The effects of flooding depth, fertilization and initial seedling size on the growth and biomass allocation of two wetland sedges, *Carex obnupta* and *Carex stipata*.

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Abstract

The effects of flooding depth, fertilization and initial seedling size on the growth and biomass allocation of two wetland sedges, *Carex obnupta* and *Carex stipata*.

Nathaniel Hough-Snee

Chair of the Supervisory Committee:
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Flooding and nutrient stress are common environmental factors that affect the composition and productivity of wetland plant communities. Wetland plants respond to flooding and either eutrophication or nutrient stress by preferentially allocating biomass to tissues that allow them to persist in stressful environments. In wetland restoration projects, hydrology and fertility are often manipulated and wetland vegetation is planted to match the designed hydrology. When wetland designers fail to achieve their desired hydrology or plants are installed at improper elevations within a wetland, plants may be exposed to higher flooding that causes a decrease in plant performance or even death. I experimentally manipulated flooding and nutrient levels in a fully crossed factorial design to examine the effects of nutrient stress and flooding on the growth and performance of two sedges, *Carex obnupta* and *Carex stipata*. Treatments were arranged across a stress gradient from high fertilization, shallow flooding to low fertilization, deep flooding. I measured plant biomass accumulation and allocation at two points within a 77-day period, calculating relative growth rates, leaf area ratio and net assimilation rates of both species

by treatment using initial plant size as a covariate in all biomass analyses. To examine the physiological processes behind these patterns in growth and allocation I measured leaf-level photosynthesis and estimated leaf chlorophyll content using SPAD. In both species total plant biomass, leaf area and proportion shoot biomass were highest in the fertilized treatments regardless of flooding stress at both harvest intervals. There was an initial plant size effect in *C. obnupta*: smaller plants grew less in response to flooding stress, especially at the first harvest point. *C. stipata* outgrew *C. obnupta* in all treatments although *C. obnupta* had a higher net assimilation rate than *C. stipata*. Gas exchange measurements showed a significant fertilization effect that allowed the less stressful high fertilization treatment to assimilate carbon more rapidly than the low fertilization, deeply flooded plants. Many of these observations may be attributable to differences in the life history and competitive strategy of the examined species. *C. obnupta* can be planted across a range of flooding conditions as a stress tolerator while *C. stipata* may be able to outgrow flooding stress by expanding leaf area rapidly through performance that suggests low oxygen escape strategy.

TABLE OF CONTENTS

Page

List of Figures ii

List of Tables iii

1.0 Introduction 1
1.1 Introduction 1
1.2 Plant Performance in the Flooded Environment 2
1.3 Nutrient Acquisition in the Flooded Environment 4
1.4 Plant Performance and Restored Wetlands 6
1.5 Plant Species and Experimental Rationale 7

2.0 Materials and Methods 9
2.1 Plant Materials and Allocation to Experimental Treatments 9
2.2 Experimental Design and Treatments 10
2.3 Plant Biomass and Allocation 13
2.4 Relative Growth Rate and Component Processes 14
2.5 Leaf Gas Exchange and Fluorescence 15
2.6 SPAD Index 16
2.7 Statistical Analyses 17

3.0 Results 19
3.1 Treatment pH and redox potentials 19
3.2 Plant Biomass and Allocation 19
3.3 Relative Growth Rate and Component Processes 36
3.4 SPAD Index 46
3.5 Leaf Gas Exchange 48

4.0 Discussion and Conclusions 58
4.1 Plant Biomass and Allocation 58
4.2 Relative Growth Rate and Component Processes 59
4.3 Leaf Gas Exchange 60
4.4 *Carex* and Stress: Conclusions and Additional Considerations 62

5.0 References 66

Appendix I: Supplemental Bar Charts 70

LIST OF FIGURES

Figure Number Page

1. Harvest I Total Biomass....20
2. Harvest I Aboveground Biomass....21
3. Harvest I Belowground Biomass....22
4. Harvest I Total Leaf Area....24
5. Harvest I Root-to-Shoot Ratio....25
6. Harvest II Total Biomass....27
7. Harvest II Aboveground Biomass....28
8. Harvest II Belowground Biomass....29
9. Harvest II Total Leaf Area....31
10. Harvest II Root-to-Shoot Ratio....32
11. Specific Leaf Area at Harvest I....41
12. Leaf Mass Ratio at Harvest I....42
13. Leaf Area Ratio at Harvest I....43
14. Specific Leaf Area at Harvest II....44
15. Leaf Mass Ratio at Harvest II....44
16. Leaf Area Ratio at Harvest II....45
17. Mean SPAD Index....47
18. *Carex stipata* Carbon Assimilation-Intercellular CO_2 Curves....50
19. *Carex obnupta* Carbon Assimilation-Intercellular CO_2 Curves....51
20. *Carex stipata* Gas Exchange....54
21. *Carex stipata* Stomatal Conductance....55
22. *Carex obnupta* Gas Exchange....56
23. *Carex obnupta* Stomatal Conductance....57

LIST OF TABLES

Table Number Page

1. Fertilization Treatment Descriptions 12
2. Factorial Treatment Descriptions....12
3. Harvest Intervals Descriptions....13
4. Mean Allocation, Total Biomass and Root-to-Shoot Ratio at Harvests I and II 34
5. ANCOVA Results for Biomass at Harvests I and II....35
6. Mean Relative Growth Rates Across Harvests....38
7. ANCOVA Results for Growth Parameters at Harvests I and II 39
8. Mean Growth Parameters at Harvests I and II....40
9. Mean SPAD Index 47
10. PERMANOVA Model Output....49
11. ANCOVA Results for Carbon Assimilation....53
12. ANCOVA Results for Stomatal Conductance....53

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DEDICATION

For Charles Thomas Snee Sr.

1.0 Introduction

1.1 *Introduction*

Wetland environments commonly exhibit fluctuations in the intensity and timing of hydrologic regimes, as well as variation in within-system nutrient loads (Shaffer et al. 1999, Mitsch and Gosselink 2007). This hydrologic and chemical variability causes a pair of potential stressors to which wetland plants must respond, flooding and nutrient stress. Flooding stress commonly governs plant distributions within wetlands, based on plant morphological and physiological adaptation and acclimation to a reduced soil environment (Kozlowski 1984, Mitsch and Gosselink 2007). Flooding reduces the amount of oxygen available to submerged portions of plants, as microorganisms consume oxygen faster than it can diffuse through flooded soils. In the absence of oxygen, microorganisms begin to use manganese, iron and nitrate as terminal electron receptors and some of these reduced elements are transformed to soluble, toxic forms capable of damaging plant tissues. The lack of oxygen also inhibits aerobic root respiration and active metabolic processes such as nutrient and water uptake and root membrane control (Laanbroek 1990).

In contrast to flooding, eutrophication, the nutrient enrichment of water bodies, increases plant productivity and nutrient uptake. Plant performance is enhanced when plants are able to sufficiently acclimate to the reduced environment and essential nutrients do not occur exclusively in toxic, reduced compounds (e.g. Rey Benayas and Scheiner 1993, Johnson and Leopold 1994, Laanbroek 1990, Merino et al. 2010). Fertile wetland conditions are also associated with higher total productivity in wetlands at the expense of plant diversity as fast-growing plants out compete stress tolerators (Bedford et al. 1999, Moore et al. 1989, Grime 1977). These community-level observations may be explained by enhanced physiological performance in enriched environments: increased leaf nitrogen content, tissue chlorophyll

content, carbon assimilation and growth (Laanbroek 1990) or specific strategies to escape stress and continue carbon assimilation and growth (Voesenek et al. 2004). Dual plant responses to flooding and eutrophication, mediated by physical and biochemical changes, may cause changes in plant photosynthesis, growth, leaf architecture and plant tissue allocation that either hinder or enhance plant performance in the wetland environment (Burdick and Mendelssohn 1990). The effects of flooding on hydrophytic vegetation have been relatively well studied and reviewed (Kozlowski 1984, Arcteca 1997, Blanch et al. 1999, Kozlowski 2002, Bailey-Serres and Voesenek 2008), while wetland plant nutrition has commonly been studied in the context of nutrient removal from wastewater and agricultural treatment wetlands (Etnier and Guterstam 1996) and changes in floristic diversity. Only rarely have flooding, nutrient enrichment and infertility been integrated into studies to see how both factors may interact to affect wetland plant growth, community assembly and functional traits (Rubio et al. 1995, Willby 2001).

1.2 *Plant Performance in the Flooded Environment*

The ability of plants to respond to flooding is determined by their ability to acquire oxygen in submerged plant portions and to expand the proportion of biomass they allocate to leaf area. The former drives respiratory energy gains in roots required for whole plant growth and nutrient and water acquisition, while the latter drives carbon gains for plant metabolism and the net assimilation rate of the plant (Lambers et al. 2003). Because oxygen and other gases diffuse 10,000 times more slowly in water than in air, flooding decreases whole plant energy balance by reducing aerobic respiration in submerged portions of plants. Submerged plants must either respire anaerobically at a net energy loss, or acclimate to the hypoxic environment through morphological mechanisms that allow them to respire aerobically (e.g. aerenchyma) or face

death due to halted cell metabolism (Jackson 1994). Plants that experience flooding acclimate in part by shifting biomass allocation to root production so the plant may acquire oxygen that can serve as the terminal electron receptor during aerobic root respiration. This allocation may also occur to create new roots with preformed aerenchyma cells (Mitsch and Gosselink 2007). Plant allocation to roots may occur at the expense of canopy growth because as total canopy biomass declines, so does the potential carbon gained through canopy photosynthesis (Mielke et al. 2003). This reduction in whole plant photosynthesis may cause a low plant energy balance (Lambers et al. 2003, Bailey-Serres and Voesenek 2008). However, even when flooding reduces leaf area expansion, plant total photosynthesis may be high due to existing high specific leaf areas that facilitate high net assimilation and relative growth rates allowing plants to 'outgrow' flooding stress (Bailey-Serres and Voesenek 2008).

In addition to changing allocation patterns, flooding has been shown to reduce leaf-level photosynthesis in many plant species (Kozlowski 1984; Mielke et al. 2003). Photosynthesis inhibition under flooding may be due to diminished root permeability in the reduced environment that prevents nutrient and water acquisition (DeLaune et al. 1998, Kozlowski 1997). This can be attributed to both stomatal and nonstomatal limitations depending on the species and timeframe in which flooding occurs (Kozlowski and Pallardy 2002, Pezeshki et al. 1996, Jones et al. 2006). Stomatal closure commonly occurs as an acclimation response to flooding after abscissic acid, ethylene or other hormones trigger reduced gas exchange. Alternately less cytokinins from the root meristem may be channeled to leaves or physiological drought may cause leaf wilting and stomatal closure in response to flooding (Kozlowski 1984, Pezeshki 2001). Regardless of the cause of stomatal closure, the result is the same: reduced leaf-level carbon assimilation and a reduced whole plant carbon balance (Kozlowski 2002). Nonstomatal

limitations to photosynthesis in the flooded environment may be driven by the energy conservation strategies of flooded plants that regulate active processes or impede nitrogen acquisition under more reduced conditions (Kozlowski 1997, Delaune et al. 1998, Pezeshki et al. 1996).

1.3 *Nutrient Acquisition in the Flooded Environment*

Plants must acquire nutrients, namely nitrogen, a key ingredient in the construction of Ribulose-1,5-bisphosphate carboxylase oxygenase (Rubisco), chlorophyll pigments and thylakoid proteins within leaves (Lambers et al. 2003). In oxidized environments with high nutrient concentrations, plants may be able to allocate more resources to leaves for the production of Rubisco, increasing net carbon gains through increased leaf-level photosynthesis. This increased plant energy balance increases leaf area ratio (Lambers et al. 2003) further facilitating rapid growth through high net canopy photosynthesis. In contrast, plants growing in nutrient-limited environments may allocate more biomass to roots, increasing root surface area to acquire nutrition (Morris and Ganf 2001, Rubio et al. 1995**)**. As in flooded environments, in nutrient poor conditions the search for limiting belowground resources may occur at the expense of leaf area expansion that allows for whole plant carbon gains. Additionally, when stressfully low nutrition is paired with flooding, interactions may further exacerbate plant stress (Pezeshki 2001). It has been well illustrated that without sufficient nutrition, nitrogen-dependent plant properties including tissue nitrogen content, photosynthesis, and growth may be reduced (Willby et al. 2001). By adding additional stressors, including flooding, to nitrogen-limited plants, plant stress may become extreme, causing plant mortality (Morris and Ganf 2001, Merino et al. 2010).

While flooding and nutrient deficiency may have negative impacts on plants, there may be positive interactions between flooding and fertilization when the flooding solution is highly enriched. Since phosphorus, potassium, magnesium and calcium, essential plant nutrients, are not reduced in anaerobic conditions, total nutrition may be increased when these elements exist in the flooding solution at high concentrations (Laanbroek 1990). To acclimate to flooding, plants also channel oxygen from leaves and shoots to submerged roots via aerenchyma, forming an oxidized rhizosphere. This oxidized zone around plant tissue may provide a buffer from anoxic conditions where nutrient compounds (namely nitrate) may remain soluble for plant uptake. When a plant is flooded in a highly-enriched solution, the plant may be able to overcome flooding stress by assimilating nitrogen from the oxidized rhizosphere to elsewhere in the plant, increasing total plant photosynthesis, plant leaf area ratio and net assimilation rate. This aboveground growth increases whole plant carbon gains and provides a positive energy balance from which belowground tissues may develop, building new aerenchymous roots that maintain the availability of oxygen for belowground respiration and potentially driving continued plant growth through low oxygen escape syndrome (Bailey-Serres and Voesenek 2008). Low oxygen escape syndrome may occur rapidly in enriched flooding conditions where continued photosynthesis allows stem architecture and aerenchyma formation to facilitate continued leaf growth (Bailey-Serres and Voesenek 2008). This escape strategy of rapidly expanding leaves when submerged has been shown to enhance the survival and growth of *Rumex palustris* in lab settings (Pierik et al. 2009) and field settings (Voesenek et al. 2004).

1.4 *Plant Performance and Restored Wetlands*

Natural wetland plant communities assemble and perform physiologically across hydrologic stress gradients of flooding and drying (Van Eck et al. 2004, Keddy 1992) and in response to nutrient loads. Wetland hydrology has been shown to govern plant community distributions within and adjacent to wetlands, often as a result of differential plant responses to wetland stressors (Keddy 1992, Keddy 2000). Hydrologic subsidies to wetlands (e.g. flood pulsing) may effectively fertilize plant communities increasing primary productivity and plant tissue concentration of essential nutrients (Barko and Smart 1978, Jordan et al. 1990), in a given wetland environment. Based on these observations, many frameworks have been created to classify natural wetlands based on hydrology (Keddy 2000) and fertility (Lugo et al. 1988), and explain wetland ecotypes and functional plant guilds (Magee 2005).

Unlike natural systems, in created and restored wetlands, those designing the wetland dictate a system's hydrology and installed plants. When designers fail to achieve their desired hydrology installed vegetation must then survive increased flooding stress when hydroperiods are longer and deeper than anticipated. In wetlands designed to receive excess nutrients from stormwater, agricultural runoff or novel soils (e.g., retired mines), nutrient loads may be variable. This variability can adversely affect plant processes when reduced eutrophication fails to deliver the anticipated loads of nutrients, or in high nutrient solutions, helping plants to overcome flooding stress by aiding nutrient acquisition. Wetland plants are commonly chosen for use in restoration and engineering projects based on their anticipated growth rates, allocation to above and/or belowground biomass, and perceived resilience to environmental stress. To select better plant species and genotypes for use in restoration, plants could be examined for their performance in simulated wetland environments prior to their use. By assessing the

physiological performance of wetland plants under a range of hydrologic and nutritional scenarios, wetland managers may more effectively select plants for restoration based on within wetland elevation, hydrology and nutrient loads increasing the probability of plant survival and project success rates.

1.5. *Plant Species and Experimental Rationale*

I established a greenhouse experiment in June 2009 at the University of Washington Center for Urban Horticulture (Seattle, WA) to assess the growth, allocation and gas exchange of two common wetland sedges, *Carex obnupta* and *Carex stipata* under a combination of two flooding treatments and two fertilization treatments. I selected these species for their regional abundance and popularity in wetland restoration projects as well as the globally widespread nature of the genus *Carex* (cyperaceae). *Carex obnupta* is an evergreen, rhizomatous sedge species that grows in wetland habitats of variable hydroperiods including coastal marshes, hydric forests, lake and stream edges and tidal riparian systems. It has been observed to occur predominantly on or near hydric organic soils that experience their deepest flooding in winter (Minore 1979). *C. obnupta* spreads rapidly through rhizomes and seed, and forms dense monocultures in many environments. *C. obnupta* is found from British Columbia to northern California, commonly west of the Cascade Mountains (Wilson et al. 2008). *Carex stipata* is a deciduous tussock forming sedge that inhabits wetland edges and other seasonally wet habitats across North America and portions of Europe and eastern Asia (Wilson et al. 2008). It is most common in open wetlands such as meadows and wet prairies but does not usually occur in deep, open water (Magee and Kentula 2005) or grow well under prolonged flooding (Ewing 1996). *C. stipata* produces a relatively heavy seed crop and its seeds have been shown to exhibit reduced

dormancy (Hough-Snee and Cooper 2011, Kettenring et al. 2007a, Kettenring and Galatowitsch 2007b). Both *C. obnupta* and *C. stipata* are currently classified as obligate wetland plant species by the United States Department of Agriculture (USDA 2010) and are commonly employed in wetland restoration for their growth, resilience, seed production, ease of propagation and commercial availability.

Although the life strategies of *C. stipata* and *C. obnupta* differ, both sedges often exist in at least seasonally inundated or high water table habitats, but at different elevations in relation to the depth and duration of flooding. My larger goal was to characterize the growth and physiological properties of both *C. stipata* and *C. obnupta* in response to flooding depth and fertilization conditions that might occur in restored or created wetlands. To assess plant performance in the simulated wetland environment, I measured growth, biomass allocation, photosynthesis and fluorescence and estimated leaf chlorophyll content using SPAD. I assessed these metrics because they at least partially define plant performance as it relates to successful survival, growth and reproduction in a given environment.

I investigated four families of hypotheses based on the aforementioned project goal:

1. Plants experiencing increased flooding will exhibit lower growth and photosynthesis rates, lower total biomass, leaf chlorophyll content and leaf area than those plants not undergoing flooding. Flooded plants will also allocate a higher proportion of their total biomass to root growth rather than shoot growth.

2. Plants experiencing fertilization will exhibit higher rates of growth and photosynthesis and higher total biomass, leaf chlorophyll content and leaf area than those plants that remain unfertilized. The root-to-shoot allocation of fertilized plants will favor shoot growth

3. When pairing flooding and fertilization, the highest levels of growth and photosynthesis will be seen in those plants experiencing shallow flooding and high fertilization. Plants experiencing deep flooding and high fertilization will grow and photosynthesize at rates lower than the shallowly flooded, fertilized plants and better than those plants experiencing shallow flooding and low fertilization and those experiencing high flooding and low fertilization respectively.

4. Between the two species, I anticipate that *C. stipata* and *C. obnupta* will perform differently based on life-history strategy. I anticipate *C. stipata* will produce leaf biomass more rapidly than *C. obnupta* because of its deciduous life strategy. Similarly, based on existing within-leaf resources in the evergreen *C. obnupta,* I anticipate that *C. obnupta* will be able to tolerate high flooding better than *C. stipata*, performing higher gas exchange and exhibiting higher leaf nitrogen (SPAD) rates regardless of treatment pairing with fertilization.

2.0 Materials and Methods

2.1 Plant Materials and Allocation to Experimental Treatments

Two hundred bare-root seedlings of *Carex stipata* and *Carex obnupta* were purchased from Fourth Corner Nurseries (Bellingham, WA) and potted on May 2nd-7th 2009. Wet weight, leaf count, length of the longest fully-developed leaf, root length and root-to-shoot ratio—all potential covariates—were measured for each seedling, before being planted into 3.8 liter (one gallon) plastic pots containing ~3.7 liters of washed builder's sand (Salmon Bay Sand and Gravel, Seattle, WA). After being potted, all plants were placed onto a greenhouse bench where

they were watered daily and monitored for mortality until June 3, 2009. Both sedge species experienced significant transplant stress and mortality. Almost all individuals of *C. stipata* exhibited leaf and shoot biomass dieback in the initial week after potting, while *C. obnupta* shed leaves to a lesser extent.

After initial seedling mortality, 72 individuals of *Carex obnupta* and 64 individuals of *Carex stipata* remained, from which 60 individuals of each species were randomly selected for inclusion in the experiment. The remaining plants of each species were destructively harvested and measured for root, shoot and stem biomass, leaf area and root to shoot allometry; this subsample of plants was used as the initial plant size baseline from which relative growth was estimated across two sets of harvest intervals. The initial proportions of biomass allocated to the below and aboveground portions of the plants were relatively homogenous within each species, so the remaining 60 plants were evenly stratified across the range of initial wet weights into four groups (N = 60, n = 15, k = 4). Each group was assigned to an experimental treatment at random (described in section 2.2 below). Initial wet weights ranged from 5.5g to 80.6g with a mean of 29.48g for *C. stipata*, and from 3.8g to 61.8g with a mean of 22.32g for *C. obnupta.* The mean wet weights of the four groups for each species did not differ significantly for either species prior to treatment initiation. A one-way Analysis of Variance (ANOVA) found $p = 0.24$ for *C. stipata* and $p = 0.94$ for *C. obnupta* testing the hypothesis that initial mean wet weights differed by treatment group.

2.2 Experimental Design and Treatments

The experimental design consisted of a fully crossed factorial experiment in which both sedge species were subjected to four experimental treatments comprised of two levels of flooding

matched with two levels of fertilization. Flooding treatments were intended to compare potential hydrologic outcomes common to wetland creation and restoration projects. The shallow flooding treatment consisted of each potted plant being placed into an individual five-liter plastic bucket (Container and Packaging Supply, Eagle, ID) and filled to a 5cm depth (≈ 600ml solution volume) with either a low or high fertilizer solution. This treatment was chosen to represent an environment where flooding results in soils inundated throughout roughly one-third of the rooting zone and a nearly dry, aerated soil surface. The high flooding treatment was representative of an environment where perennial flooding occurs: individual plants were allocated to five-liter plastic buckets that were then filled to a depth 4 cm above the substrate level in the 3.8-liter pots (≈ 5000ml solution volume). High and low fertilization treatments were initiated at each flooding level. 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 (summarized in Table 1). These fertilization levels provided nutrient concentrations that were thought to be potentially limiting (low) or in excess of the minimum amounts required for plant growth. Prior to plant harvests on day 74, I measured redox potential and pH of all pots using a platinum electrode and calomel reference electrode (Campbell Scientific) and pH probe respectively (Campbell Scientific). Herein I refer to the treatment combinations with two letter codes: HD, HS, LD, LS (Table II).

