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By Donovan J. Moxley

Entitled Interactive Effects of Elevated CO_2 and Salinity on Three Common Grass Species

For the degree of Master of Science

Is approved by the final examining committee:

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INTERACTIVE EFFECTS OF ELEVATED CO_2 AND SALINITY ON THREE COMMON GRASS SPECIES

A Thesis

Submitted to the Faculty

of

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by

Donovan J. Moxley

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For everyone I have missed, forgotten, or ignored.

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IU Bloomington, spending time teaching or researching only to bring both home with me, finishing a second Bachelor's degree on the side and, of course, writing this thesis.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Moxley, Donovan J. M.S., Purdue University, August 2012. Interactive Effects of Elevated CO_2 and Salinity on Three Common Grass Species. Major Professor: Xianzhong Wang.

Carbon dioxide (CO_2) level in the atmosphere has increased steadily since Pre-Industrial times. The need for a better understanding of the effects of elevated CO_2 on plant physiology and growth is clear. Previous studies have focused on how plants are affected by either elevated CO_2 or salinity, one of many environmental stresses for plants. However, little research has been focused on the interaction of these two factors. In my project, three common grass species were exposed to both elevated CO_2 and salinity, so that the effects of either of these factors and the interaction of the two on these species could be examined. The CO_2 levels were set to 400 μmol mol^{-1}, close to the current concentration, or 760 μmol mol^{-1}, projected to be reached by the end of this century. Salt solutions of 0, 25, 50, 75, and 100 mM NaCl with $CaCl_2$ at lower rates (1% of each respective molarity for NaCl) were used to water the grasses, which are unlikely to experience prolonged exposure to salt conditions beyond this range in their natural habitats. The three common grass species studied in my experiment were Kentucky bluegrass (*Poa pratensis* L.) and red fescue (*Festuca rubra* L.), both C_3 cool season grasses, as well as buffalo grass (*Buchloe dactyloides* (Nutt.) Engelm.), a C_4 warm season

grass. Each treatment had five replicates, bringing the total number of experimental pots to 150. Various growth parameters were monitored, and all data was statistically analyzed for statistical significance. My results showed that elevated CO_2 had a stimulating effect on most growth parameters, particularly when plants were given more time to grow. In a 100-day growth experiment, CO_2 affected the number and dry biomass of plants of all species, regardless of their C_3 or C_4 photosynthetic pathways. Salinity consistently inhibited germination and growth at all stages, from germination through plant emergences, numbers of established plants, and dry biomasses at harvest. Interactive effects of CO_2 and salinity did occur, though often in seemingly specific instances rather than forming clear and consistent trends. My findings suggested that growth of common grasses would be enhanced by the rising level of CO_2 in the atmosphere, but the effect would be modified by environmental stresses, such as salinity.

CHAPTER 1. INTRODUCTION

Earth's environment is changing. One of the most important global changes is the steady increase in atmospheric carbon dioxide (CO_2) concentration. Virtually all plants will be affected by this change, as the increase is occurring uniformly worldwide. Other environmental changes, potentially stressful to plants, are occurring as well. It is thus important to have a better understanding of how plants respond to other stresses as CO_2 continues to increase in the atmosphere. Although the effects are localized to particular regions or locations, salinity stresses on plants occur globally. Many studies have focused on the effect of elevated CO_2 or salinity stress on plants. However, very little research has been done to investigate the interaction of these two major factors. In my project, I studied the effects of both elevated CO_2 concentration and increasing salinity as well as the interaction of the two factors on common grass species.

1.1 Plant Responses to Atmospheric Carbon Dioxide

Atmospheric CO_2 is likely to be double the Pre-Industrial levels by the end of the 21st century, reaching as much as 700 μmol mol^{-1} (IPCC 2007). As of June 2012, CO_2 measured at Mauna Loa Observatory, Hawaii, exceeded 395 μmol mol^{-1} (Tans and Keeling 2012). As the concentration increases at a pace to reach substantially elevated levels, the need to understand the potential responses of plant species is clear.

Elevated CO_2 levels result in increases in photosynthetic rate, plant biomass, and rate of development (Curtis and Wang 1998, Garcia-Sanchez and Syvertsen 2006, Geissler et al. 2009a, Mateos-Naranjo et al. 2010). The responses are not uniform, however, as unique characteristics of plant species and functional groups can change the magnitudes and types of responses to elevated CO_2.

Most studies of CO_2 enrichment have focused on C_3 species, in which growth has been stimulated by elevated CO_2 a vast majority of the time (Poorter 1993, Wand et al. 1999). While the magnitude of response is species-specific, growth increases and aboveground biomass tend to be greater at elevated CO_2. Aboveground dry biomass and total biomass have been found to increase in C_3 species, including increases of 31% among forbs, 18-24% among legumes, and 28% among trees (Reich et al. 2001, Ainsworth and Long 2005).

