larger individual *Carices*, especially of evergreen species with thick leaves (low SLA), may compete more effectively as plant tissue resources help individuals to rapidly increase whole plant photosynthesis and growth under stress (Poorter et al., 2009).

The reduced LAR and LMR observed in the LD and LS treatments are consistent with other studies that have shown SLA to limit growth in infertile environments (Poorter and Remkes, 1990). Similar to our findings, it has been shown that coupling nutrient stress with other stressors can significantly reduce LAR in emergent wetland plants (Morris and Ganf, 2001). For example, as the duration and depth of flooding increased in *Genipa americana* seedlings, LMR

Fig. 1. Factorial response plots illustrating the differences in total biomass, leaf area, belowground biomass, aboveground biomass and root:shoot ratio between flooding and fertilization treatments and species at harvest two. Lower-case letters at each bar indicate statistical differences between treatment combinations within each species. Upper-case letters indicate statistically significant interspecific differences between *C. obnupta* and *C. stipata* within a given treatment combination.

Table 1
Final biomass allocation weights, leaf area, and root-to-shoot ratio ANCOVA results for h_2 significant ($p < 0.05$) probability values are reported below.

Growth parameter	Species	Initial wet weight	Flooding	Fertilization	Flooding–fertilization interaction
Wet weight	<i>C. obnupta</i>	$p < 0.01$	–	$p < 0.01$	–
	<i>C. stipata</i>	–	–	$p < 0.01$	$p = 0.023$
Leaf area	<i>C. obnupta</i>	$p < 0.01$	–	$p < 0.01$	–
	<i>C. stipata</i>	–	–	$p < 0.01$	$p < 0.01$
Aboveground biomass	<i>C. obnupta</i>	$p < 0.01$	–	$p < 0.01$	–
	<i>C. stipata</i>	–	–	$p < 0.01$	$p < 0.01$
Total biomass	<i>C. obnupta</i>	$p < 0.01$	–	$p < 0.01$	–
	<i>C. stipata</i>	–	$p = 0.01$	$p < 0.01$	$p < 0.01$
Belowground biomass	<i>C. obnupta</i>	$p < 0.01$	$p = 0.05$	$p < 0.01$	–
	<i>C. stipata</i>	–	–	$p < 0.01$	–
Root-to-shoot ratio	<i>C. obnupta</i>	–	$p = 0.01$	$p < 0.01$	–
	<i>C. stipata</i>	–	$p < 0.01$	$p < 0.01$	$p = 0.05$
Leaf area ratio	<i>C. obnupta</i>	–	–	$p < 0.01$	–
	<i>C. stipata</i>	$p = 0.06$	–	$p < 0.01$	–
Specific leaf area	<i>C. obnupta</i>	–	–	$p = 0.044$	–
	<i>C. stipata</i>	–	$p = 0.02$	–	–
Leaf mass ratio	<i>C. obnupta</i>	$p = 0.04$	$p = 0.02$	$p < 0.01$	–
	<i>C. stipata</i>	–	$p = 0.01$	$p < 0.01$	–

and LAR both decreased (Mielke et al., 2003). Flooding depth influences SLA in numerous species (Jung et al., 2010), while leaf area, specifically LAR, of which SLA is a component process, may drive plant growth (Evans and Poorter, 2001; Shipley, 2006).

4.2. *Carex* and stress: Linking stress responses to plant life history strategies

C. obnupta and *C. stipata* had distinct responses to flooding and fertilization in their growth, morphology, and biomass allocation. The relationships between plant persistence across fertility and flooding gradients commonly show tradeoffs between growth and resource acquisition in productive environments and the effective conservation of resources under stressful conditions (Poorter and Remkes, 1990; Rubio et al., 1995; Willby et al., 2001). As each species experiences amenable conditions for growth – highly fertile, flooded conditions for *C. stipata* and fertile, saturated conditions for *C. obnupta* – it may increase total photosynthetic biomass, whole plant photosynthesis and relative growth rates. High growth rates correspond to high LARs and low R:S indicative of high canopy carbon gains rather than belowground tissue allocation for acclimating to flooding and nutrient stress. It is possible that both species initially expanded their leaf areas in response to flooding stress, but the costs of building new evergreen leaves amid limited oxygen within flooded treatments forced *C. obnupta* to grow less rapidly by the end of the trial. Another deciduous wetland species, *Rumex palustris*, has been shown to preferentially elongate petioles and expand leaf area in response to flooding (Bailey-Serres and Voesenek, 2008; Pierik et al., 2008; Voesenek et al., 2004), allowing the plant to acclimate to flooding over long timeframes. These observations help to explain not only the life strategy and realized habitats of both sedges, but also provide insights to each species' range of potential niches across these gradients.