Numerous steps were taken to maintain consistent growing conditions during the experiment. Flooding and fertilization treatments were discarded and replaced with fresh treatments on a weekly basis. To compensate for water loss by evapotranspiration between solution replacements, solution levels were topped off to their 5cm and 24cm depths daily using deionized water. Plants were evenly spaced on greenhouse benches to prevent canopy effects

and were systematically rearranged weekly when treatment solutions were changed. Greenhouse temperatures ranged between 17 and 29 °C and the light period consisted of artificial light levels set at 14 hours per day at ≈ 120 μ mol^{-1} $meter^2$ $second^{-1}$ throughout the duration of the experiment. Artificial light accompanied natural light that was as high as 170 μ mol^{-1} $meter^2$ $second^{-1}$.

Table 1: Fertilization treatment summary in ml $liter^{-1}$ and μ mol^{-1}

Compound	High fertilization concentration (ml $liter^{-1}$)	Low fertilization concentration (ml $liter^{-1}$)	Element	High fertilization concentration μ mol^{-1}	Low fertilization concentration μ mol^{-1}
KNO_3	3.0ml	0.75ml	N	8,000	2,000
$Ca(NO_3)$	2ml	0.5ml	K	6,000	1500
$NH_4H_2PO_4$	1ml	0.25ml	Ca	4,000	1,000
$MgSO_7H_2O$	0.5ml	0.125ml	P	2,000	500
Micronutrients	1.0ml	0.25ml	S	1,000	250
Iron	0.25ml	0.0675ml	Mg	1,000	250

Table 2: Flooding and fertilization factorial treatment summary and abbreviations used in the text.

	Deep Flooding	**Abbreviation in text**	**Shallow Flooding**	**Abbreviation in text**
High Fertilization	Submerged plant soil surface 4cm below water line, 24cm deep; half strength Hoagland's solution; in-text abbreviation	**HD**	Plants grown submerged up to the 5cm depth; half strength Hoagland's solution; in-text abbreviation	**HS**
Low Fertilization	Submerged plant soil surface 4cm below water line, 24cm deep; one-eighth strength Hoagland's solution; in-text abbreviation	**LD**	Plants grown submerged up to the 5cm depth; one-eighth strength Hoagland's solution; in-text abbreviation	**LS**

2.3 Plant Biomass and Allocation

I destructively harvested plants at two points during the experiment, measuring plant leaf area, whole plant wet weight, and dry weights by root, shoot and stem proportions. Seven seedlings of each species from each treatment combination (28 plants per species) were harvested on the July 3rd, growing day 31 of the experimental trial. The remaining 64 plants, 32 of each species, were harvested on August 23rd, growing day 77. Wet weight of each plant was recorded before being dissected into three sections: stems, shoots and roots and rhizomes. I dried separated biomass from each plant to a constant weight at 80°C for 72 hours and used dry weight values in the estimation of classical growth parameters (section 2.4) between the initiation of the experiment and the first and second harvests, and between the first and third harvests. I refer to those plants harvested at treatment allocation as the first harvest (t_0), those harvested on day 31 as the second harvest (t_1), and those harvested at day 77 as the third harvest (t_2). With the exception of leaf area and wet weight, all biomass results presented herein use dry biomass values unless otherwise noted.

Table 3: Harvest intervals used in the calculation of relative growth rate and associated parameters.

Harvest Interval	**Quantity plants sampled**		**Date of Harvest (growing day)**
	Carex obnupta	***Carex stipata***	
Harvest One (t_0)	4	4	Prior to experimental treatment allocation (day zero)
Harvest Two (t_1)	28	28	July 3rd (day 31)
Harvest Three (t_2)	32	32	August 23rd (day 77)

2.4 Relative Growth Rate and Component Processes

I calculated leaf area ratio (LAR), leaf mass ratio (LMR) and specific leaf area (SLA) at t_1 and t_2 to assess the relationship between leaf area and biomass allocation to roots or shoots at each harvest. LMR is defined as the proportion of leaf biomass to total plant biomass while SLA is the ratio of leaf area to leaf biomass. I also estimated mean relative growth rate (RGR), net assimilation rate (NAR), LAR, LMR and SLA across three distinct harvest intervals: t_0-t_1, t_0-t_2, t_1-t_2. I used the classical plant analysis formulae of Causton and Venus (1981), as employed by Hunt et al. (2002) to estimate RGR as a product of component plant processes using whole plant dry weight, the focal parameter in plant growth analysis, and leaf area. The relationship between RGR and component parameters is as follows:

$$\mathrm{RGR} = \mathrm{NAR} \times \mathrm{LAR}$$

$$\mathrm{LAR} = \mathrm{SLA} \times \mathrm{LMR}$$

$$\mathrm{RGR} = \mathrm{NAR} \times \mathrm{SLA} \times \mathrm{LMR} \qquad (1)$$

$$(1/W)/(\mathrm{d}W/\mathrm{d}t) = (1/L_A)(\mathrm{d}W/\mathrm{d}t) \times L_A/L_W \times L_W/W$$

t = time

W = total dry weight per plant*

L_A = total leaf area per plant*

L_W = total leaf dry weight per plant

d = delta

To estimate RGR across one harvest interval t_1 to t_2, I calculate, parameter R (Fisher 1921):

$$R \approx (\log_e W_2 - \log_e W_1)/(t_2 - t_1) \qquad (2)$$

To estimate NAR across one harvest interval, I calculate *E* (Williams 1946):

$$E \approx [(W_2 - W_1)(\log_e L_{A2} - \log_e L_{A1})] / [(L_{A2} - L_{A1})(t_2 - t_1)] \quad (3)$$

To estimate LAR across one harvest interval, I calculate LAR (*f*') at each harvest point (Causton and Venus 1981) and then divide by the number of harvest intervals:

$$f' \approx \exp[\log_e f + ½ \vee (\log_e f)] \quad (4)$$

$$F \approx ½ (f'_1 + f'_2) \quad (5)$$

Parallel methods may be used to estimate SLA and LMR across harvest intervals.

The approach of Hunt et al. (2002) does not require the pairing of individual plants, nor does it require repeated non-destructive measurements from which growth parameters are estimated. This approach allows for the estimation of the mean RGR and NAR for a set of plants harvested at two separate points (one harvest interval), providing a 95% confidence interval and standard error value (SE) for each parameter (Hunt et al. 2002). The formulae I used to estimate RGR and component parameters are fully summarized in Hunt et al. (2002), originating from Causton and Venus (1981), Fisher (1921), and Williams (1946). The formulae and proofs for the variance components are excluded here, but are found in Hunt et al. (2002).

2.5 Leaf gas exchange

I examined the photosynthesis and fluorescence of four randomly selected *C. obnupta* and *C. stipata* from within each treatment group with an LI-6400 infrared gas analyzer (IRGA) and Li-6400-40 leaf chamber fluorometer (Li-Cor Biosciences, Lincoln, Nebraska) on August 18-23, days 67 through 72 of the experiment. Measurements were blocked across day by treatment and species, with all measurements occurring between 7:00am and 1:00pm. I measured gas

exchange across a range of CO_2 levels to create curves of photosynthetic carbon assimilation (A) against intercellular CO_2 mole fraction (C_i).

I exposed the third youngest, fully expanded leaf of each plant to eleven ambient CO_2 levels between 100 and 1500 μmol mol^{-1} to investigate potential biochemical limitations to carbon assimilation within each treatment group. Within the A/C_i curves there were two measurements at 400 μmol mol^{-1} after the latter of which point measurements of maximum gas exchange and stomatal conductance were analyzed independently of A/C_i curves. This measurement was taken to provide an adequate acclimation time to the 400 μmol mol^{-1} CO_2 for analysis of stomatal conductance. CO_2 levels between the reference and leaf chambers were matched at the beginning of each CO_2 step. All parameters, including chlorophyll fluorescence were recorded concurrently at A/C_i stabilization or after three minutes, whichever occurred first. I took all measurements at a saturating light level of 1500 μmol m^{-1} s^{-1} holding temperature constant at 25º C with a flow rate of 200 liters per minute. I also assessed electron transport rates (ETR), quantum yield of CO_2 and quantum yield of photosystem II (PSII) at each CO_2 step. ETR and the quantum yield to CO_2 and PSII are parameters used in finding the difference between morphological and biochemical limitations to photosynthesis. By plotting quantum yield of PSII against the quantum yield of CO_2, investigators may see if the plant is biochemically (Rubisco regeneration) limited or morphologically (carbon dioxide assimilation/stomatal conductance) limited. These results, although not discussed within the text are presented in Appendix II.

2.6 SPAD Index

I assessed the relative chlorophyll content of one fully expanded mature leaf on all remaining plants prior to the final harvest using a chlorophyll meter (SPAD-502, Minolta Corporation,

Japan). At growing day 70 I took five chlorophyll measurements on one leaf of each plant, and averaged the values to determine each plant's average SPAD value. SPAD-502 is a device that can be used to quickly and non-destructively provide a proxy index of leaf chlorophyll content. SPAD-502 has been shown to effectively determine the effects of fertilization (Kaakeh et al. 1992), disease and other stressors on crop plants. Monje and Bugbee (1992) and Markwell and others (1995) have shown a strong relationship between SPAD index and leaf chlorophyll concentration in greenhouse and horticultural settings while *Phragmites australis* responses to flooding stress have also been assessed with SPAD (Mauchamp and Methy 2004). I report my results based on meter output rather than leaf chlorophyll concentration.

2.7 Statistical Analyses

I used a two-way factorial analysis of variance (ANOVA) to test for differences in pH, redox potential and leaf SPAD index. For all harvested biomass parameters, and calculated LAR, ULR, SLA I used a two-way factorial analysis of covariance (ANCOVA) to test for differences in treatment group means using plant initial wet weight as the covariate. I used type II sums of squares in all analyses because the model contained two-way interaction effects. I performed between group comparisons with Tukey contrasts. Both ANCOVA and Tukey contrast results were considered significant if $p < 0.05$. Prior to analyses, data were assessed for normality and constant variance using Shapiro-Wilk and Bartlett's tests respectively and log transformed as necessary to ensure homogeneity of group variances and data normality. All data and figures herein are presented untransformed.

I analyzed photosynthesis across the range of ambient CO_2 levels using PERMANOVA (Anderson 2001), a multivariate hypothesis testing procedure that uses distance matrices to

assess differences between multiple groups. I tested the null hypothesis that A/C_i curves do not differ between flooding and fertilization combinations. This approach to testing repeated-measures multivariate photosynthesis data has been recommended by Potvin et al. (1990) using MANOVA. The more recently developed PERMANOVA is robust to departures from multivariate MANOVA test assumptions (multivariate normality, etc) and still provides an *F*-statistic, R^2 value and *p*-value analogous to those found in ANOVA or MANOVA output. All tests used 10,000 permutations of the data and Euclidean distance for creation of distance matrices from the initial gas exchange-ambient CO_2 matrices. Data was permuted across the entire data set per the recommendation of Anderson (2003). Main effects PERMANOVA tests were followed by PERMANOVA contrasts to identify which treatment combinations significantly differed from one another. These tests relied upon 10,000 permutations across the entire data set. All statistical analyses were performed in the R statistical package (R Core Development Team) version 2.11.1. CO_2 point measurement data were analyzed using 2x2 factorial ANCOVA with SPAD as the covariate. Post-hoc correlations were run between SPAD and gas exchange and stomatal conductance to elucidate the effects for observed leaf nutrition on gas exchange rates.

Mean growth rate and component parameters were calculated *sensu* Hunt et al. (2002) and the 95% confidence interval output was used to assess between group differences in relative growth and net assimilation rates. These methods are discussed further in section 2.4 above.

3.0 Results

3.1 *Treatment pH and Redox Potentials*

Redox potential did not differ between treatment groups for either species at day 74 ($p = 0.40$). At the experimental midpoint pH ranged from 6.9 to 10.36 and was higher in *C. obnupta* than in *C. stipata*. ANOVA showed that fertilization significantly increased pH in both species ($p < 0.001$) while the fertilization-flooding interaction was also significant in *C. obnupta* ($p = 0.037$). In *C. obnupta* pH significantly differed between HD and LS and between HD and LD. In *C. stipata* pH significantly differed between HD and LD and between HS and LD. HD exhibited the highest pH of all treatments in both species.

3.2 *Plant Biomass and Allocation*

3.2.1 *Harvest one*

For both species, initial wet weight and fertilization had significant effects on total biomass. The HD treatment exhibited the highest total biomass (8.8 g) while LS had the lowest biomass (7.13 g) for *C. obnupta*; HS and LD were the highest (20.56 g) and lowest (9.24 g) dry biomass groups for *C. stipata*. Tukey comparisons showed that all treatment groups to be identical for *C. obnupta* while in *C. stipata* the HD and HS groups were significantly higher than the LD group. All ANCOVA results for harvests one and two are summarized in Table 4 while between-group comparisons are summarized in Table 5.

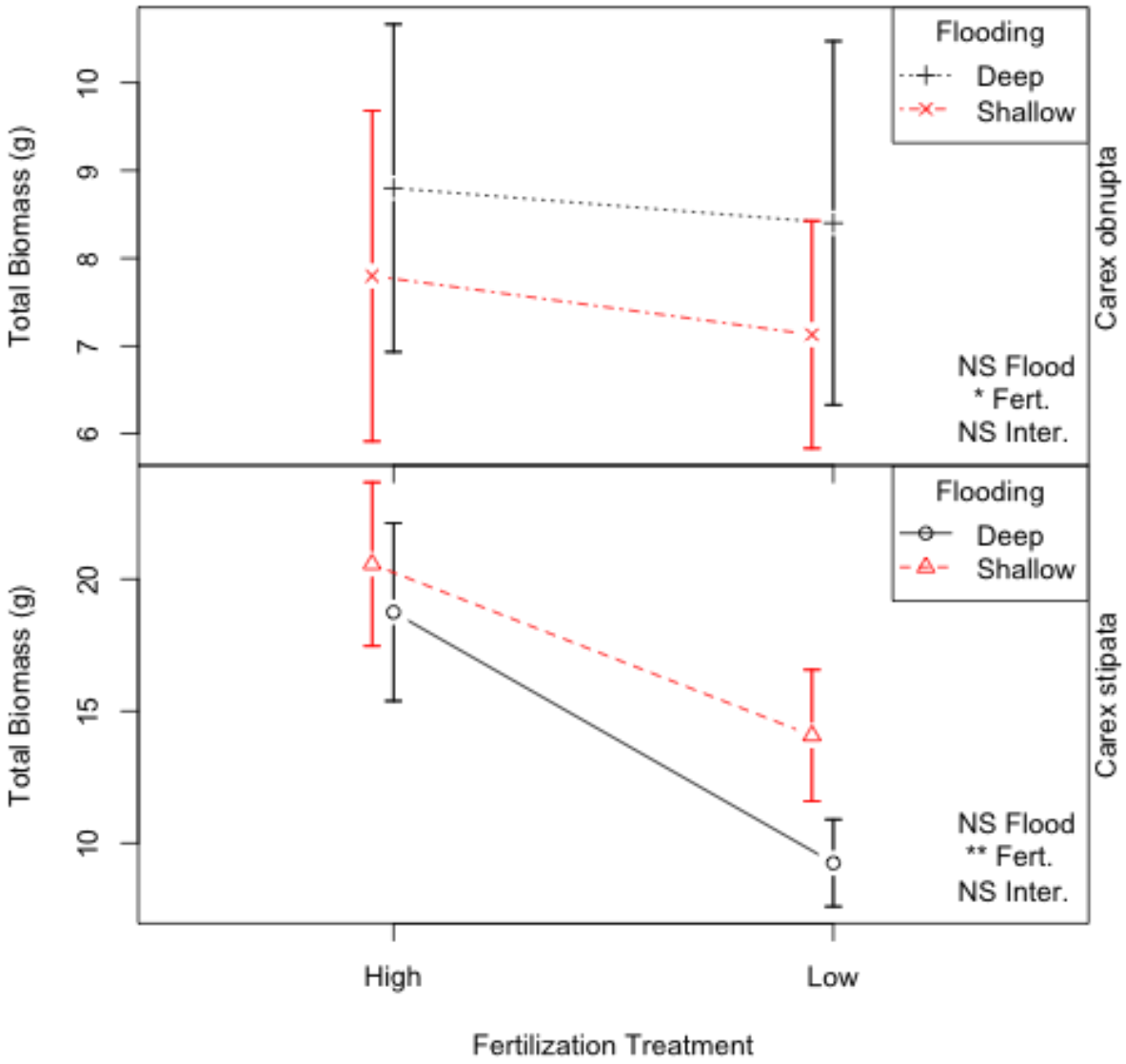

Figure 1: Total sedge biomass for *C. obnupta* and *C. stipata* from t_1. I observed significant fertilization effects in both species with fertilized treatments accumulating the most biomass in *C. stipata* and the deeply flooded treatments accumulating the most biomass regardless of fertilization treatment. Bars are ± standard error.

Aboveground biomass was highest in HD for both species with mean weights of 5.11g and 10.61 g for *C. obnupta* and *C. stipata* respectively. LD had the lowest biomass treatment for both *C. obnupta* (3.90g) and *C. stipata* (3.31g). HD and HS showed significantly higher aboveground biomass than LD and LS in *C. stipata* while *C. obnupta* showed no differences between

treatment groups. These differences were attributable to significant initial wet weight and fertilization effects for both species.

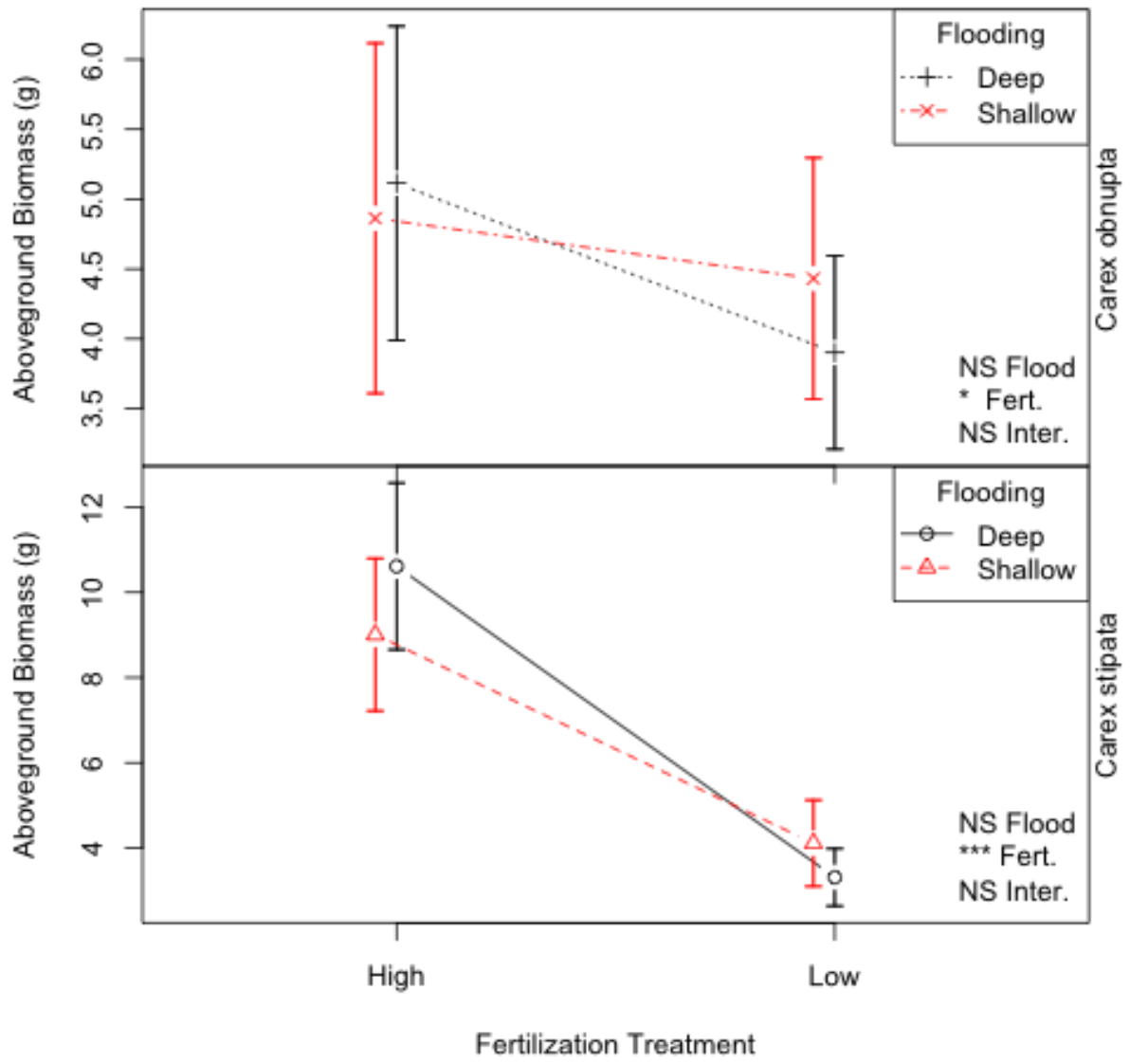

Figure 2: Aboveground biomass allocation for *C. obnupta* and *C. stipata* from t_1. Fertilization effects were found to significantly drive aboveground biomass growth for both species while flooding showed no trends or significant effects. Bars are ± standard error.

Both species' belowground biomass was significantly affected by initial wet weight while flooding also significantly affected the amount of belowground biomass in *C. stipata*. There

were no between group differences in belowground biomass for *C. obnupta*, but Tukey contrasts for *C. stipata* showed significant differences between HS (11.56 g) and LD (5.93 g).

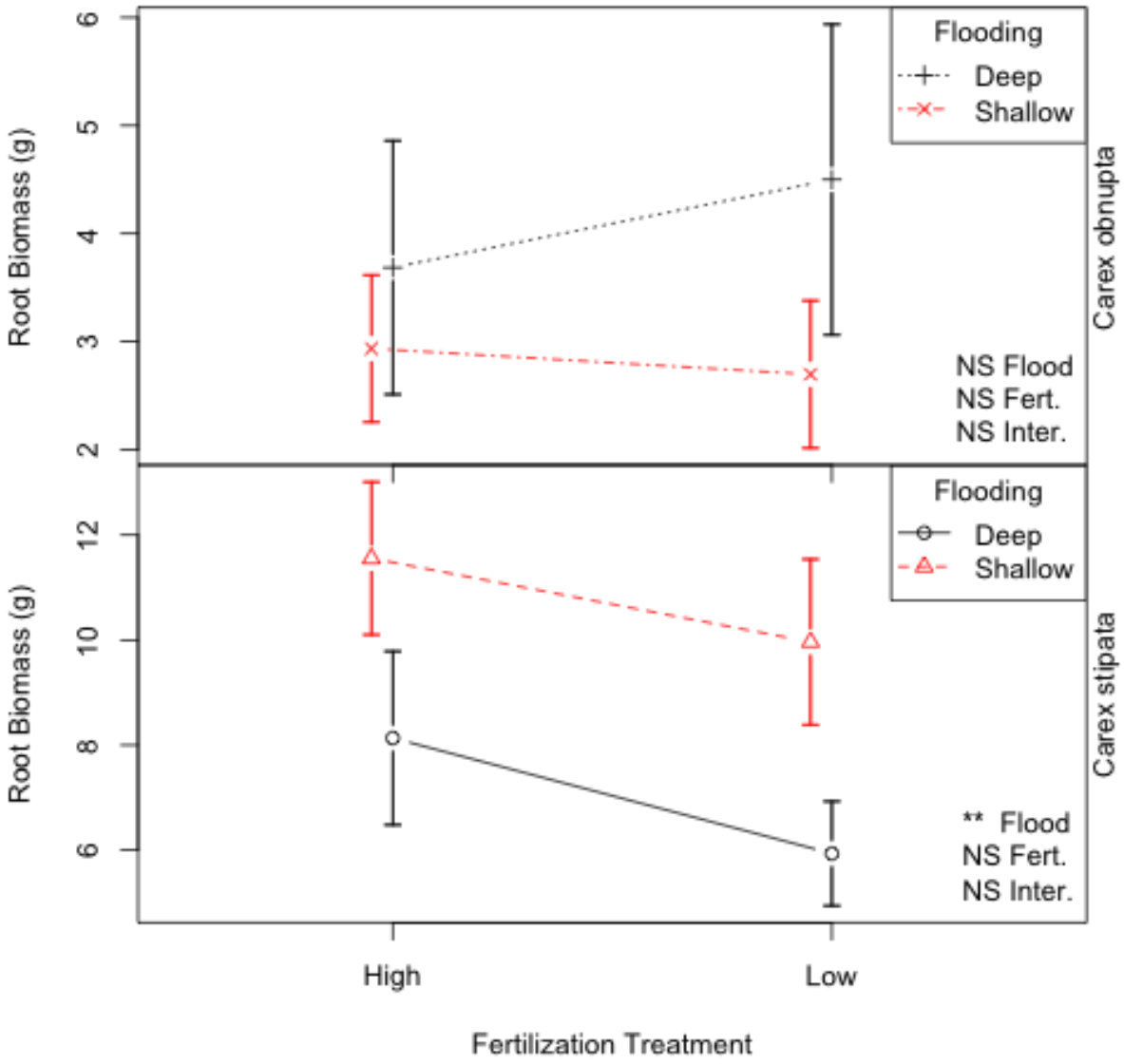

Figure 3: At t_1 belowground biomass allocation for *C. obnupta* showed no statistically significant response to any treatments although the most stressful treatment (LD) showed the highest allocation to roots. *C. stipata* showed that flooding significantly reduced root growth at t_1. Bars are ± standard error.

Fertilization had a significant effect on leaf area in both *C. stipata* and *C. obnupta* while initial wet weight also had an effect on *C. obnupta*. HS exhibited the highest leaf area in *C. obnupta* (220.59 cm^2) while HD, LD and LS had leaf areas of 200.78, 100.87 and 76.21 cm^2 respectively. HD had the highest leaf area for *C. stipata* (1,478.89 cm^2), significantly higher than HS (1,228.43 cm^2), while HS was significantly higher than LS (411.25 cm^2) and LD (445.22 cm^2).

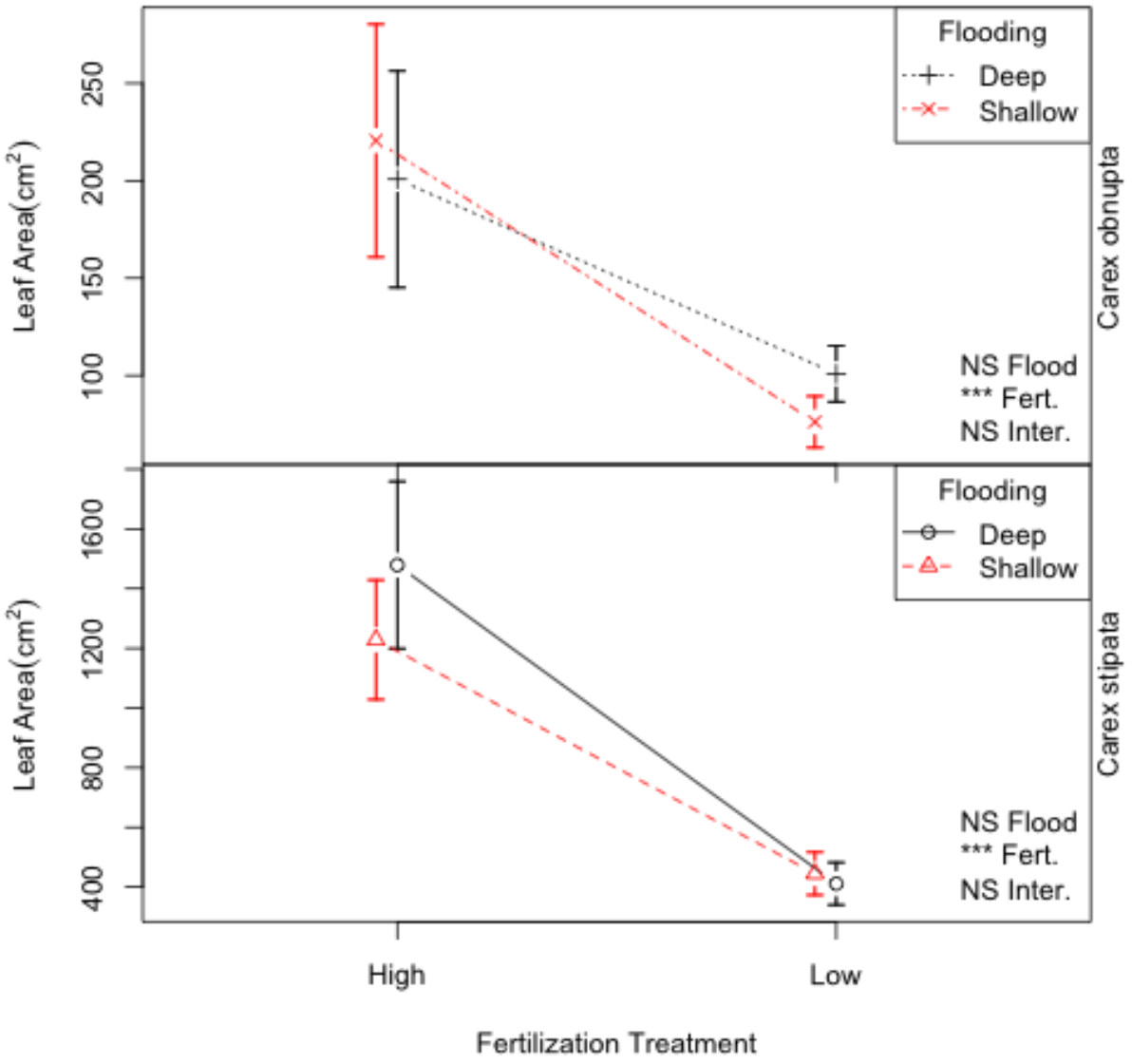

Figure 4: Total leaf area for both *C. obnupta* and *C. stipata* were significantly influenced by fertilization regardless of flooding treatment at t_1. Bars are ± standard error.

C. obnupta root-to-shoot ratio was not significantly affected by any of the experimental variables; both flooding and fertilization significantly affected *C. stipata* root-to-shoot ratio. LS

(2.913) and HS (1.391) exhibited significantly higher root-to-shoot ratios than both LS and HD (0.812).

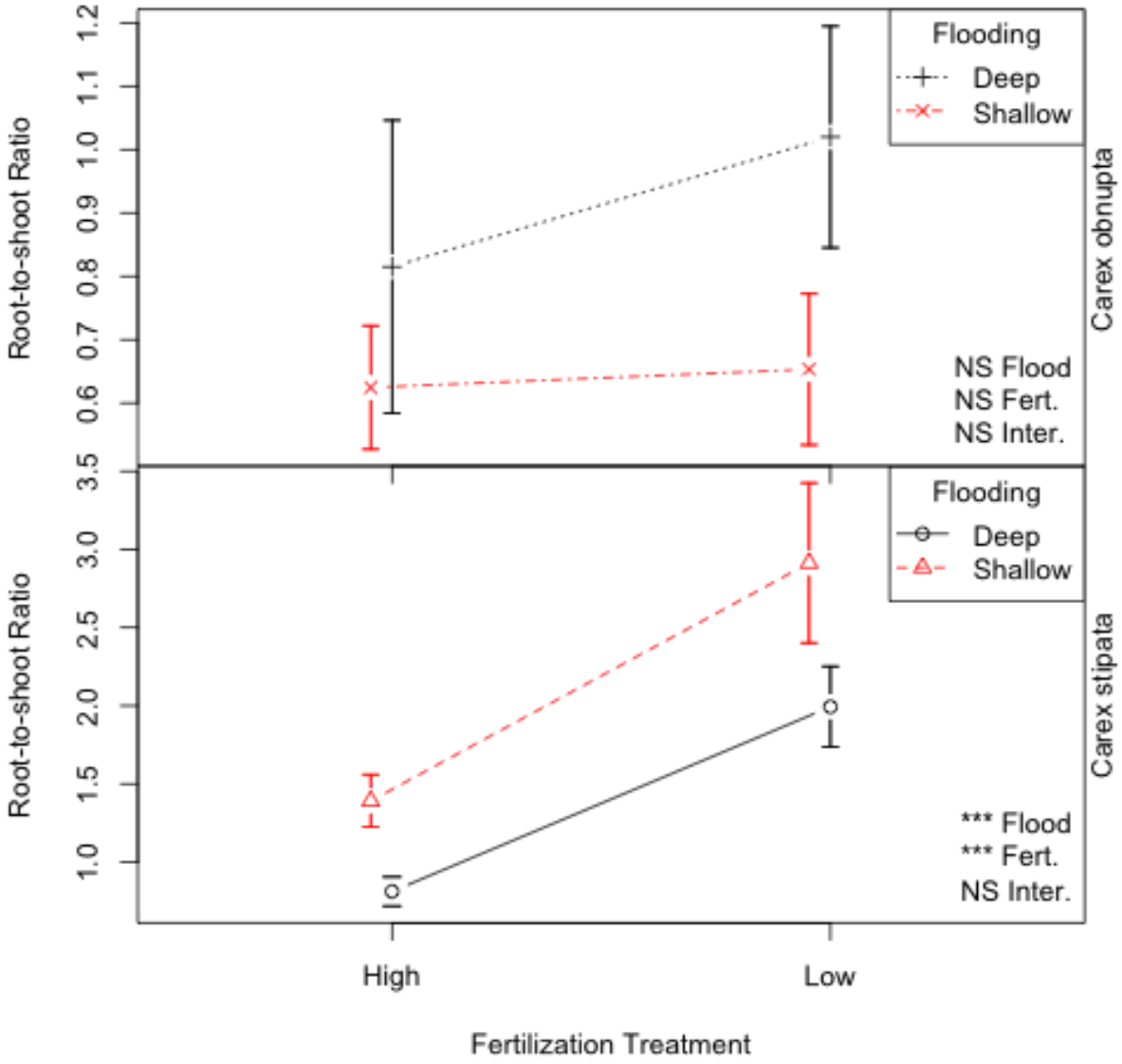

Figure 5: Root-to-shoot ratio trends in fertilization were similar for both *C. obnupta* and *C. stipata* at t_1. Only *C. stipata* showed significant flooding and fertilization effects with nutrient-stressed plants putting out more roots as a proportion of total biomass. *C. obnupta* had higher root allocation in deeply flooded plants whereas *C. stipata* showed the opposite. Bars are ± standard error.

3.1.2 Harvest Two

Both *C. obnupta* and *C. stipata* total biomass were positively driven by significant fertilization effects ($p < 0.0005$ for both species), while plant initial wet weight had a significant positive effect ($p < 0.0005$) on *C. obnupta* and flooding-fertilization interaction had a significant positive effect on *C. stipata* ($p = 0.0150$) total biomass. Total biomass was highest for *C. obnupta* in HS (43.25 g) and lowest in LS (17.49 g), and highest in HD (127.73 g) and lowest in LD (22.14 g) for *C. stipata*.

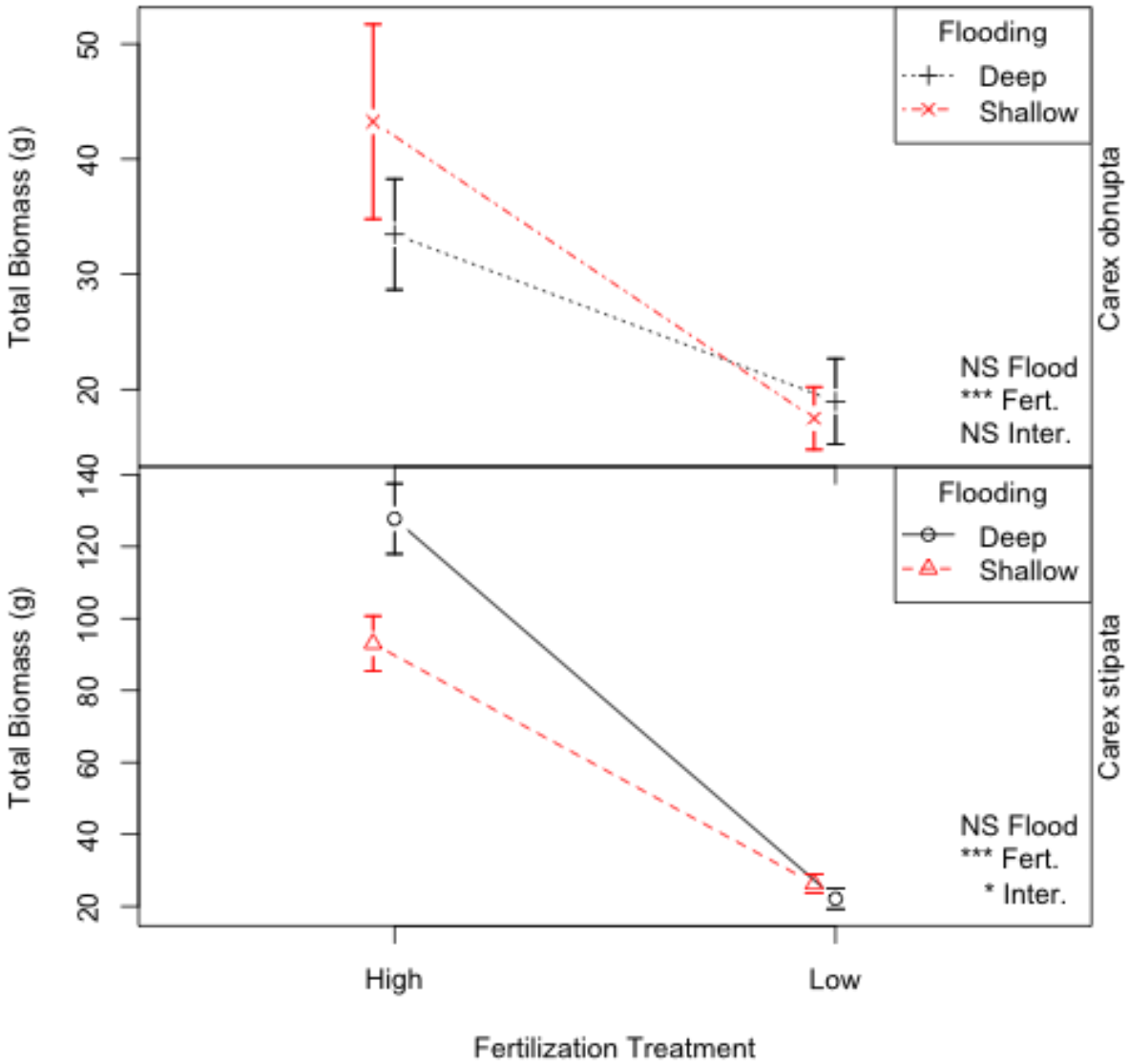

Figure 6: Fertilization drove total biomass for both *C. obnupta* and *C. stipata* from t_2. There was an additional significant interaction term in *C. stipata* that shows the deeply flooded plants at high nutrients accumulated more biomass while the shallowly-flooded, nutrient-limited plants accumulated less biomass. Bars are ± standard error.

C. stipata aboveground biomass showed significant flooding ($p = 0.0106$), fertilization ($p < 0.0005$) and flooding-fertilization interaction ($p = 0.0004$) effects, while *C. obnupta* was significantly affected by initial wet weight ($p < 0.0005$) and fertilization ($p < 0.0005$). HS (22.83

g) and HD (66.91 g) had the highest aboveground biomass while LS (7.34 g) and LD (6.10 g) had the lowest aboveground biomass for *C. obnupta* and *C. stipata* respectively.

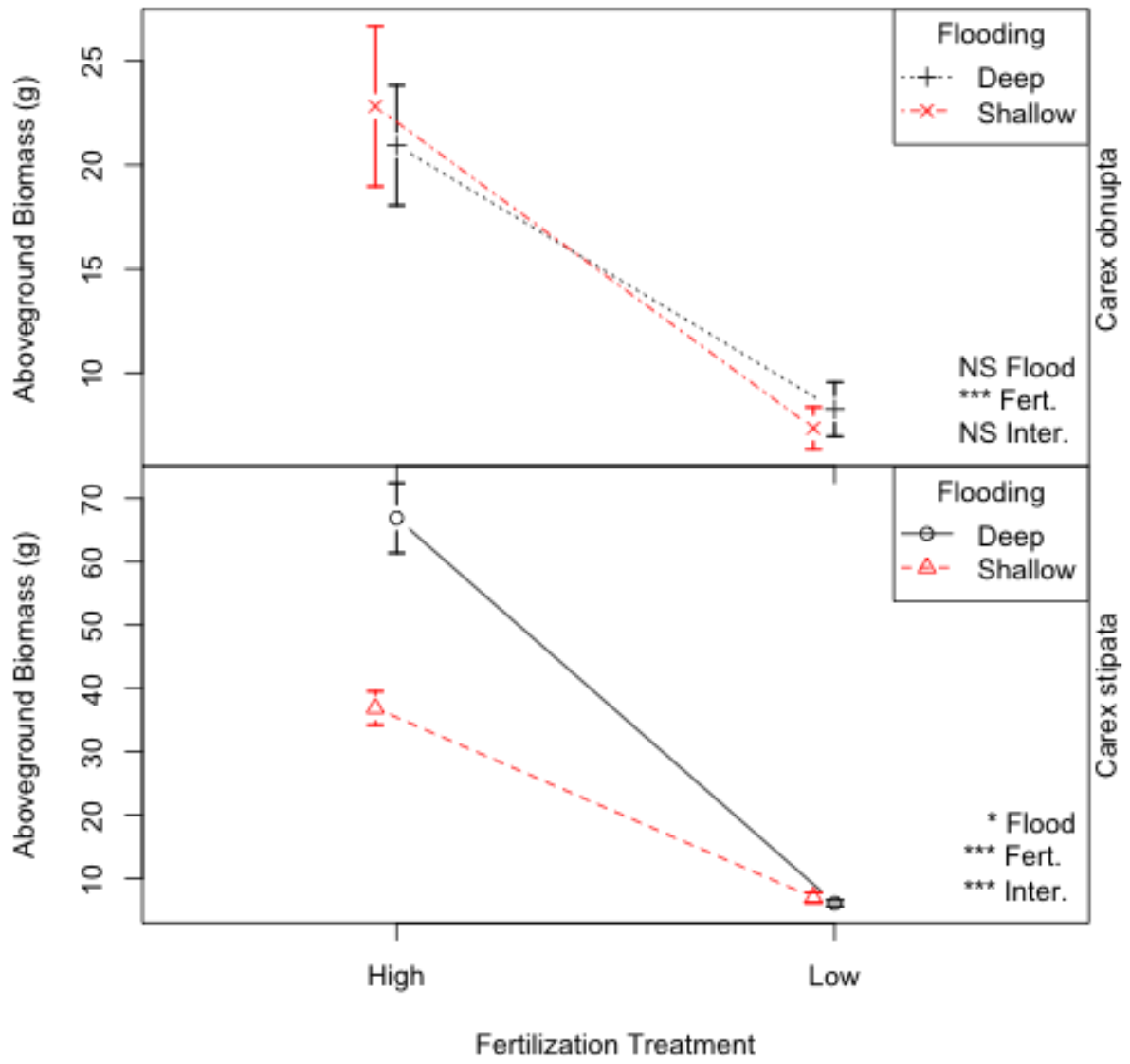

Figure 7: At t_2 aboveground biomass allocation for *C. obnupta* was driven by fertilization whereas *C. stipata* was affected by flooding, fertilization and the interaction; heavily flooded and fertilized *C. stipata* plants accumulated the most biomass and nutrient stressed and heavily flooded accumulated the least. Bars are ± standard error.

Fertilization was a significant driver of belowground biomass in both sedges ($p < 0.005$), while initial wet weight ($p < 0.0005$) and flooding level ($p = 0.0499$) also affected *C. obnupta* belowground biomass accumulation. The highest belowground biomass levels were found in HS (20.43 g) and HD (60.81 g) for *C. obnupta* and *C. stipata*; the lowest belowground biomass levels for *C. obnupta* and *C. stipata* were LS (10.15 g) and LD (16.04 g) respectively.