The dynamic wetland environments that sedges inhabit may expose plants to additional stressors such as drying, sediment accumulation or herbivory that further shape their functional traits and select for specific life history strategies. For example, as the distance to water table decreased, transplants of *Carex utriculata* were shown to survive at greater rates in riparian meadows than those plants that never experienced root-zone flooding (Sarr and Dudley, 2008). Drying following flooding also reduces gas exchange in *C. stipata* (Ewing, 1996). Post anoxic injury is common in many plant species following flooding, so perhaps to better define the environmental limitations to *C. obnupta* and *C. stipata* performance, these species should be subject to flooding and drying regimes similar to

the hydrographs of many wetlands where they commonly occur (Ewing, 1996). If wetland hydrology has shifted to extreme wet and dry periods, like many urban and disturbed wetlands, the dual stressors of flooding and drying will likely affect many plant life stages, including reproduction. For example, hydrology might strongly affect the germination of *C. stipata* seed, its most common reproductive strategy. Elucidating the limitations to seedling emergence in response to seasonal flooding and water table draw-downs would be of great use in predicting how altered hydroperiods might change wetland plant communities by altering suitable habitat availability over time. Further investigation could examine how each species responds to flooding and fertilization from germination onward and over longer timeframes. The temporal scale at which rhizomatous species such as *C. obnupta* expand is often longer than the duration of this trial, allowing for limited inference on how flooding and fertilization might influence the reproduction dynamics and long-term population demographics of the species.

While short-duration flooding may not be a limiting factor for the successful growth of larger, established individuals of *C. obnupta* and *C. stipata*, flooding stress is exacerbated by nutrient limitations that reduce leaf level and whole plant photosynthesis, leaf area expansion, and plant growth in both species. *C. stipata*, while widely distributed, may only be dominant in environments where it can rapidly expand its leaf area and increase plant total biomass to outgrow stress early. *C. stipata* may be more adversely affected by flooding during the seedling stage immediately following seed germination. If plants adapted to outgrow flooding are too small to extend a 'straw' above floodwater surfaces and continue gas exchange, then they may not persist in that environment. The capability to outgrow flooding is only particularly useful in situations where flooding is shallow and prolonged (Voesenek et al., 2004), such as the HD and LD treatments within this study. *C. stipata* performs poorly in infertile environments, showing a stress-tolerant–ruderal life history strategy (Grime, 1977) that may not perform well in low-nutrient wetlands such as fens and bogs.

C. obnupta shows a competitive-stress tolerator strategy (*sensu* Grime, 1977) as it persists in flooded environments with high nutrient levels that allow for the creation and maintenance of efficient, long-lived evergreen leaves. Paired flooding stress and nutrient limitation may affect small *C. obnupta* individuals more adversely than large individuals. Unlike *C. stipata*, *C. obnupta* lacks the ability to rapidly increase specific leaf area and expand total photosynthetic area to overcome flooding stress. This may also explain why these species are not often found in the same types of wetlands. Our SLA results imply that *C. stipata* may also compete

Fig. 2. Factorial response plots illustrating the leaf area ratio, specific leaf area, and leaf mass ratio of each species across flooding and fertilization treatments at final harvest. Error bars are standard error of the mean. Lower-case letters adjacent to each bar indicate statistical differences between treatment combinations within each species at each harvest. Upper-case letters indicate statistically significant interspecific differences between *C. obnupta* and *C. stipata* within a given treatment combination.

well with invasive plants that employ similar ruderal strategies. *C. obnupta*, which can expand through large rhizome networks, may expand more slowly, but may be able to persist under some invasion pressure—in part because of the flooded environments that it inhabits and partly because competing species may not be able to recruit into dense rhizomatous monocultures.

This study investigated how individuals of two *Carex* species responded to prolonged flooding and nutrient subsidy or stress. We identified that *C. obnupta* has a stress-tolerating strategy that relies on existing canopy biomass to ensure consistent growth and

C. stipata uses a rapidly growing low-oxygen escape strategy to out-grow stress. Each species' functional responses to environmental stress help to explain their realized niches. Additionally these stress responses illustrate both species potential niches in novel wetland environments. Our findings have implications for how each species competes within communities and persist through new environmental filters like urban hydrology or agricultural nutrient subsidies. By understanding how these species respond to stress, inference can be made on how sedge-dominated wetland communities might change under different anthropogenic stressors.

Fig. 3. Net assimilation rates of all species-treatment combinations on a per unit leaf mass (A) and per unit leaf area basis (B). *Carex obnupta* had higher leaf construction costs than *Carex stipata*, photosynthesizing at a higher rate per unit leaf area. Letters indicate significant differences in means between species at a given treatment combination. Error bars are standard error of the mean.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.aquabot.2014.03.001>.

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