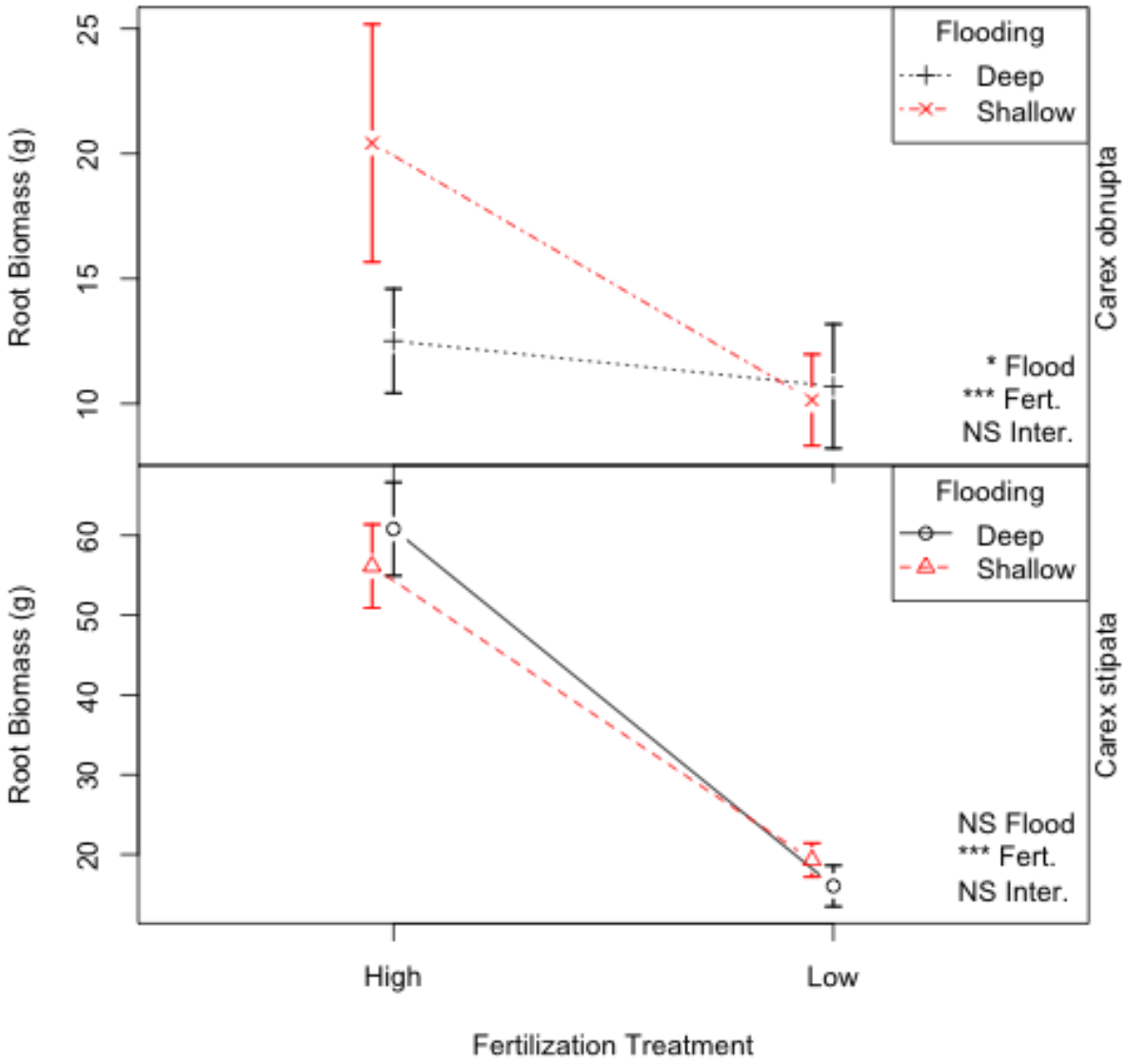

Figure 8: Belowground biomass growth for both *C. obnupta* and *C. stipata* at t_2 were driven by fertilization and fertilization while *C. obnupta* root production was negatively related to flooding. Bars are ± standard error.

Fertilization had a significant effect on leaf area in both species ($p < 0.0005$); initial wet weight also had an effect on *C. obnupta* ($p < 0.0005$) while the flooding-fertilization interaction had an effect on *C. stipata* leaf area ($p = 0.0002$). HS (837.36 cm^2) and HD (7464.14 cm^2) exhibited the highest mean leaf areas for *C. obnupta* and *C. stipata* respectively, while the lowest

mean leaf areas were LS (255.48 cm^2) and LD (668.52 cm^2) for *Carex obnupta* and *Carex stipata* respectively.

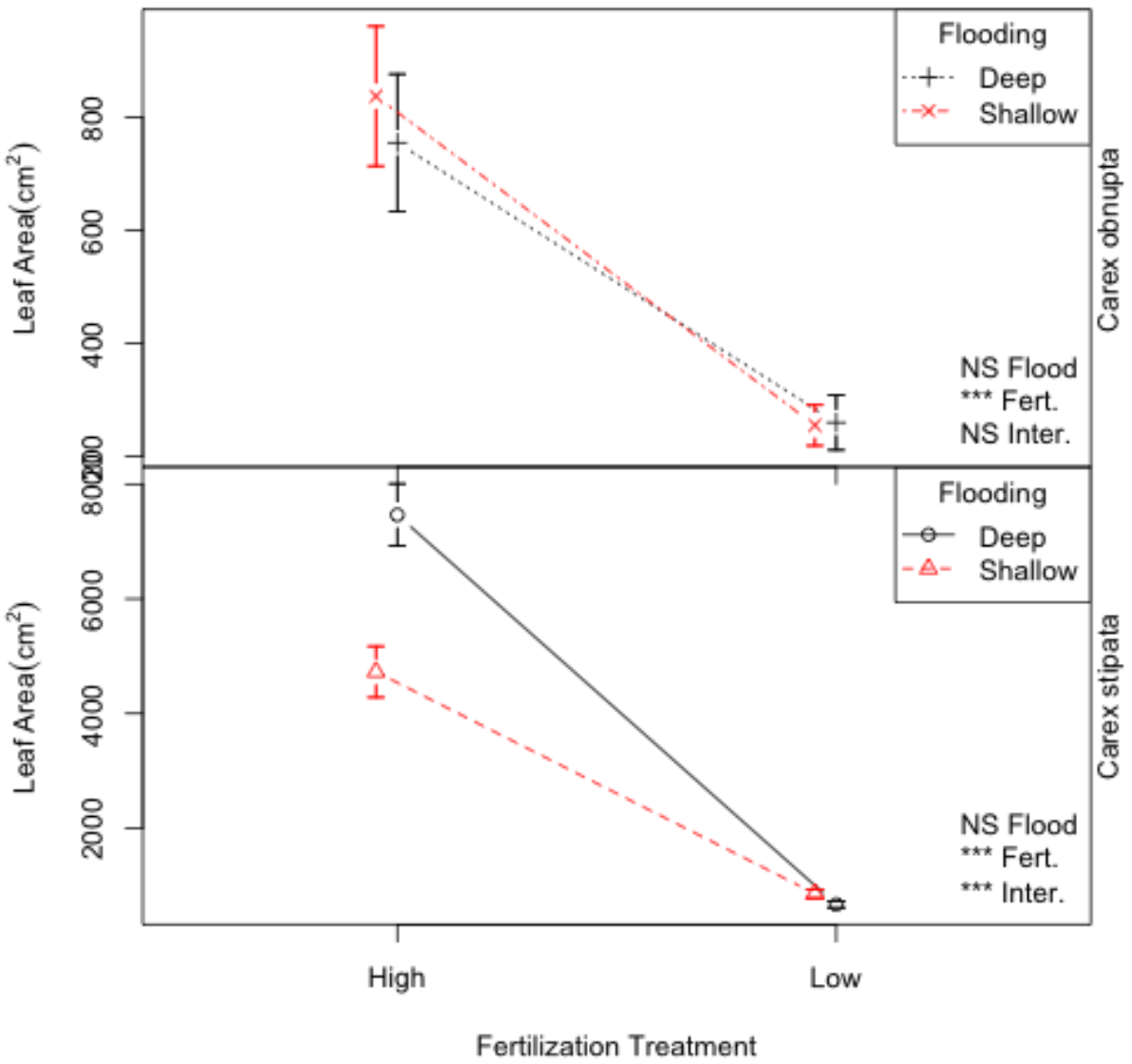

Figure 9: Total leaf area for both *C. obnupta* and *C. stipata* at t_2 were driven by fertilization. The most stressful treatment, LD showed the least leaf area as a result of the significant interaction term in *C. stipata*. Bars are ± standard error.

Both flooding and fertilization had significant effects on root to shoot ratio for both species ($p < 0.01$ for both factors) while the flooding-fertilization interaction marginally affected the *C. stipata* root-to-shoot ratio ($p = 0.0501$). Plants in the LS treatment had the highest proportion of roots (*C. obnupta* = 1.397; *C. stipata* = 2.927) while HD had the highest proportion shoots (*C. obnupta* = 0.588; *C. stipata* = 0.927).

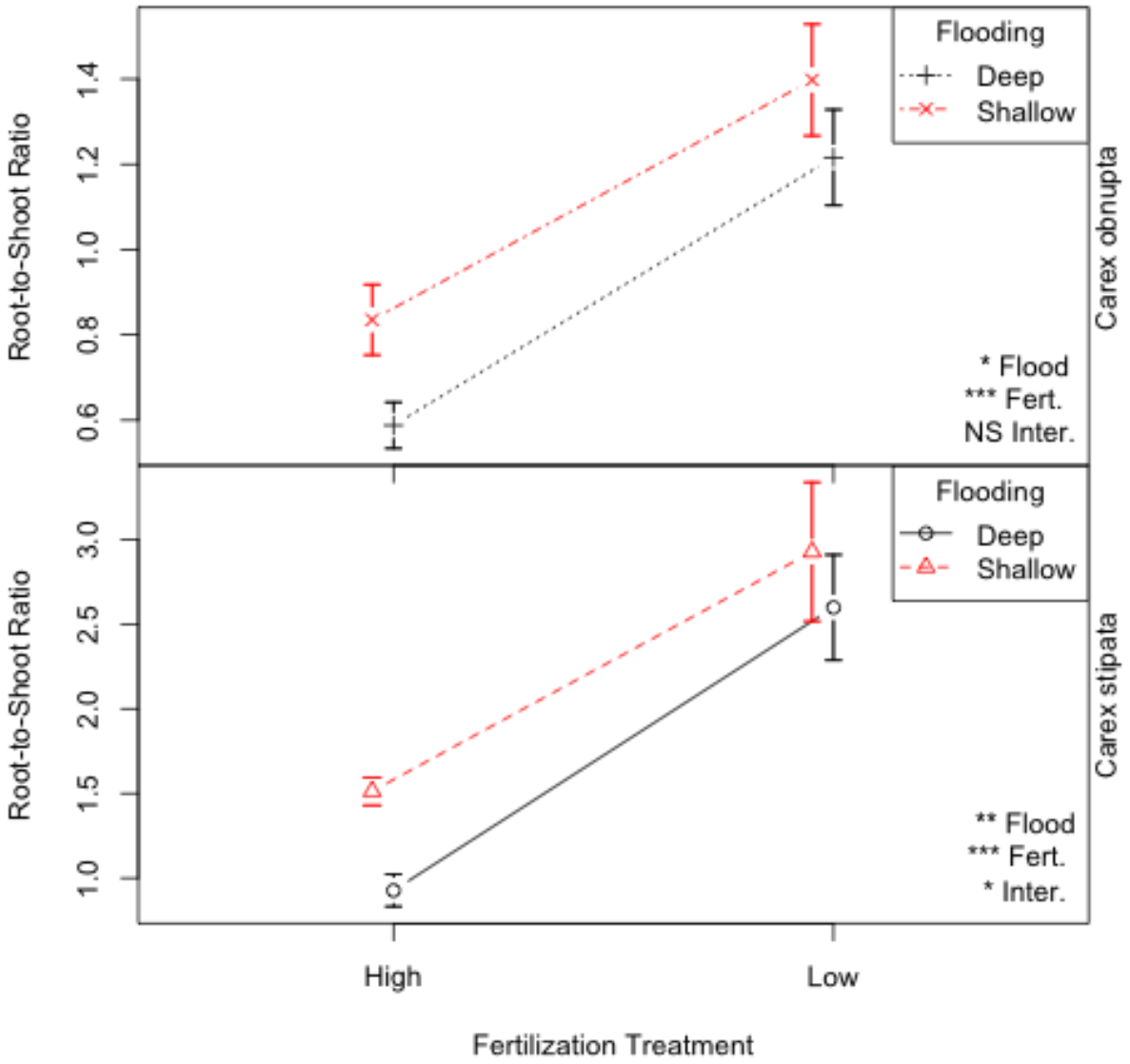

Figure 10: Flooding and fertilization at t_2 identically drove trends in the root-to-shoot ratio of *C. obnupta* and *C. stipata*. Bars are ± standard error.

Table 4: Growth and allocation parameter mean values ± standard error. Subscript letters indicate group membership.

Harvest	*Species*	*Fertilization*	*Flooding*	*Total Biomass (gs dry weight)*	*Aboveground biomass (gs dry weight)*	*Belowground biomass (gs dry weight)*	*Leaf Area (cm^2)*	*Root to Shoot Ratio*	*Wet Weight (gs)*
Harvest I (t_1)	***Carex obnupta***	*High*	*High*	$8.80\pm1.87_{a}$	$5.11\pm1.12_{a}$	$3.69\pm1.17_{a}$	$200.78\pm55.60_{ab}$	$0.815\pm0.231_{a}$	$50.46\pm10.91_{a}$
		High	*Low*	$7.80\pm1.89_{a}$	$4.86\pm1.25_{a}$	$2.94\pm0.68_{a}$	$220.59\pm59.78_{a}$	$0.625\pm0.097_{a}$	$52.11\pm16.58_{a}$
		Low	*High*	$8.40\pm2.07_{a}$	$3.90\pm0.69_{a}$	$4.50\pm1.44_{a}$	$100.87\pm14.38_{bc}$	$1.019\pm0.175_{a}$	$44.73\pm10.05_{a}$
		Low	*Low*	$7.13\pm1.30_{a}$	$4.43\pm0.86_{a}$	$2.70\pm0.68_{a}$	$76.21\pm13.21_{c}$	$0.653\pm0.119_{a}$	$37.17\pm5.60_{a}$
	Carex stipata	*High*	*High*	$18.744\pm3.363_{a}$	$10.611\pm1.957_{a}$	$8.133\pm1.653_{ab}$	$1478.887\pm280.078_{c}$	$0.812\pm0.093_{c}$	$157.657\pm32.192_{a}$
		High	*Low*	$20.563\pm3.099_{a}$	$9.007\pm1.790_{a}$	$11.556\pm1.455_{a}$	$1228.426\pm200.111_{a}$	$1.391\pm0.168_{a}$	$151.657\pm26.167_{a}$
		Low	*High*	$9.244\pm1.648_{b}$	$3.310\pm0.674_{b}$	$5.934\pm0.993_{b}$	$411.249\pm71.048_{b}$	$1.993\pm0.258_{ab}$	$87.514\pm17.934_{a}$
		Low	*Low*	$14.073\pm2.492_{ab}$	$4.110\pm1.012_{b}$	$9.963\pm1.576_{ab}$	$445.22\pm71.81_{b}$	$2.913\pm0.511_{b}$	$107.657\pm24.614_{a}$
Harvest II (t_2)	***Carex obnupta***	*High*	*High*	$33.46\pm4.81_{a}$	$20.95\pm2.89_{a}$	$12.51\pm2.09_{ab}$	$755.06\pm121.15_{a}$	$0.588\pm0.054_{a}$	$175.16\pm29.46_{ac}$
		High	*Low*	$43.25\pm8.46_{a}$	$22.83\pm3.86_{a}$	$20.43\pm4.76_{a}$	$837.36\pm123.75_{a}$	$0.835\pm0.083_{ab}$	$233.21\pm48.59_{a}$
		Low	*High*	$18.95\pm3.72_{b}$	$8.25\pm1.30_{b}$	$10.7\pm1.83_{a}$	$260.25\pm48.48_{b}$	$1.216\pm0.112_{bc}$	$109.94\pm23.60_{b}$
		Low	*Low*	$17.49\pm2.72_{b}$	$7.34\pm1.01_{b}$	$10.15\pm1.83_{b}$	$255.48\pm36.04_{b}$	$1.397\pm0.130_{c}$	$105.96\pm14.48_{bc}$
	Carex stipata	*High*	*High*	$127.73\pm9.77_{a}$	$66.91\pm5.52_{c}$	$60.81\pm5.85_{a}$	$7464.14\pm540.85_{c}$	$0.927\pm0.094_{c}$	$792.10\pm64.23_{a}$
		High	*Low*	$93.03\pm7.66_{a}$	$36.89\pm2.67_{a}$	$56.14\pm5.25_{a}$	$4726.47\pm444.29_{a}$	$1.153\pm0.083_{a}$	$615.41\pm37.39_{a}$
		Low	*High*	$22.14\pm2.85_{b}$	$6.10\pm0.45_{b}$	$16.04\pm2.58_{b}$	$668.52\pm54.04_{b}$	$2.597\pm0.311_{b}$	$144.76\pm8.87_{b}$
		Low	*Low*	$26.28\pm2.60_{b}$	$6.99\pm0.82_{b}$	$19.29\pm2.11_{b}$	$859.36\pm77.19_{b}$	$2.927\pm0.410_{b}$	$174.79\pm21.84_{b}$

Table 5: Final biomass allocation weights, leaf area, and root-to-shoot ratio ANCOVA results for harvest I (t_1) and II (t_2) and two-way ANOVA results for SPAD at t_2; only significant ($p < 0.05$) probability values are reported below.

Growth Parameter			**Initial Wet Weight**	**Flooding**	**Fertilization**	**Flooding Fertilization Interaction**
Wet Weight	t_1	*C. obnupta*	$p = 9.011 \times 10^{-9}$	ns	p = 0.0052	ns
		C. stipata	p = 0.01061	ns	p = .01205	ns
	t_2	*C. obnupta*	$p = 5.849 \times 10^{-5}$	ns	$p = 2.475 \times 10^{-5}$	ns
		C. stipata	p = 0.07534	ns	$p < 2 \times 10^{-16}$	p = 0.02324
Leaf Area	t_1	*C. obnupta*	$p = 3.217 \times 10^{-4}$	ns	$p = 9.14 \times 10^{-5}$	ns
		C. stipata	ns	ns	$p = 7.839 \times 10^{-7}$	ns
	t_2	*C. obnupta*	$p = 7.822 \times 10^{-7}$	ns	$p = 3.021 \times 10^{-12}$	ns
		C. stipata	ns	ns	$p = 2.2 \times 10^{-16}$	p = 0.0002330
Total biomass	t_1	*C. obnupta*	$p = 4.447 \times 10^{-1}$	ns	p = 0.03401	ns
		C. stipata	p = 0.0313	ns	p = 0.00234	ns
	t_2	*C. obnupta*	p = 5.414 ns 10^{-7}	ns	$p = 2.378 \times 10^{-8}$	ns
		C. stipata	ns	ns	$p = 5.416 \times 10^{-15}$	p = 0.01495
Aboveground biomass	t_1	*C. obnupta*	$p = 7.538 \times 10^{-6}$	ns	p = 0.0412	ns
		C. stipata	p = 0.07491	ns	$p = 5.269 \times 10^{-5}$	ns
	t_2	*C. obnupta*	$p = 5.969 \times 10^{-8}$	ns	$p = 7.203 \times 10^{-13}$	ns
		C. stipata	ns	p = 0.010573	$p < 2.2 \times 10^{-16}$	p = 0.0003548
Belowground biomass	t_1	*C. obnupta*	$p = 9.098 \times 10^{-7}$	ns	ns	ns
		C. stipata	0.03170	0.00381	ns	ns
	t_2	*C. obnupta*	$p = 1.079 \times 10^{-5}$	p = 0.04999	p = 0.0007962	ns
		C. stipata	ns	ns	$p = 1.236 \times 10^{-10}$	ns
Root-to-Shoot Ratio	t_1	*C. obnupta*	ns	ns	ns	ns
		C. stipata	ns	0.002865	3.275×10^{-6}	ns
	t_2	*C. obnupta*	ns	p = 0.01335	$p = 1.443 \times 10^{-6}$	ns
		C. stipata	ns	p = 0.004035	$p = 1.257 \times 10^{-8}$	p = 0.050053
SPAD Index	t_2	*C. obnupta*	ns	$p = 1.762 \times 10^{-8}$	p = 0.00928	ns
		C. stipata	ns	$p = 2.00 \times 10^{-6}$	ns	p = 0.03277

3.3 Relative Growth Rate and Component Processes

3.3.1a Relative growth rate, net assimilation rate and leaf area ratio estimates between $t_0 - t_1$

In the harvest interval between days zero and 31, *C. stipata* showed relative growth rates higher than *C. obnupta* at all treatment levels except LD, in which *C. stipata* had a negative RGR (-0.0008 g/g/day). Estimates of the component growth parameters that drive RGR were variable in both species and all component parameters (LAR, NAR) had high standard error values (Table 6). The only apparent trend in NAR was that *C. stipata* grew more rapidly than *C. obnupta* in all treatments except HS where *C. obnupta* had a higher NAR value than *C. stipata* (Table 6).

3.3.1b RGR, NAR and LAR estimates between $t_0 - t_2$

HD had the highest RGR for *C. stipata* between days zero and 77 (0.0359 g/g/day), while HS had the highest RGR for *C. obnupta* (0.0242 g/g/day). The lowest RGR rate for *C. obnupta* was LS (0.0130 g/g/day) and the lowest RGR for *C. stipata* was LD (0.0121 g/g/day). Between days zero and 77 *C. obnupta* showed higher NAR than *C. stipata* (Table 6), with HS showing the highest rate (0.0012 g/cm^2/day for *C. obnupta* and 0.0007 g/cm^2/day for *C. stipata*) and LD showing the lowest rates in both *C. obnupta* (0.0007 g/cm^2/day) and *C. stipata* (0.0005 g/cm^2/day). For both species, the HD treatment had the highest LAR (22.5211 cm^2/g for *C. obnupta* and 54.1248 cm^2/g for *C. stipata*) and LD had the lowest LAR (18.1910 cm^2/g for *C. obnupta* and 40.2842 cm^2/g for *C. stipata*).

3.3.1c RGR, NAR and LAR estimates between $t_1 - t_2$

Between days 31 and 77 HD had the highest RGR (0.0441 g/g/day), NAR (0.0007 g/cm^2/day) and LAR (70.7464 cm^2/g) for *C. stipata* while HS had the highest RGR (0.0387 g/g/day), NAR (0.0021 g/cm^2/day) and LAR (25.4158 cm^2/g) for *C. obnupta*. For *C. obnupta* the lowest RGR (0.0192 g/g/day) and NAR (0.0012 g/cm^2/day) were found in the LD treatment while LS

exhibited the lowest average RGR (0.0152 g/g/day), NAR (0.0005 g/cm^2/day) and LAR (33.9021 cm^2/g) values for *C. stipata*. LS had the lowest LAR (13.4150 cm^2/g) for *C. obnupta*.

Table 6: Relative growth rate, net assimilation rat e, specific leaf area, leaf mass ratio and leaf area ratio estimated across three different harvest intervals, t_0-t_1, t_1-t_2 and t_0-t_2. Mean values are displayed below ± standard error. All values were calculated using equations in section 2.4 and Appendix I. There were no statistically significant differences across any treatment groups.

Growth Parameter	Fertili-zation	Flooding	Day 0-31 (t_0)		Day 31-77 (t_1)		Day 0-77 (t_2)	
			C. obnupta	*C. stipata*	*C. obnupta*	*C. stipata*	*C. obnupta*	*C. stipata*
Relative Growth Rate (g/g/day)	*H*	*D*	0.0075 ±0.0225	0.0235±0.0246	0.0313±0.0156	0.0441±0.0113	0.0218±0.0068	0.0359±0.0083
	H	*S*	0.0046 ±0.0247	0.0273±0.0235	0.0387±0.0194	0.0345±0.0105	0.0242±0.0090	0.0316±0.0084
	L	*D*	0.0024 ±0.0242	-0.0008±0.0262	0.0192±0.0190	0.0207±0.0140	0.0134±0.0085	0.0121±0.0090
	L	*S*	0.0019 ±0.0194	0.0140±0.0245	0.0204±0.0142	0.0152±0.0115	0.0130±0.0072	0.0147±0.0085
Net Assimilation Rate ($g/cm^2/day$)	*H*	*D*	-0.0002 ±0.0009	0.0004±0.0004	0.0017±0.0008	0.0007±0.0002	0.0010±0.0004	0.0007±0.0002
	H	*S*	0.0007 ±0.0017	0.0005±0.0004	0.0021±0.0011	0.0007±0.0002	0.0012±0.0006	0.0007±0.0002
	L	*D*	0.0002 ±0.0008	0.0018±0.0005	0.0012±0.0013	0.0007±0.0004	0.0007±0.0006	0.0005±0.0003
	L	*S*	0.0003 ±0.0014	0.0317±0.0023	0.0016±0.0012	0.0005±0.0003	0.0008±0.0005	0.0005±0.0002
Specific Leaf Area (cm^2/g)	*H*	*D*	55.3276±29.4659	231.7882± 87.5915	22.9161±8.7035	70.7464±19.5055	51.5523±15.100	197.2682±77.087
	H	*S*	54.8702±21.5913	224.3829± 85.6648	25.4158±11.127 4	56.5096±14.2684	52.5416±16.223	200.2068±77.691
	L	*D*	44.0593±16.0044	232.5274± 94.0164	14.7988±6.3068	39.5007±11.5130	47.6237±15.936	191.6322±77.518
	L	*S*	40.4106±14.6437	213.3310± 96.9130	13.4150±4.4502	33.9021±10.7552	49.3214±15.068	196.5940±77.843
Leaf Mass Ratio ($g\ g^{-1}$)	*H*	*D*	0.4515 ±0.1883	0.2965 ±0.1108	0.4857±0.1882	0.3502±0.0932	0.4463±0.1007	0.2974±0.0960
	H	*S*	0.4836 ±0.1609	0.2632 ±0.1018	0.4966±0.1732	0.2863±0.0724	0.4252±0.1195	0.2668±0.0876
	L	*D*	0.4345 ±0.1374	0.2238 ±0.0925	0.4136±0.1411	0.2019±0.0559	0.3912±0.1057	0.2218±0.0859
	L	*S*	0.4322 ±0.1172	0.2156 ±0.1027	0.4029±0.1149	0.1943±0.0748	0.3829±0.0981	0.2224±0.0886
Leaf Area Ratio (cm^2/g)	*H*	*D*	22.2776 ±9.2635	64.8932±22.9990	22.916±8.7035	70.7464±19.5055	22.5211±7.0322	54.1248±17.3788
	H	*S*	18.4904 ±7.6665	54.9538±19.4373	25.4158±11.127 4	56.5096±14.2684	21.7438±7.5004	49.8273±16.6219
	L	*D*	25.5545±11.2729	47.4881±17.7951	14.7988±6.3068	39.5007±11.5130	18.1910±6.3651	40.2842±16.3215
	L	*S*	16.6589±6.1433	41.2506±17.7555	13.4150±4.4502	33.9021±10.7552	18.6386±6.4481	40.9231±15.8407

3.3.2 Leaf area rate, specific leaf area and leaf mass ratios at days 31 and 77

At t_1 I found that LAR was significantly driven by fertilization in both *C. stipata* and *C. obnupta*, while initial plant weight and the flooding-fertilization interaction drove LAR in *C. obnupta*. In *C. stipata*, flooding also had a significant effect on LAR (Table 7 and 8). SLA at t_1 was driven by fertilization and flooding-fertilization interaction in *C. obnupta*, while *C. stipata* was only significantly driven by initial seedling size. LMR at t_1 was driven by initial wet weight in *C. obnupta* and both flooding and fertilization in *C. stipata*.

For plants harvested at t_2, LAR was significantly driven by fertilization in both species although initial wet weight was close to significant for both species (Table 6). At t_2 SLA was driven by flooding in *C. stipata* and fertilization in *C. obnupta*. Initial wet weight, flooding and fertilization significantly affected LMR in *C. obnupta* at t_2 while flooding and fertilization were significant drivers of LMR in *C. stipata* at the same harvest.

Table 7: ANOVA probability values for LAR, SLA and LMR growth parameters calculated at days 31 (t_1) and 77 (t_2). *p*-values less than 0.05 are indicated by an ns symbol.

Growth Parameter			**Initial Wet Weight**	**Flooding**	**Fertilization**	**Flooding Fertilization Interaction**
Leaf Area Ratio	t_1	*C. obnupta*	0.01116	ns	1.489×10^{-5}	0.03789
		C. stipata	ns	0.0003396	5.915×10^{-8}	ns
	t_2	*C. obnupta*	ns	ns	9.864×10^{-6}	ns
		C. stipata	0.06053	ns	8.801×10^{-9}	ns
Specific Leaf Area	t_1	*C. obnupta*	ns	ns	0.00014	0.02850
		C. stipata	0.05359	ns	ns	ns
	t_2	*C. obnupta*	ns	ns	0.04424	ns
		C. stipata	ns	0.01575	ns	ns
Leaf Mass Ratio	t_1	*C. obnupta*	0.02841	ns	ns	ns
		C. stipata	ns	0.005619	8.37×10^{-6}	ns
	t_2	*C. obnupta*	0.04023	0.01944	1.697×10^{-6}	ns
		C. stipata	ns	0.01157	2.738×10^{-8}	ns

Table 8: Growth parameters calculated at days 31 (t_1) and 77 (t_2) ± standard error; letters indicate significant group differences from Tukey's Least Significant Difference Test.

Harvest	*Species*	*Fertilization*	*Flooding*	*Leaf Area Ratio* (cm^2/g)	*Specific Leaf Area* (cm^2/g)	*Leaf Mass Ratio* ($g\ g^{-1}$)
Harvest I (t_1)	*Carex obnupta*	*High*	*High*	$21.109 \pm 1.925_{ab}$	$38.134 \pm 5.502_{a}$	$0.597 \pm 0.063_{a}$
		High	*Low*	$27.151 \pm 2.621_{a}$	$43.320 \pm 3.652_{a}$	$0.626 \pm 0.030_{a}$
		Low	*High*	$14.419 \pm 2.069_{bc}$	$27.619 \pm 2.803_{ab}$	$0.516 \pm 0.040_{a}$
		Low	*Low*	$11.086 \pm 1.058_{c}$	$18.628 \pm 2.548_{b}$	$0.624 \pm 0.045_{a}$
	Carex stipata	*High*	*High*	$79.058 \pm 5.335_{a}$	$141.090 \pm 6.686_{a}$	$0.562 \pm 0.031_{a}$
		High	*Low*	$60.177 \pm 4.174_{ab}$	$140.552 \pm 5.467_{a}$	$0.429 \pm 0.027_{ab}$
		Low	*High*	$44.959 \pm 1.212_{b}$	$134.965 \pm 13.444_{a}$	$0.346 \pm 0.022_{bc}$
		Low	*Low*	$33.271 \pm 4.013_{c}$	$122.957 \pm 11.276_{a}$	$0.278 \pm 0.029_{c}$
Harvest II (t_2)	*Carex obnupta*	*High*	*High*	$22.690 \pm 1.405_{a}$	$35.574 \pm 1.482_{ab}$	$0.635 \pm 0.021_{a}$
		High	*Low*	$20.835 \pm 1.426_{a}$	$37.561 \pm 1.656_{a}$	$0.553 \pm 0.025_{ab}$
		Low	*High*	$13.972 \pm 0.794_{b}$	$30.689 \pm 1.647_{b}$	$0.459 \pm 0.021_{bc}$
		Low	*Low*	$15.049 \pm 1.247_{b}$	$35.218 \pm 2.038_{ab}$	$0.427 \pm 0.025_{c}$
	Carex stipata	*High*	*High*	$59.634 \pm 3.924_{a}$	$113.266 \pm 5.663_{a}$	$0.528 \pm 0.027_{c}$
		High	*Low*	$50.933 \pm 2.703_{a}$	$126.996 \pm 5.156_{a}$	$0.401 \pm 0.012_{a}$
		Low	*High*	$31.908 \pm 2.652_{b}$	$109.846 \pm 4.509_{a}$	$0.291 \pm 0.023_{b}$
		Low	*Low*	$33.326 \pm 2.209_{b}$	$126.164 \pm 7.085_{a}$	$0.270 \pm 0.022_{b}$

For both t_1 and t_2 LAR, SLA and LMR differed between treatment combinations for one or both species. At t_1 HS and HD had the highest LAR, SLA and LMR for *C. obnupta* and *C. stipata* respectively. LS had the lowest SLA and LAR for both species, and the lowest LMR for *C. stipata*, while HS had the lowest LMR for *C. obnupta*. At t_2 LAR was highest in treatments HD and HS and lowest in LD. There were no between treatment differences in SLA at t_2 for *C. stipata*. For *C. obnupta* at t_2, SLA was highest in HS and lowest in LD, while neither HD nor LS differed significantly from the two groups. Mean values for LAR, SLA and LMR for all treatment combinations are summarized in Table 8 above and in Figures 11-16 below.

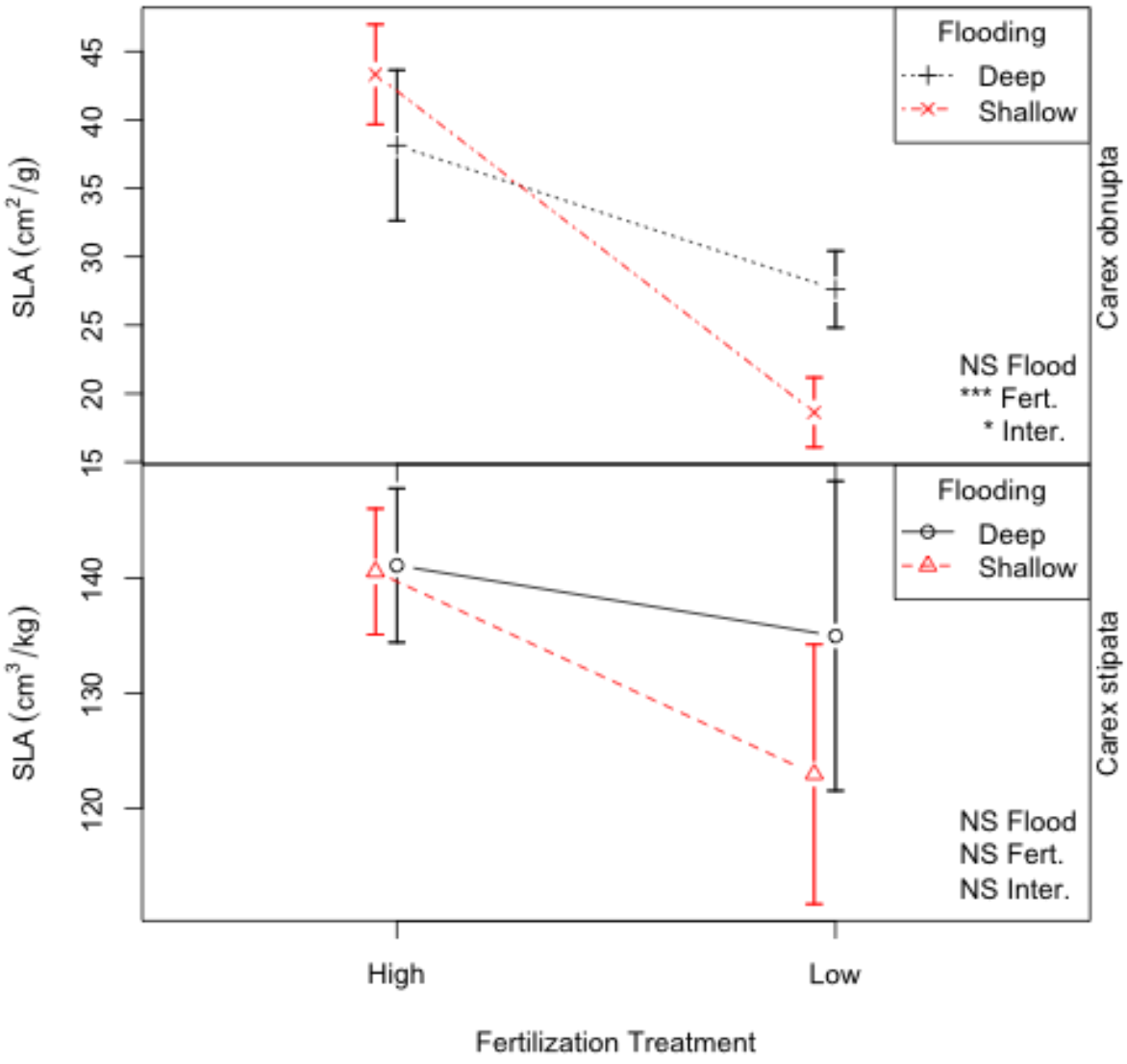

Figure 11: Specific leaf area at t_1 was driven by fertilization and the interaction between flooding and fertilization in *C. obnupta*. Bars are ± standard error.

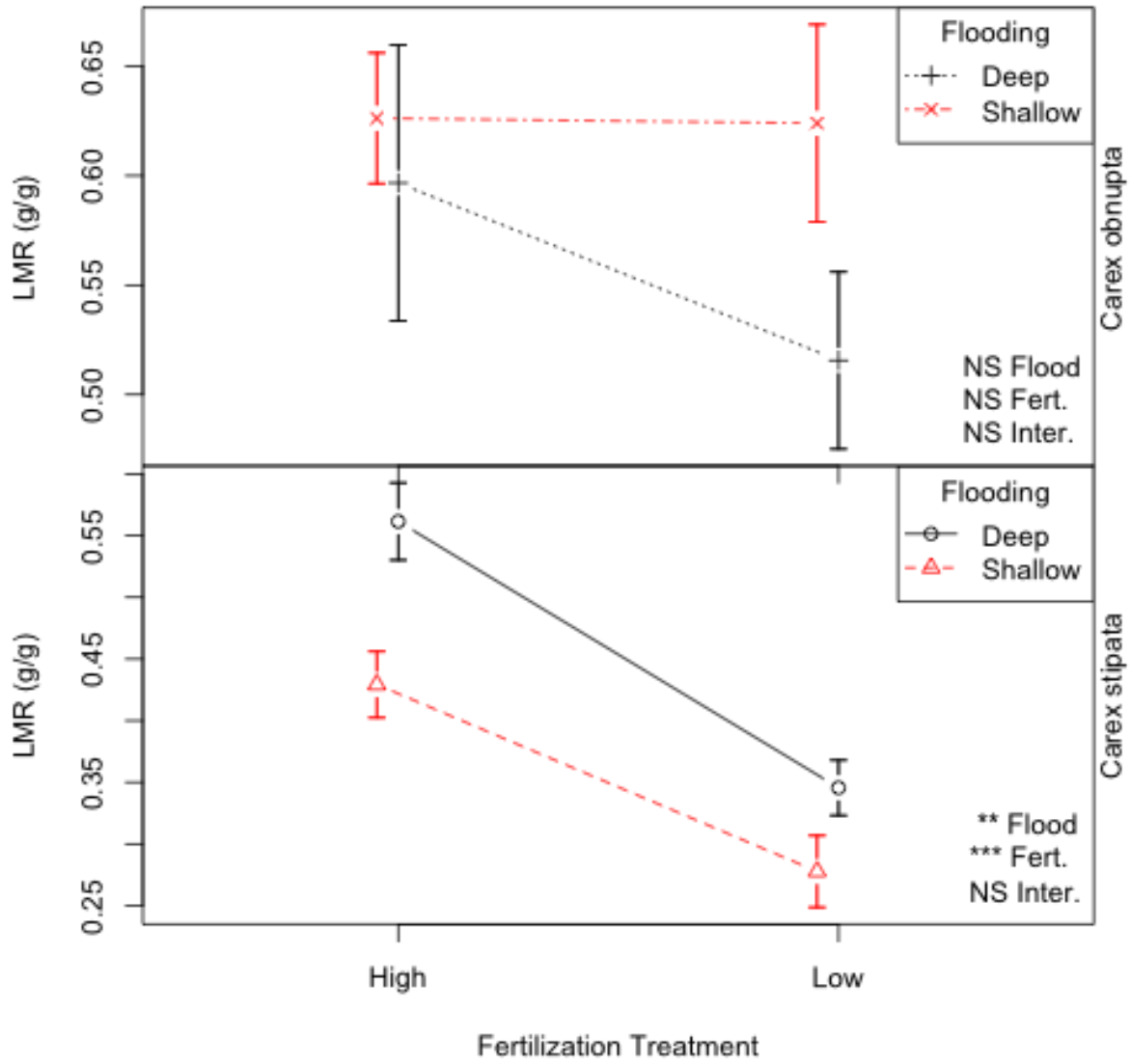

Figure 12: Leaf mass ratio at t_1 was positively influenced by fertilization and flooding in *C. stipata*. Bars are ± standard error.

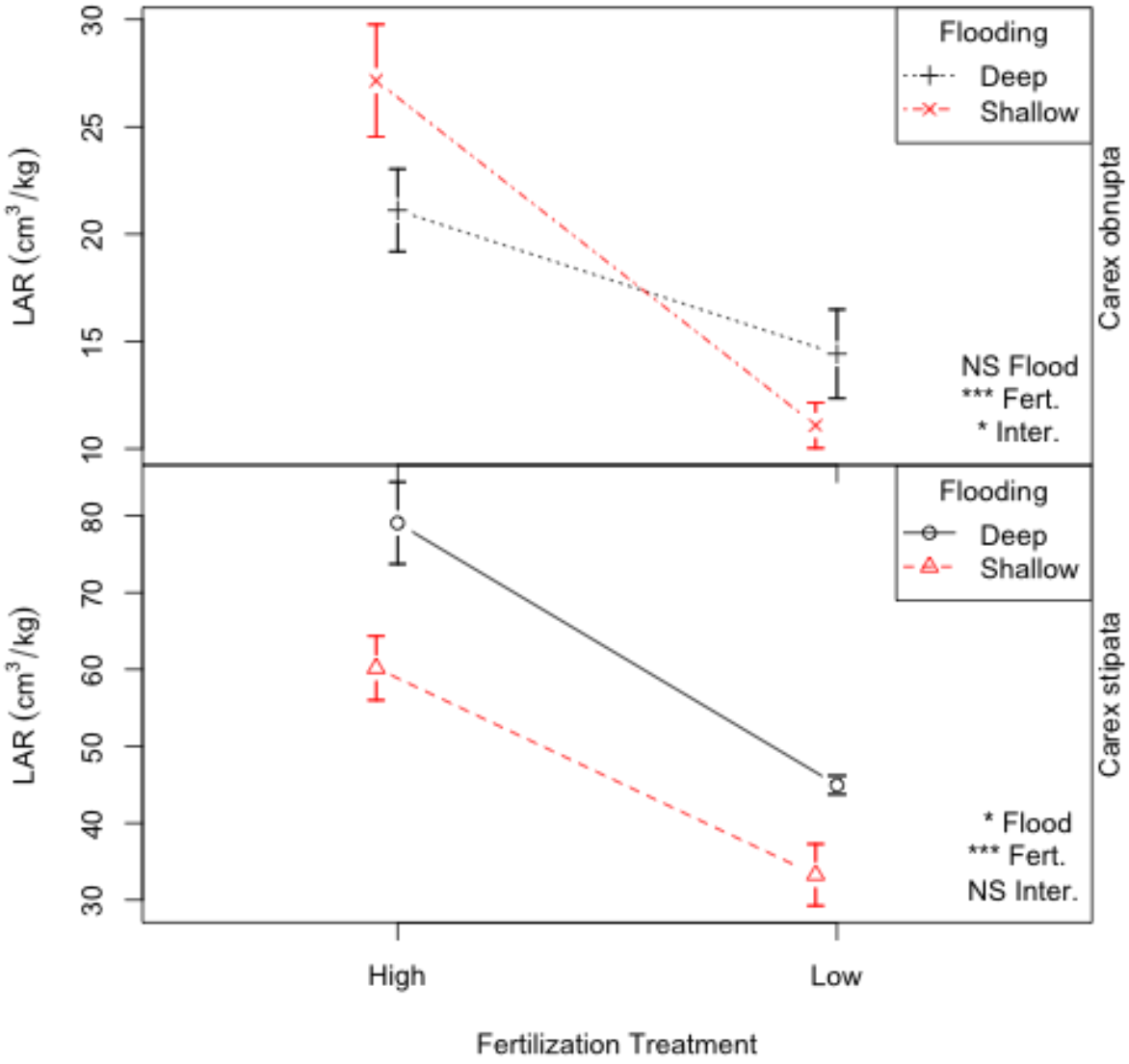

Figure 13. Leaf area ratio at t_1 was positively affected by fertilization in both species while flooding positively affected LAR in *C. stipata*. Bars are ± standard error.

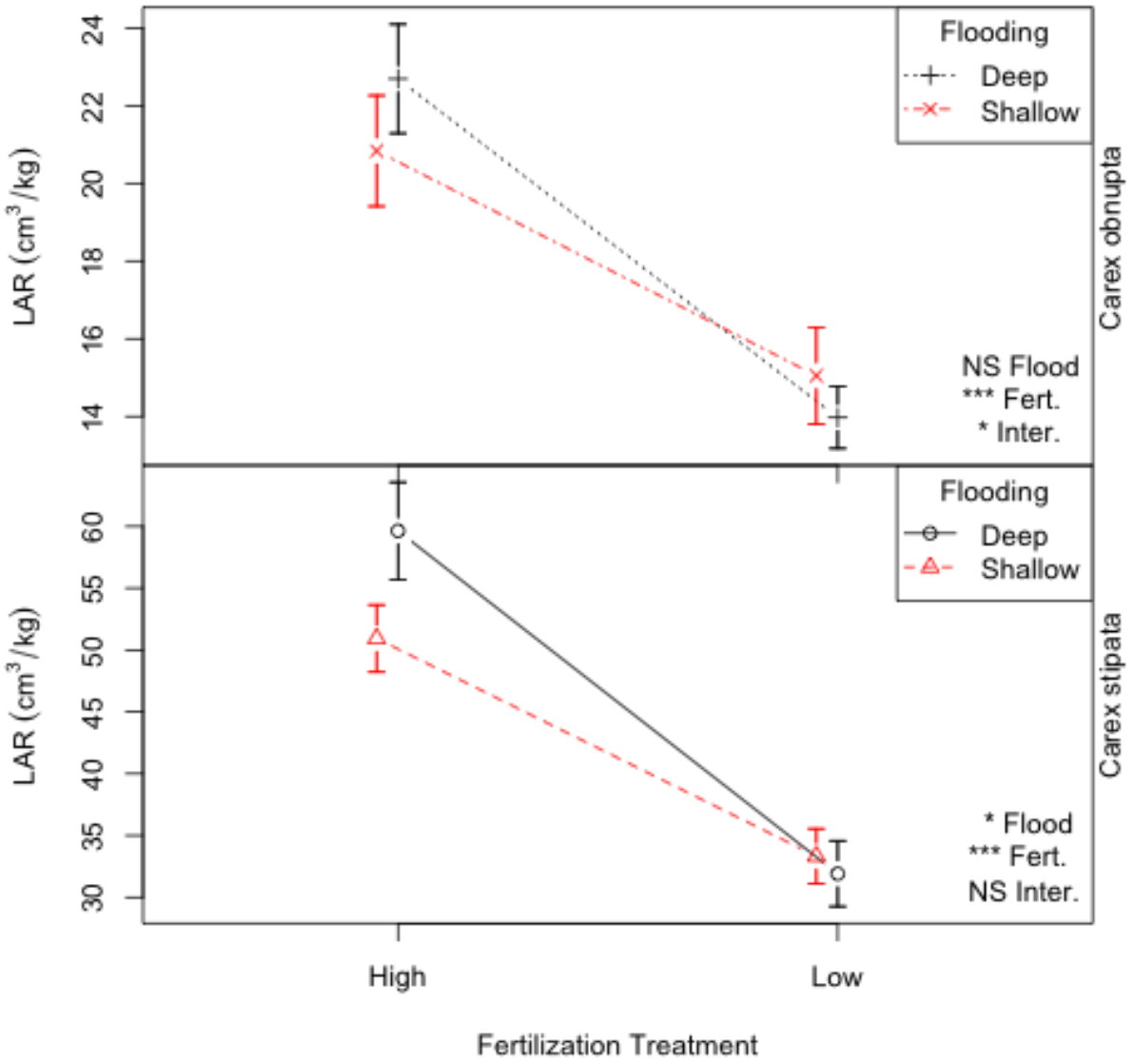

Figure 14: Leaf area ratio at t_2 peaked under high fertilization in both species. Bars are ± standard error.

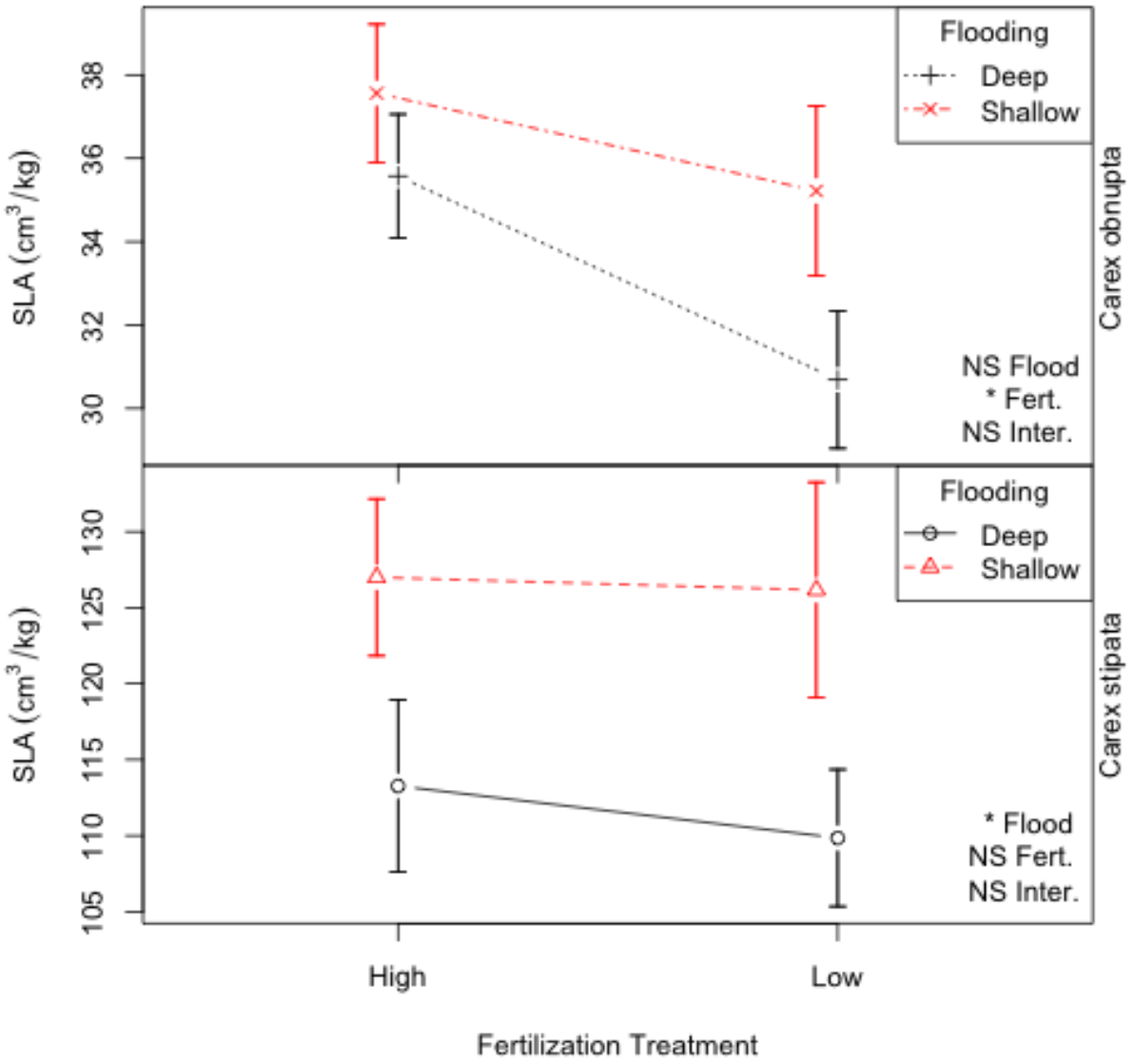

Figure 15: Specific leaf area at t_2 was driven upward by shallow flooding in both species and fertilization in *Carex obnupta*. Bars are ± standard error.

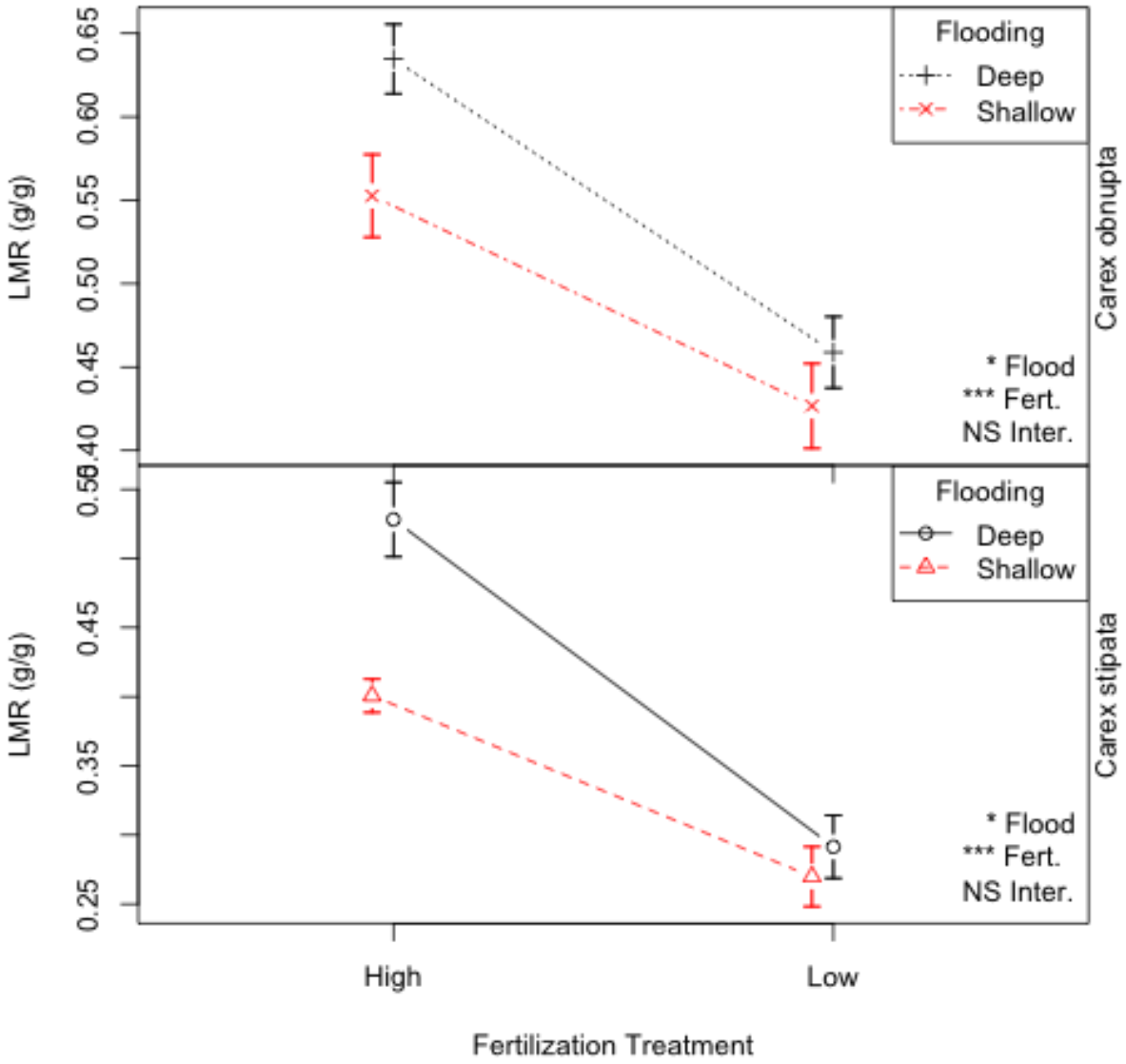

Figure 16: Leaf mass ratio in both species was driven by flooding and fertilization at t_2. Bars are ± standard error.

3.4 SPAD Index

Carex obnupta exhibited higher SPAD levels than *Carex stipata* regardless of treatment. For both species, I found flooding to have a significant effect on SPAD index (probability values summarized in Table 9). For *C. stipata*, there was a significant interaction effect between flooding and fertilization levels, while *C. obnupta* showed a significant fertilization effect. HS

showed the lowest chlorophyll index for both species while the highest indices were LD and HD for *C. obnupta* and *C. stipata* respectively (Figure 17).

Table 9: Estimated chlorophyll content by SPAD. Values are in SPAD units and ± standard error of the mean for each treatment.

Species	***Fertilization***	***Flooding***	***SPAD Value (Unitless)***
Carex obnupta	*High*	*High*	$55.9775 \pm 2.27_{b}$
	High	*Low*	$44.585 \pm 1.05_{a}$
	Low	*High*	$57.8125 \pm 0.54_{b}$
	Low	*Low*	$48.9025 \pm 0.88_{a}$
Carex stipata	*High*	*High*	$28.485 \pm 0.74_{b}$
	High	*Low*	$15.765 \pm 1.90_{a}$
	Low	*High*	$25.98 \pm 1.17_{bc}$
	Low	*Low*	$19.49 \pm 1.14_{ac}$

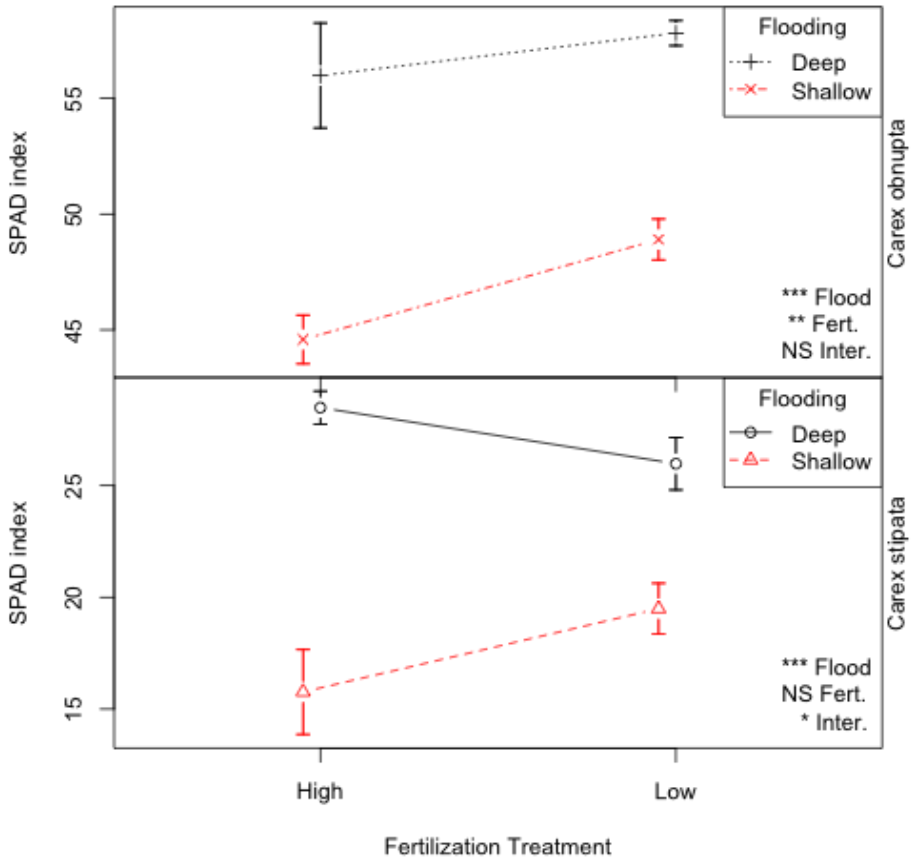

Figure 17: Mean SPAD values increased with deep flooding in both species. Fertilization reduced SPAD values in shallowly flooded plants. Bars are ± standard error.

3.5 Leaf Gas Exchange

Gas exchange A/C_i curves differed both between sedge species and between factorial treatment groups (Figures 18 and 19). *Carex stipata* exhibited lower rates of gas exchange than *Carex obnupta* within all treatment groups and CO_2 concentrations. In *Carex stipata*, HS and HD showed the greatest initial slopes on their A/C_i curves, indicating the highest initial carboxylation efficiency. HS and HD showed the highest compensation points at which photosynthesis became RuBP limited. LS showed a lower initial carboxylation efficiency and point of RuBP limitation than those of HD and HS, but was higher than LD for both metrics. LD showed the lowest rates of gas exchange, lowest carboxylation efficiency, and was limited by RuBP regeneration and electron transport at lower C_i values within the A/C_i curve.

Carex obnupta gas exchange exhibited similar patterns between treatment groups as those found in *C. stipata* but at higher rates. HS showed the highest rates of gas exchange, initial carboxylation efficiency, and was RuBP regeneration limited at higher A and C_i levels than other treatment groups. HD had the second highest gas exchange levels in the RuBP-limited region of the A/C_i curve, but showed lower carboxylation efficiency at low C_i levels than HS. LS showed the second highest carboxylation efficiency, but assimilated less carbon at higher C_i levels. LD showed both the lowest initial carboxylation efficiency and compensation point at which RuBP limited carbon assimilation.

PERMANOVA results on these curves showed that as the result of significant fertilization effects ($p = 0.019$) the least stressful treatment (HS) exhibited significantly higher gas exchange than the more stressful treatments (LD, $p = 0.010$) in *C. obnupta*. *C. stipata* showed a statistically significant fertilization effect ($p = 0.049$) although gas exchange did not

differ between any treatment groups. The trend in *C. stipata* was similar to that found in *C. obnupta* where the greatest between group differences in gas exchange curves were between HS and LD ($p = 0.064$). All PERMANOVA results are summarized in Table 10.

Table 10: PERMANOVA model output for gas exchange curves show a significant fertilization effect in both species. * Indicates a statistically significant *p*-value at the $\alpha = 0.05$ level.

Species	Factor	Mean Squares	F-statistic	R^2	Probability (> F)
C. stipata	Fertilization	141.9695	4.5198	0.2843	0.0487*
	Flooding	8.0548	0.2564	0.0161	0.7105
	Fertilization × Flooding	3.7907	0.1207	0.0076	0.9124
	Residuals	31.41068	--	0.6920	--
C. obnupta	Fertilization	337.53	4.8594	0.2605	0.0190*
	Flooding	181.12	1.7005	0.0912	0.1887
	Fertilization × Flooding	6.36	0.0916	0.0049	0.9706
	Residuals	6.95	--	0.6434	--

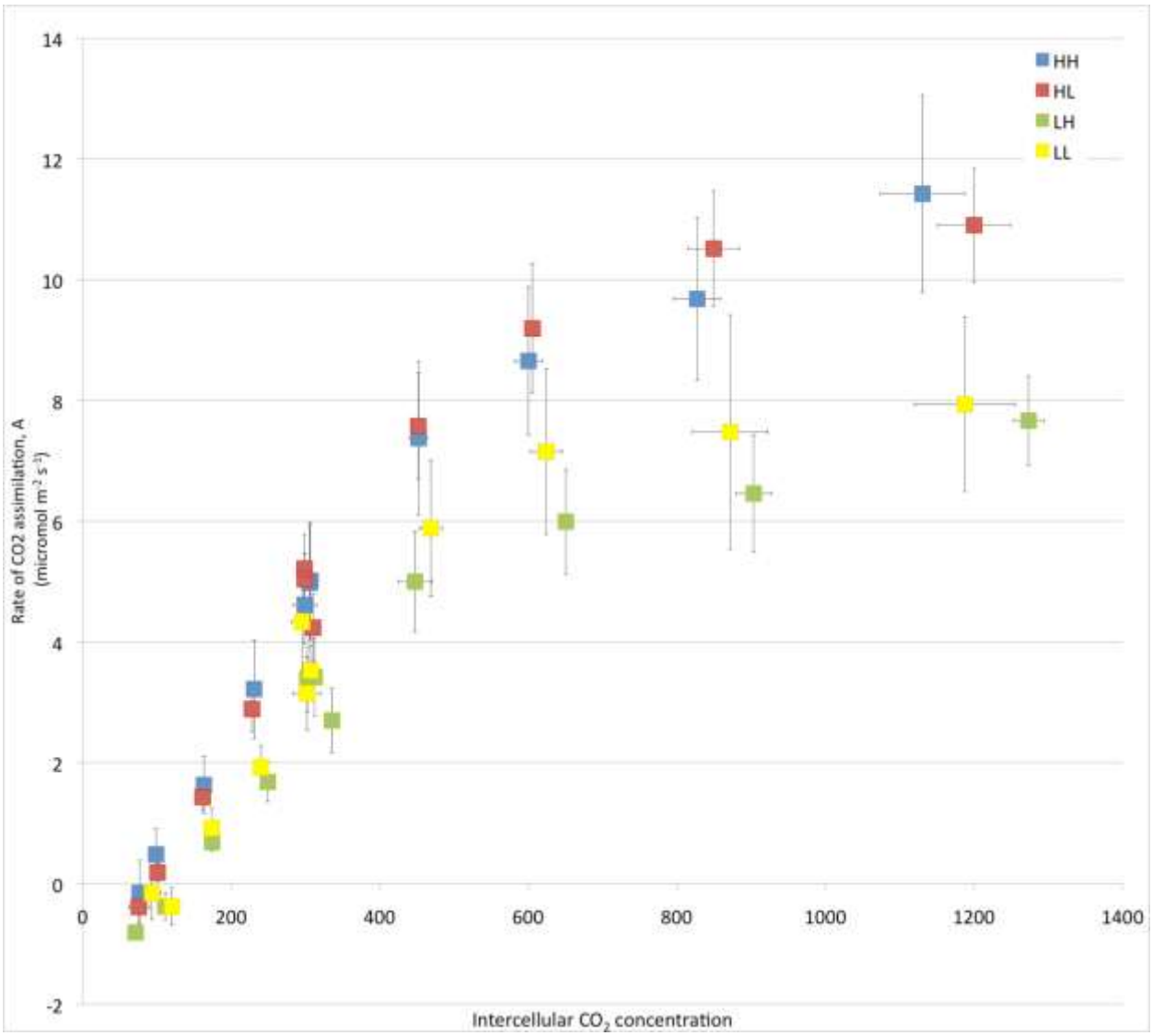

Figure 18: Carbon Assimilation plotted against intercellular CO_2 concentration for *C. stipata*. Two-way error bars are ± standard error for their respective axis.

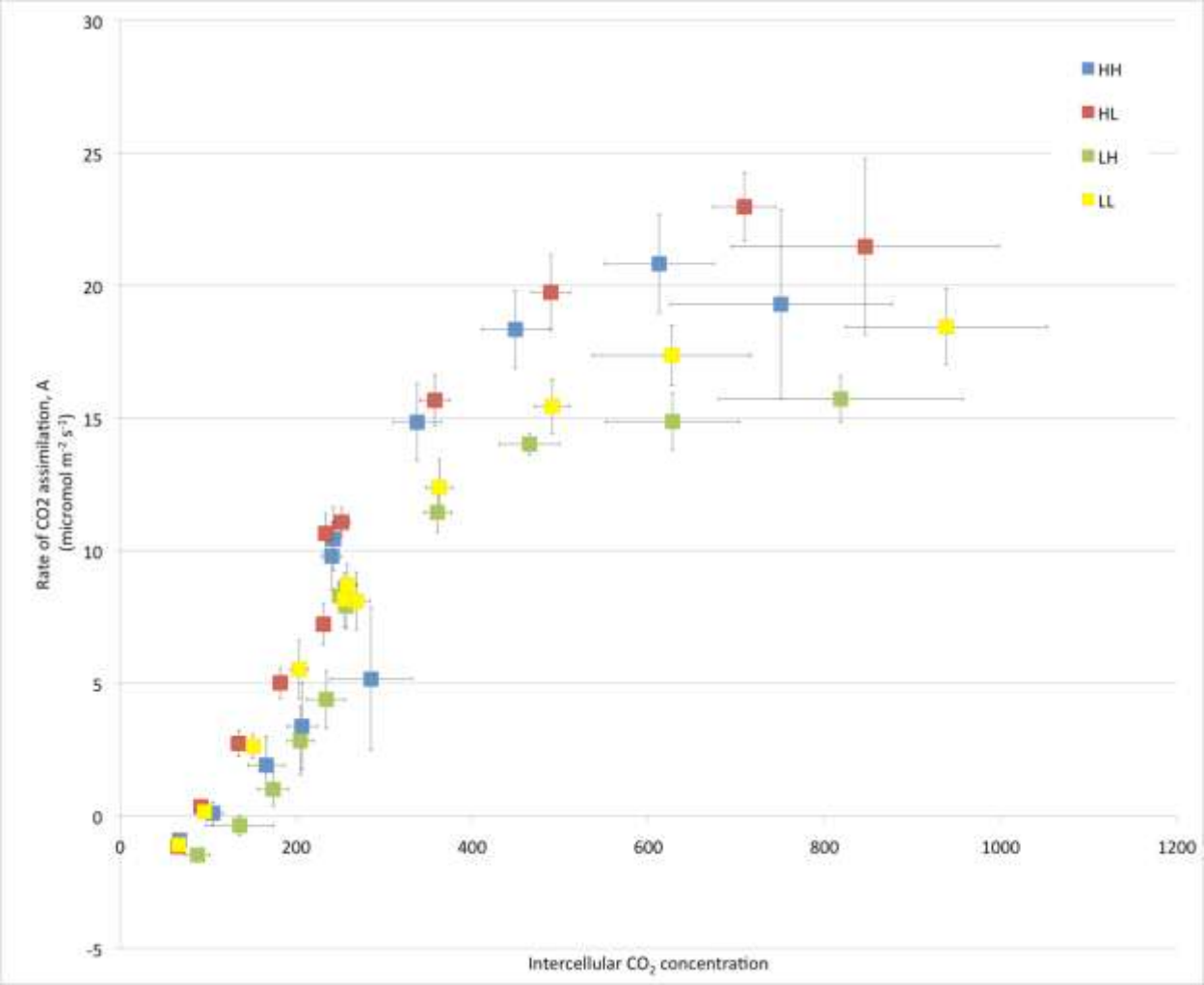

Figure 19: Carbon assimilation plotted against intercellular CO_2 concentration for *C. obnupta*. Two-way error bars are ± standard error for their respective axis.

Point measurements of gas exchange at the 400μmol mol^{-1} level showed the least stressful nutrient treatments (HS, HD) to have the highest gas exchange in both species. While low sample size prevented these trends from being statistically significant, a positive trend between gas exchange and SPAD was observed in all treatments for both species excluding LD for *C. stipata*. This anomaly observation may have been a product of a product of a deleted observation due to measurement error that further reduced sample size. Although statistically non-significant, stomatal conductance in both species was generally positively related to SPAD regardless of treatment. A flooded treatment in each species—HD in *C. obnupta* and LD in *C. stipata*—showed a negative relationship between SPAD and stomatal conductance. I believe this to be a function of low sample sizes and the aforementioned deleted observation in LD for *C. stipata*. The flooding and fertilization treatments did not significantly affect A or g_s in *Carex stipata*. The fertilization treatment significantly affected gas exchange in *C. obnupta* but between group comparisons were not statistically significant. The resulting ANCOVA *F*-statistics and *p*-values are presented in Tables 11 (*A*) and 12 (g_s) and figures 20-23 present the gas exchange and stomatal conductance data by treatment.

Table 11: ANCOVA table for gas exchange by treatment combination and SPAD values at 400μmol mol^{-1}

Species	Factor	Mean Squares	F-statistic	Probability (> F)
C. stipata	Fertilization	5.1418	2.1498	0.1733
	Flooding	1.0494	0.4388	0.5227
	Fertilization × Flooding	0.0078	0.0033	0.9555
	SPAD	1.9914	0.8326	0.3830
	Residuals	2.3917	--	--
C. obnupta	Fertilization	15.840	5.4596	0.03941*
	Flooding	0.5256	0.1812	0.67858
	Fertilization × Flooding	2.0127	0.6937	0.42262
	SPAD	9.4480	3.2563	0.09857
	Residuals	2.9014	--	--

Table 12: ANCOVA table for leaf stomatal conductance by treatment combination and SPAD values at 400 μmol mol^{-1}

Species	Factor	Mean Squares	F-statistic	Probability (> F)
C. stipata	Fertilization	0.0016	2.1270	0.1754
	Flooding	0.0000	0.0295	0.8671
	Fertilization × Flooding	0.0000	0.0013	0.9714
	SPAD	0.0012	1.6262	0.2311
	Residuals	0.0007	--	--
C. obnupta	Fertilization	0.0007	0.8243	0.3834
	Flooding	0.0000	0.0465	0.8333
	Fertilization × Flooding	0.0000	0.0001	0.1784
	SPAD	0.0019	2.0668	0.9908
	Residuals	0.0009	--	--

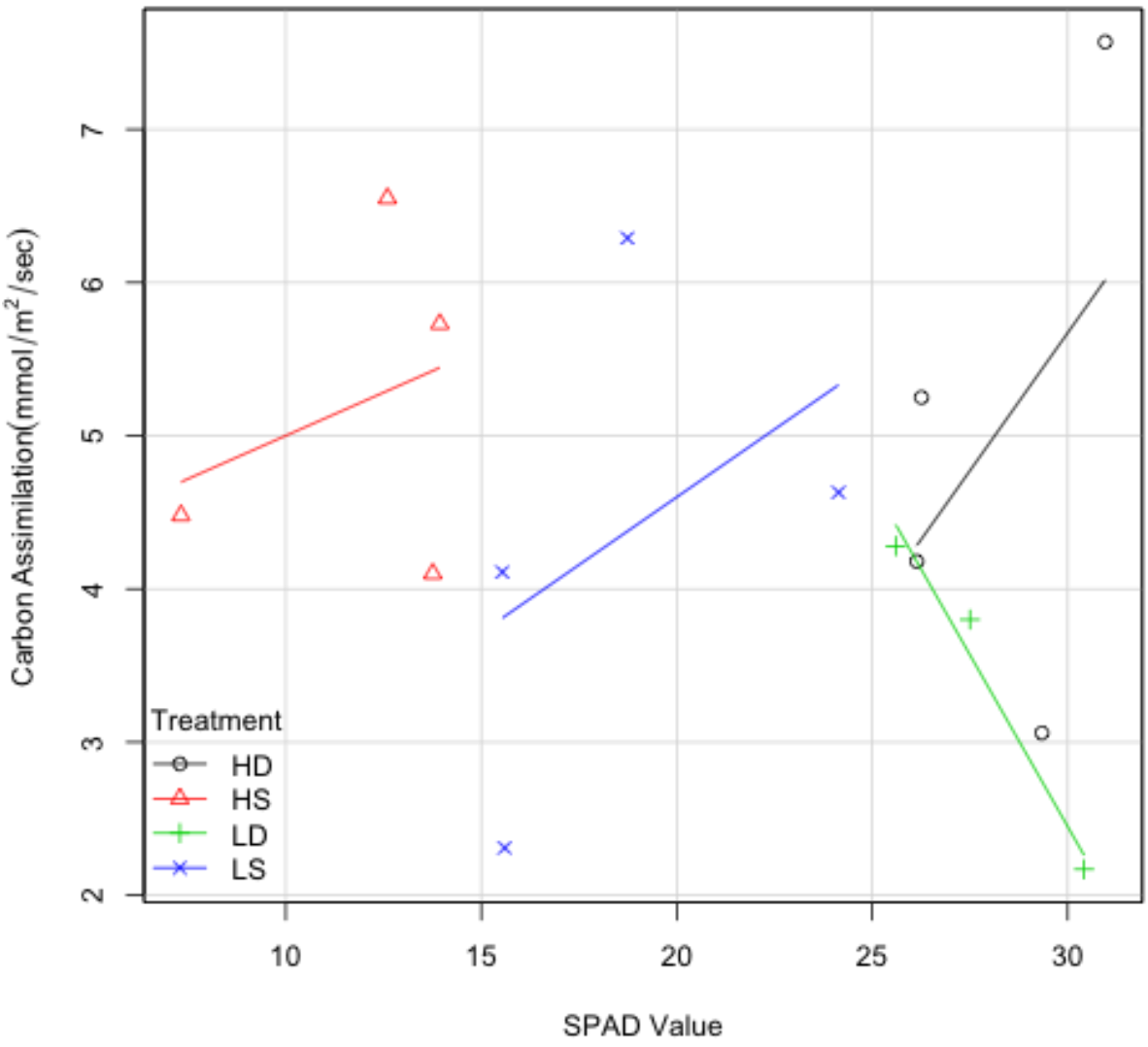

Figure 20. A scatterplot of *C. stipata* carbon assimilation plotted by treatment group and against SPAD value shows the between-group trends in stomatal conductance and trends in relation to SPAD value. These trends were not statistically significant and no between group differences existed. Trend lines are least squares regression fit between A and SPAD.

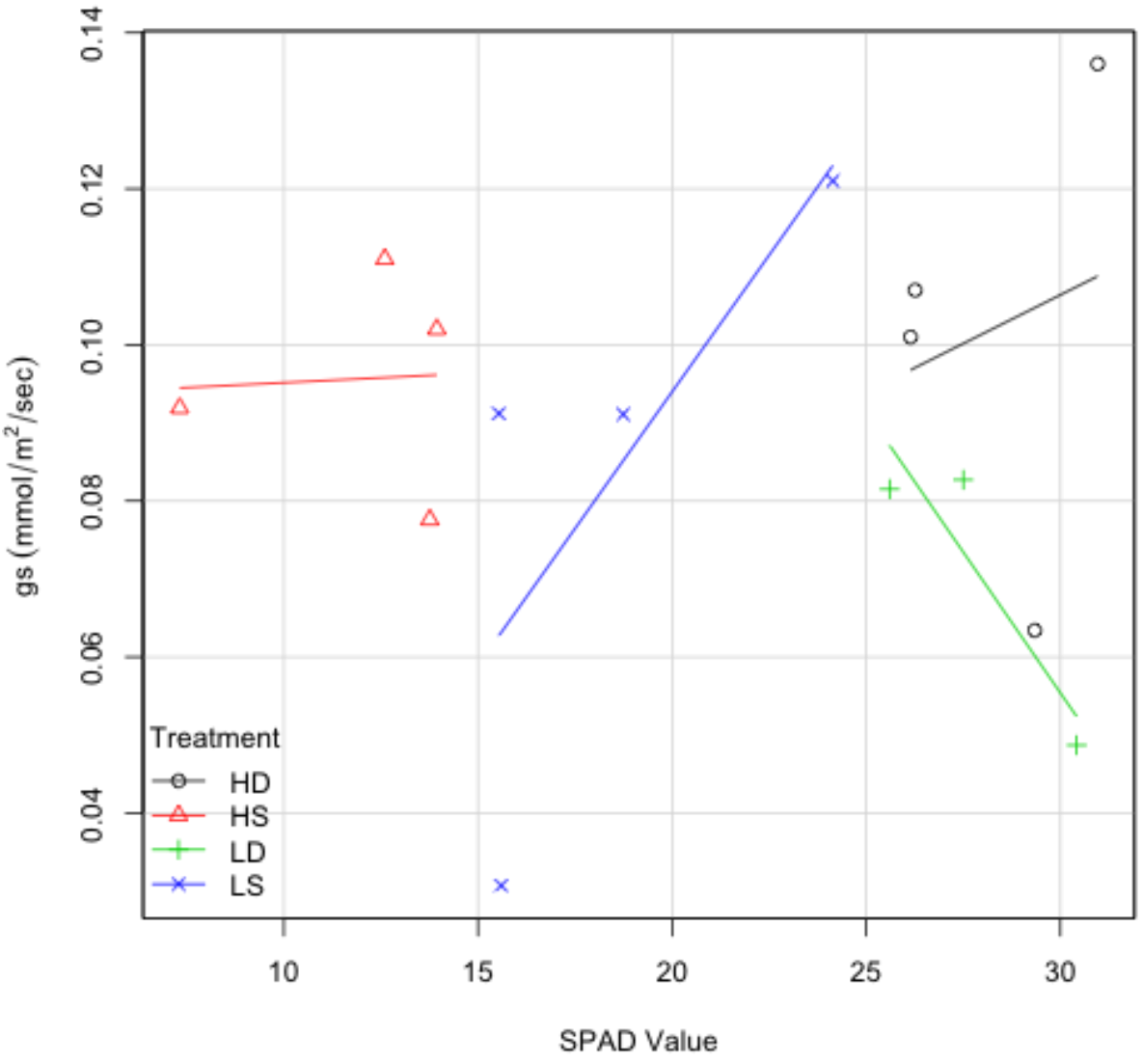

Figure 21. A scatterplot of *C. stipata* stomatal conductance (g_s) plotted by treatment group and against SPAD value shows statistically non-significant between-group trends in stomatal conductance and trends in relation to SPAD value. Trend lines are least squares regression fit between g_s and SPAD.

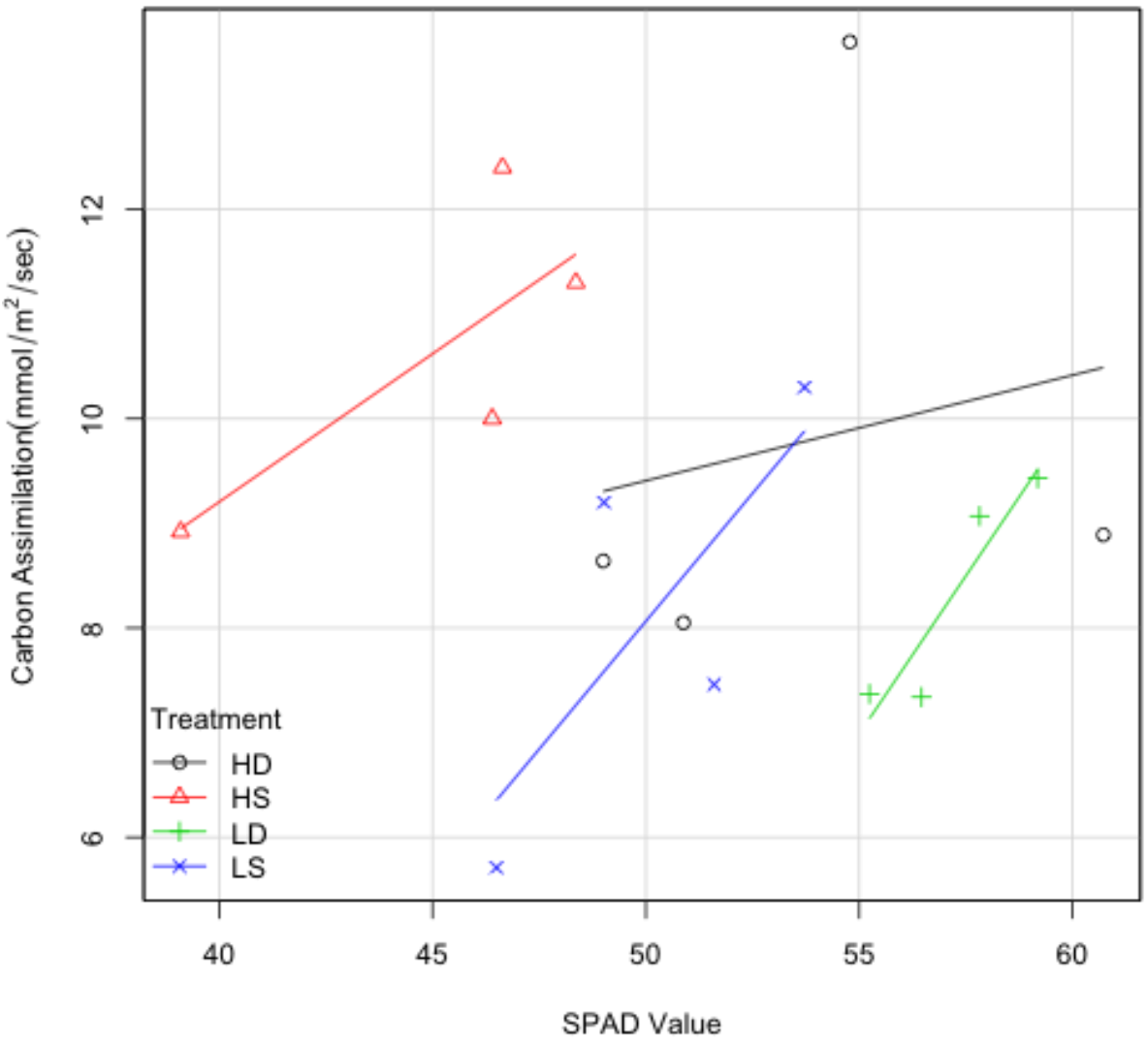

Figure 22. A scatterplot of *C. obnupta* carbon assimilation (A) plotted by treatment group and against SPAD value shows a significant fertilization effect on A and a statistically non-significant positive trend between SPAD and A. Trend lines are least squares regression fit between A and SPAD.

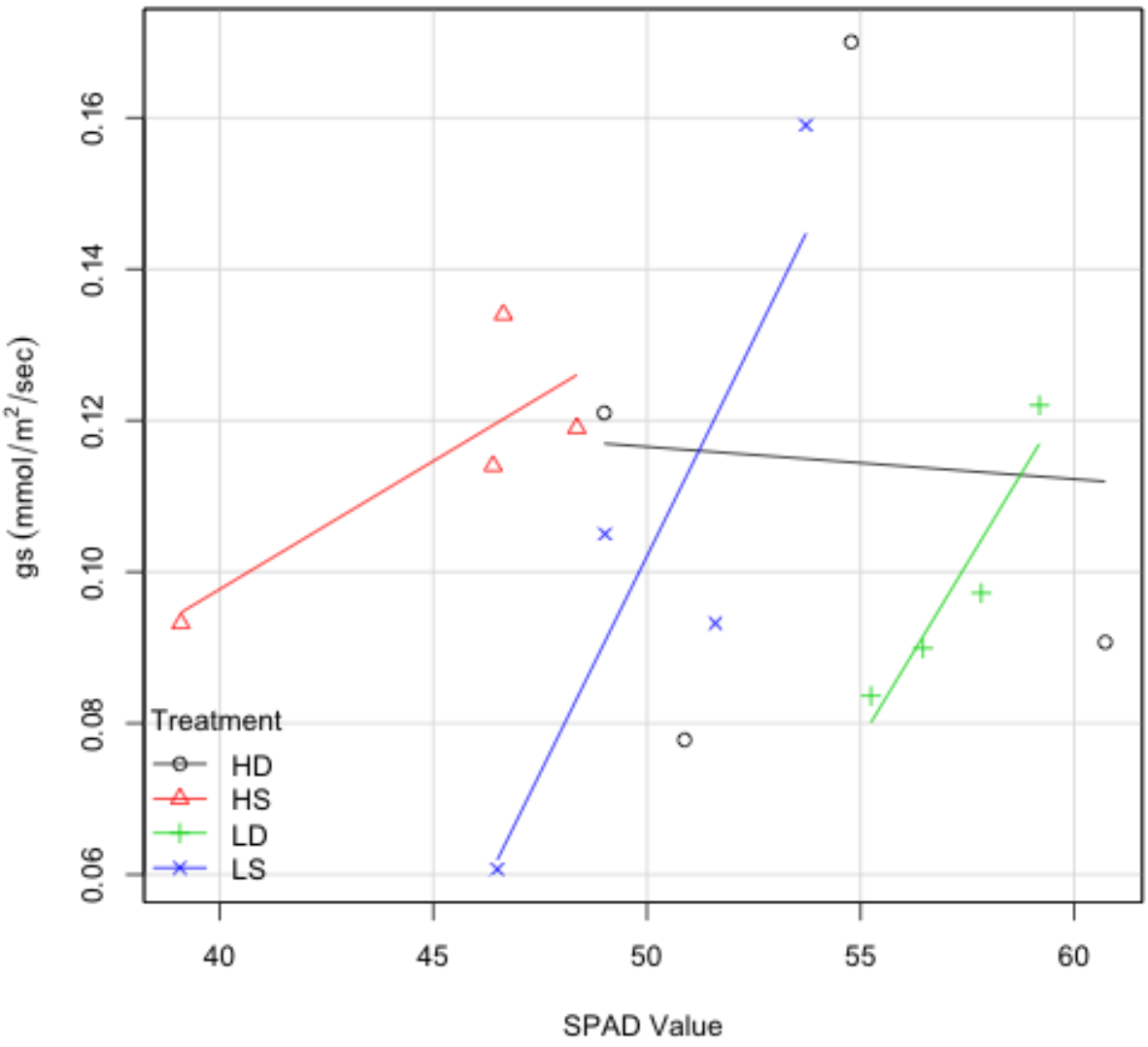

Figure 23: A scatterplot of *C. obnupta* stomatal conductance plotted by treatment group and against SPAD value shows the between-group differences in stomatal conductance and trends in relation to SPAD value. These trends were not statistically significant and no between group differences existed. Trend lines are least squares regression fit between g_s and SPAD.

4.0 Discussion and Conclusions

4.1 Plant Biomass and Allocation

Both species showed increases in total biomass throughout the study duration, although the allocation of this biomass to above and belowground growth in response to stress (treatments) varied between species. *C. obnupta* showed no treatment difference between the ratio of belowground to aboveground biomass at t_1, but, however at t_2 the most stressful treatments (LS and LD) showed flooding and fertilization increased allocation towards roots over shoots. *C. stipata* showed a similar trend in which the most fertile treatments (HD, HS) allocated biomass preferentially to shoots, while the more stressful treatments (LD, LS) tended to produce more roots. These results are consistent with the findings of Visser and others (2000) where four sedges, *C. Limosa, C. Sempervirens, C. rostrata* and *C. davalliana*, that were submerged and partially flooded increased their allocation to belowground biomass compared to untreated plants. The allocation to aboveground biomass in the more fertile HD and HS treatments allowed both *C. obnupta* and *C. stipata* to rapidly expand their leaf areas compared to those sedges in the LS and LD treatments. Flooding depth has been shown to influence SLA in numerous species (Violle 2010) while plant leaf area, specifically LAR, of which SLA is a component process, may drive plant growth (Lambers et al. 2003; Shipley 2006 should be noted in contrast). These observations indicate that fertilization seems to exert a stronger effect on leaf expansion and area in *C. obnupta* and *C. stipata* rather than flooding. *C. stipata* showed a particular tendency for rapid leaf expansion in HD at t_1, with more biomass allocated to shoots than roots and large fertilization and flooding driven gains in leaf area, aboveground biomass and leaf area ratio. Additionally, I observed a fertilization driven rapid expansion of leaf area in both species at both harvest times regardless of flooding level that shows both species are able to

effectively utilize excess soil resources to overcome belowground plant stress, with *C. stipata* employing this strategy at both harvest intervals and across fertilization treatments.

Initial plant size had a significant effect on plant final wet weight, leaf area, total biomass, and above and below ground biomass *C. obnupta* at both t_1 and t_2. Initial plant wet weight at treatment initiation was positively correlated to plant leaf area at both harvest intervals for the evergreen *C. obnupta*. The allocation cost of building leaves is much higher for evergreen species than deciduous species (Aerts 1995). Accordingly initial plant size had a more significant effect on final *C. obnupta* size, including leaf area at both harvest intervals, than on final biomass and leaf area in the deciduous *C. stipata*. This effect was more pronounced at t_1 leading us to believe the initial size at which plants experience inundation may be a major driver behind *C. obnupta* growth in wetland environments, where a greater proportion of plant total biomass is subjected to flooding stress. The size of individuals may also drive plant survival and growth as smaller plants may have lower energy reserves to borrow from during morphological acclimation to flooding and nutrient stress. Much like my results, Steed and DeWald (2003) found the size of transplanted *Carex nebrascensis, C. rostrata* and *C. languinosa* to be positively correlated with plant growth and survival in a high elevation meadow.

4.2 Relative Growth Rate and Component Processes

I observed that between t_0 and t_2 and between t_1 and t_2 the RGR and NAR of *C. obnupta* and *C. stipata* were highest in HS and HD respectively. Where nutrients were limiting (LS) or where flooding and nutrient stress were paired (LD), RGR and NAR were reduced in both *C. obnupta* and *C. stipata*. The ability of each species to increase leaf area under flooded conditions was related to the observed differences in RGR and component processes. High LAR in the HD and

HS treatments corresponded to high RGR and NAR, a function of total canopy and whole plant photosynthesis. The reduced LAR and LMR that I observed in LD and LS are consistent with other studies that showed SLA to limit growth in infertile environments (Poorter and Remkes 1990, Ceulemans 1989). Morris and others (2001) also illustrated that coupling nutrient stress with other stressors can significantly reduce LAR in emergent wetland plants. In a similar study, as the duration and depth of flooding increased in *Genipa Americana* seedlings, LMR and LAR both decreased (Mielke 2003). The between species differences in RGR, NAR and LAR indicates that growth may occur most rapidly in flooded environments for *C. stipata*, while *C. obnupta* performs well in fertile, but not highly flooded settings. These observations correspond with the environments inhabited by *C. obnupta* and *C. stipata*: *C. stipata* commonly grows in riparian mineral substrates and moist prairies where short-duration flooding can be frequent, while *C. obnupta* is more commonly found in organic soils with ephemeral hydrology (Minore 1969, Franklin and Dyrness, 1988). *C. stipata's* growth rates, when paired with its preferential expansion of roots may help to explain its ruderal strategy of colonizing disturbed sites and open light environments.

4.3 Leaf Gas Exchange

I anticipated that both species would exhibit reduced photosynthesis under more extreme flooding conditions (LD, HD) and that this effect would be further exacerbated by nutrient stress. This expectation was validated as *C. obnupta* and *C. stipata* showed reduced gas exchange under combined nutrient and flooding stress (LD). I hypothesized that HD would show the next lowest gas exchange as a result of flooding stress, but instead found that LS had the second lowest gas exchange rates for both species. *C. obnupta* and *C. stipata* in the HD treatment had gas exchange rates slightly lower than those in the HS treatment, which implies that nutrient deficiency is

equally as detrimental to gas exchange as flooding. Those plants grown with free access to nutrients (HD, HS) showed the highest average quantum yields of both CO_2 and PSII and highest average electron transport rates, implying that in LD and LS treatments, nitrogen limitation reduced photosynthesis in both the carboxylation and RuBP limited portions of the A/C_i curves (Figures 21, 22, 23). In addition to trends in A/C_i curves, I observed a fertilization effect on gas exchange but no effect on stomatal conductance (*C. obnupta* A/C_i data for HD, HS). This leads me to postulate that biochemical limitations from a lack of nutrition (LS) may play a stronger role in inhibiting carbon gains than morphological responses such as flooding-induced stomatal closure. SPAD was positively correlated to both g_s and *A* in both species although not statistically significant. Disentangling SPAD and leaf level gas exchange leads me to believe that although nutrition was limiting (LD, LS) both species may increase the quality of their leaves in response to stress (flooding) by increasing tissue N ocntent but are then morphologically limited by stomata in how much carbon they can assimilate when flooded. This outcome is most probable in *C. stipata* as we observed a nearly significant flooding term in our gas exchange PERMANOVA results.

The low physiological performance of plants within LD correspond to the low RGR and NAR observed between t_1 and t_2 and the low LAR, SLA and LMR observed at t_2. These results show that in flooded and infertile environments both poor leaf-level gas exchange and reduced allocation to leaf area may drive reduced growth and total biomass accumulation. While the drivers of this correlation are uncertain, it appears to potentially be a combination of biochemical and morphological limitations depending on species and treatment combinations.

4.4 Carex and Stress: Conclusions and Additional Considerations

Both *Carex obnupta* and *Carex stipata* responded differently to flooding and fertilization in their growth, biomass allocation, leaf nitrogen content and physiological performance. The relationships between plant presence and survival across both fertilization and flooding gradients commonly show tradeoffs between growth and resource acquisition in productive environments and the effective conservation of resources under infertile and/or stressful conditions (Poorter and Remkes 1992, Rubio et al. 1995, Willby et al. 2001). As each species experiences optimal conditions for growth—highly fertile, flooded conditions for *C. stipata* and fertile, saturated conditions for *C. obnupta*—each species may increase their leaf-level gas exchange and increase their net assimilation and relative growth rates. These gas exchange and growth rates correspond to high leaf area ratios and low root to shoot ratios that indicate a shift towards canopy level carbon gains rather than acclimating to flooding and nutrient stress within the root zone. It is possible that both species initially expanded their leaf areas in response to flooding stress, but the eventual costs of evergreen leaves and limiting resources of the more stressful treatments forced *C. obnupta* to grow less rapidly by the end of my trial. *Rumex palustris* has been shown to preferentially elongate petioles and expand leaf area in response to flooding (Pierik et al. 2009, Bailey-Serres and Voesenek 2008, Voesenek et al 2004), allowing the plant to acclimate to flooding over long timeframes. These observations may help to explain not only the life strategy and realized habitats of both sedges species, but also provide insights to wetland managers on the appropriate conditions under which both species may be installed.

While I have framed my experiment around the stresses of flooding and nutrient stress as potential limiting factors to the growth and physiological performance of *C. obnupta* and *C. stipata* in natural and restored environs, the dynamic environments that sedges inhabit may

expose plants to additional stressors such as drying. For example, Sarr and Dudley (2008) found that as the distance to water table decreased, transplants of *Carex utriculata* survived at greater rates in riparian meadows than those plants that never experienced flooding in any portion of the root zone. Ewing (1996) showed that drying following flooding reduced 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 for restoration practitioners, both species should be subjected to a regime of flooding and drying similar to the hydrologic patterns found in many urban and agricultural watersheds (Ewing 1996). The temporal scale at which restoration success may be measured is often longer than the duration of my trial, allowing us to make only limited inference on how flooding and fertilization might influence the long-term success of both species in field-based restoration.

While at low level flooding may not be a limiting stress factor for *C. obnupta* and *C. stipata*, flooding stress is exacerbated by nutrient limitations that reduce leaf level carbon assimilation and component photosynthetic processes, leaf chlorophyll content, allocation to leaf area and whole plant growth in both species (treatment LD). Restoration practitioners may overcome these limitations by matching *C. obnupta* and *C. stipata* to restoration sites with hydrology and nutrient budgets conducive to each species' growth and establishment. *C. stipata*, can be planted into wet (shallow flooding in a portion of the root zone or slightly above the stem, fertile sites that allow *C. stipata* to rapidly expand its leaf area and increase plant total biomass. *Carex stipata* may also be adversely affected by flooding at smaller sizes 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 survive in that environment.

Voesenek and other (2004) noted that the ability to outgrow flooding is only particularly useful in situations where flooding is shallow and prolonged, such as the HD and LD treatments within this study. Inherently, if wetland hydrology is flashy like that used in Ewing (1996), then *C. stipata* may not be able to overcome the dual stressors of flooding and drying. *C. stipata* is a poor performer in infertile environments and may not perform well in wetland restorations without sufficient nutrients e.g. fens and bogs.

C. obnupta should be planted in less flooded environments with high nutrient levels that allow the creation of cost-intensive evergreen leaves. In the evergreen *C. obnupta*, paired flooding stress and nutrient limitation may affect small plants more adversely than large plants. *C. obnupta* should be planted as larger stock when being introduced to either infertile or flooded conditions, because it lacks the ability to rapidly expand leaf area to overcome flooding stress. *C. stipata* has the ability to expand its leaf area more rapidly than *C. obnupta,* and should be considered an effective plant for wetland restoration and revegetation, especially in denuded sites conducive to the ruderal strategy of *C. stipata*. This result implies that planted *C. stipata* may also compete well with invasive plants that employ similar strategies. *C. obnupta* may be an effective choice for wetland revegetation across a range of hydrologic scenarios due to its ability to rapidly expand through rhizomes.

This study did not investigate some key drivers of plant competition and survival under stressful conditions. Because this study used relatively large seedlings of both species, I did not identify germination or early growth limitations that might limit the natural or reproductive ranges of either species. This limitation would likely more strongly effect *C. stipata* that relies largely on seed for reproduction. Elucidating the limitations to seedling emergence in response to flooding would be of great use to restoration practitioners as seed is an inexpensive and

efficient way of introducing plants to a site, especially at large scales. Further investigation should be performed on how each species responds to flooding and fertilization over longer timeframes from seed germination to multiple years to confirm differences in the stress tolerating strategy of *C. obnupta* and *C. stipata*'s low oxygen escape strategy. Because genetics also play a role in the flood tolerance of populations of each species based on their local adaptations to microclimate and flooding in a given environment (Bailey-Serres and Voesenek 2008), these effects should be investigated before restoration plant selection is made. Selecting seed from plants occurring in higher water tables and depths has shown local responses to environmental stress and should also be investigated if the propagation of a large quantity of plants with a specific stress tolerance is desired.

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Appendix I. Supplemental Bar Charts

All plots herein are alternative representations of the interaction plots found in figures 1-17 in the text.

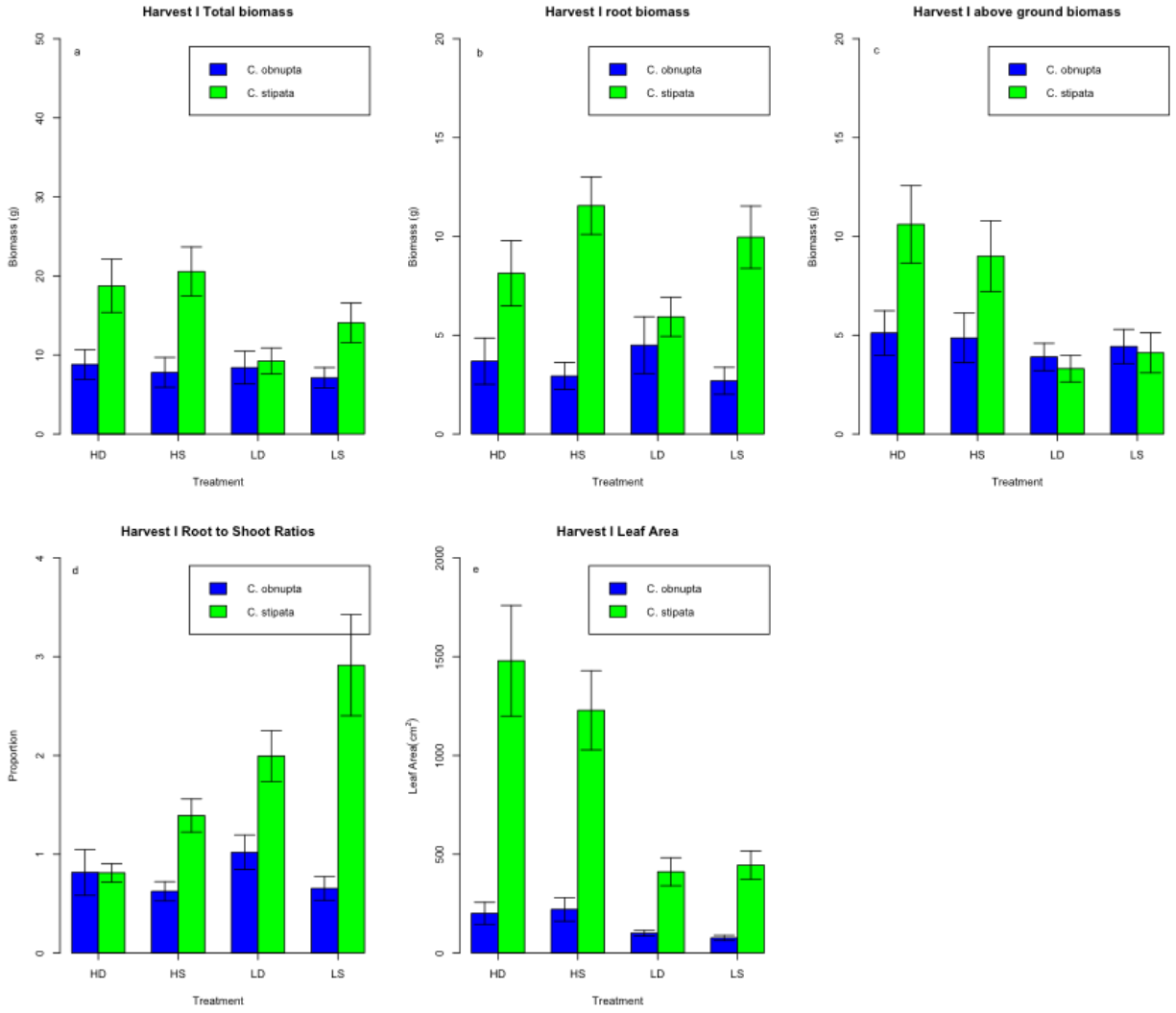

Figure A1: Biomass parameters at t_1. These plots correspond to Figures 1-5 in the text.

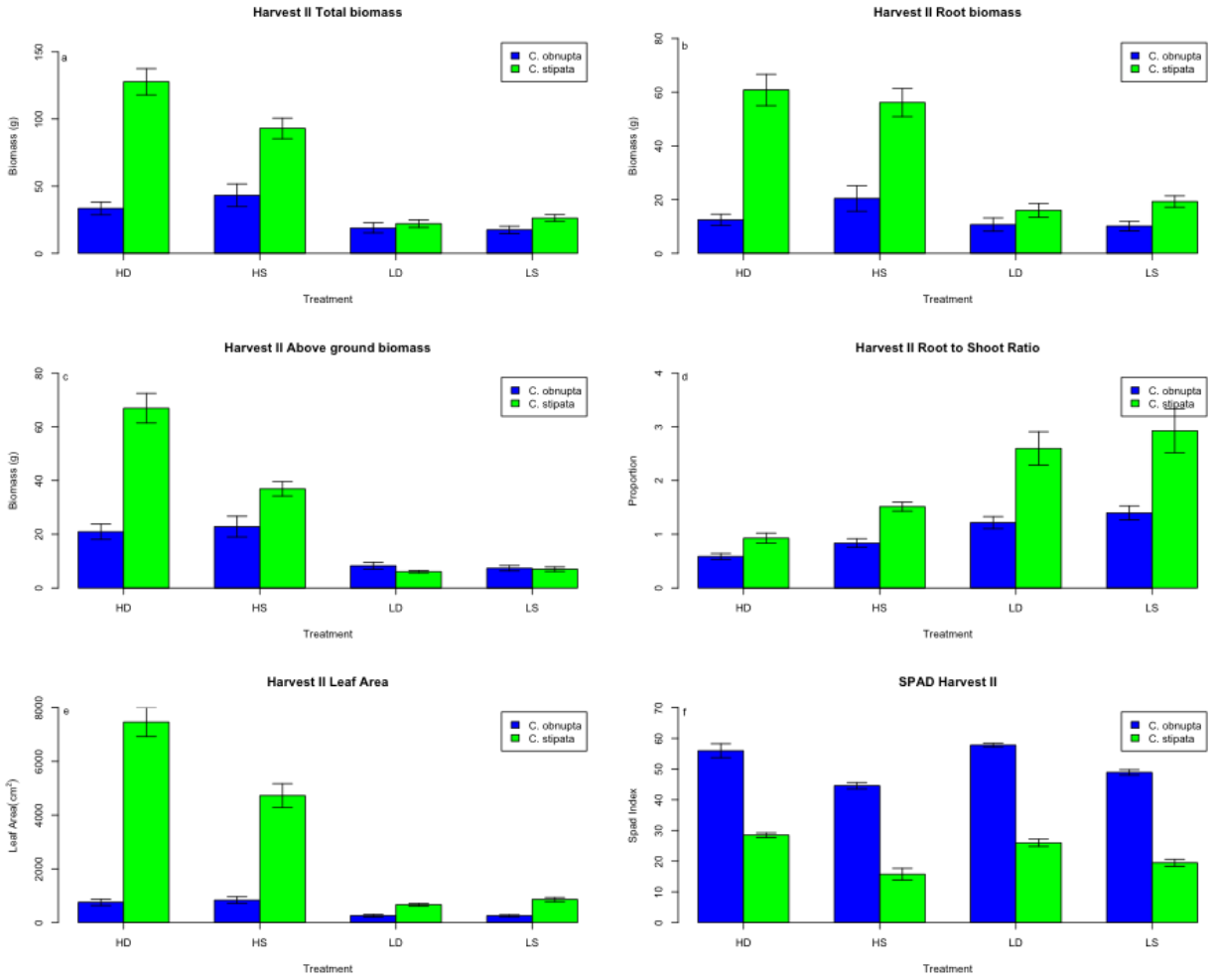

Figure A2: Biomass parameters and SPAD index at t_2. These plots correspond to Figures 6-11 in the text.

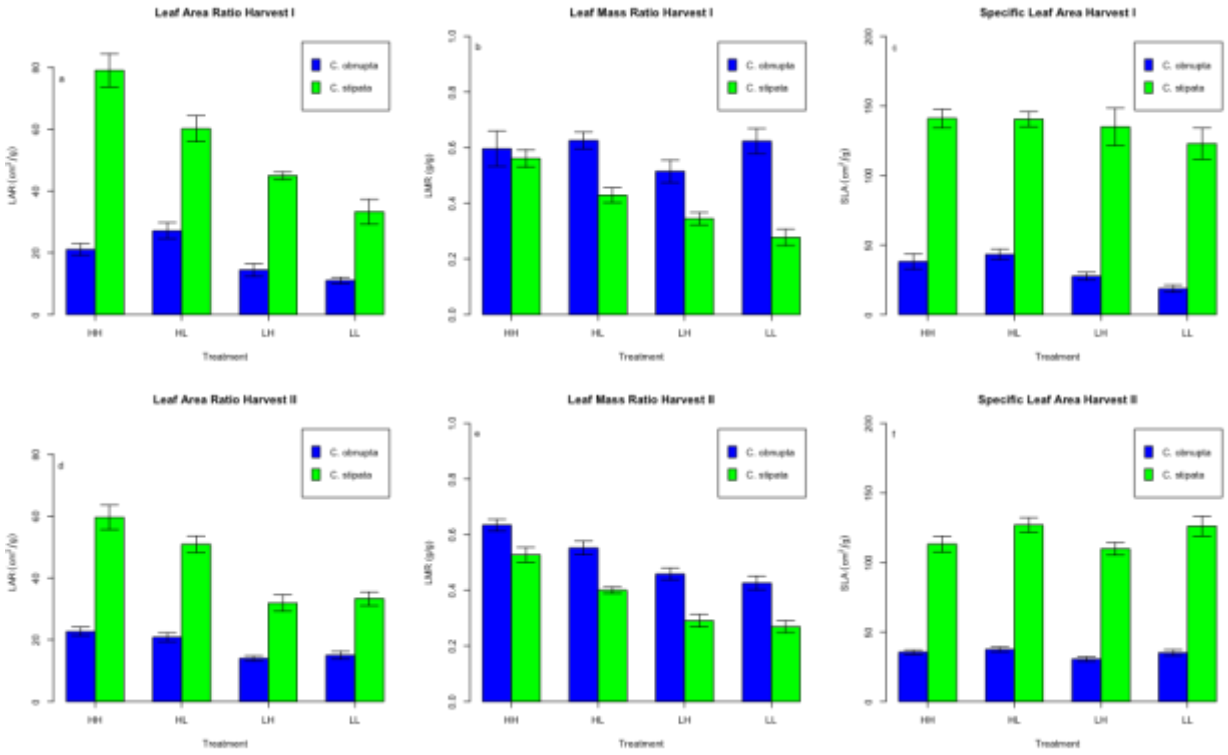

Figure A3: Leaf area ratio, leaf mass ratio, specific leaf area at t_2 (plots a-c) and t_3 (d-f). These plots correspond to Figures 12-17 in the text.