As these results suggest, functional groups had varied responses to elevated CO_2. The C_3 grasses tended to be stimulated as well, but the magnitude of response varies. At elevated CO_2, C_3 grasses experienced aboveground biomass increases of 10-38%, belowground biomass increases up to 44%, and total biomass increases of 9-44% (Wand et al. 1999, Reich et al. 2001, Ainsworth and Long 2005, Wang et al. 2008).

Much less research has been conducted to look at C_4 species, which occur less often than C_3 species, and the mixed results to date render the understanding of C_4 plant responses to CO_2 unclear. Temperate C_4 grasses are important components of North American prairies (Wand et al. 1999). Included in my experiments were grass species representing both the C_3 and C_4 photosynthetic pathways.

The enhancing effects of CO_2 observed in C_3 plants are not so marked in many C_4 species. This likely reflects the differences between enzymes which help produce the three- and four-carbon products of the initial steps of C_3 and C_4 photosynthesis, respectively. The enzyme ribulose bisphosphate (RuBP) carboxylase in C_3 photosynthesis has a low affinity for CO_2, but as atmospheric CO_2 increases the plants assimilate carbon more efficiently. In C_4 photosynthesis, the enzyme phosphoenol pyruvate (PEP) carboxylase has a higher affinity for CO_2, resulting in higher concentrations of CO_2 within bundle sheath cells than gained from atmospheric diffusion. The Calvin-Benson cycle thus operates more efficiently in C_4 plants than in C_3 plants at ambient CO_2. The C_4 plants devote less leaf tissue to photosynthesis, though, and thus have lower potential photosynthetic rates. As atmospheric CO_2 increases, the C_4 plants become saturated with CO_2 at a lower concentration than the C_3 plants, causing the growth benefits of increasing CO_2 to cease in C_4 plants but continue in C_3 plants. Aboveground biomass in C_4 plants has been found to be unresponsive to CO_2 enrichment while total biomass of C_4 grasses was even reduced at elevated CO_2 (Reich et al. 2001, Ainsworth and Long 2005). While these results suggest major differences among C_3 and C_4 species, contradicting claims have suggested that these differences are not as substantial as generally perceived (Poorter 1993, Wand et al. 1999). Because results have been inconsistent in the past, C_3 and C_4 species were grown side-by-side in my experiments so that the results might be used to better inform the current debate.

1.2 Plant Responses to Salinity Stress

Like other species, grasses are subject to salinity stresses, which occur when salt in the soil accumulates for a variety of reasons. Despite competition for water resources

and common saline conditions of the soil and water, the amount of turfgrass use in arid or semiarid regions continues to increase. In some cases, source turfgrass facilities are even developed near sources of salt water. Salts from fertilizers or from deicing sidewalks, highways, or airport runways can also reach and stress grasses (Wu and Lin 1994, Zhang et al. 2012).

Some plants, described as halophytic, experience maximal growth under slightly saline conditions. Dry biomass increases in halophytic estuarine species have been observed at over 100 mM NaCl, yet the photosynthetic differences between salt-tolerant and salt-sensitive species remain uncertain (Maricle et al. 2007, Mateos-Naranjo et al. 2010). The growth of non-halophytic species has been shown to decrease with an increase in salinity (Ball and Munns 1992).

Similar to the effects of CO_2, the effects of salinity are less understood among C_4 plants than among C_3 plants (Maricle et al. 2007). The C_4 species *B. dactyloides* has specifically been described as a moderately salt sensitive species, with significant variation among clones within particular populations. It is native to the Great Plains (Wu and Lin 1994, Zhang et al. 2012), often naturally occurring in arid conditions, where drought stresses are sometimes associated with salinity stress (Wu and Lin 1994). Though it has shown moderate salt tolerance during growth and maturity, *B. dactyloides* has shown salt sensitivity during germination (Zhang et al. 2012). Thus, while the reactions of all plants to salt stress will be useful, the responses of the C_4 *B. dactyloides* should be especially relevant.

1.3 Plant Responses to Interactive Effects of Carbon Dioxide and Salinity

The importance of studying plant growth responses to the interactive effects of elevated CO_2 and salinity has been expressed in peer-reviewed literature (Bray and Reid 2002). The effects of elevated CO_2 on plants are often considered opposite to those of salt stress (Geissler et al. 2009b). Because of the opposite impacts, it is reasonable to expect that negative effects of increasing salinity might be mitigated by elevated CO_2. Current data both supports and refutes this expectation. However, too few observations exist to lend to a strong conclusion regarding the impact of CO_2 and salinity interaction on plant photosynthesis and growth (Poorter and Perez-Soba 2001). This experiment was designed to observe and explain interactive effects between these two factors.

In both C_3 and C_4 species, elevated atmospheric CO_2 can help alleviate some of the negative effects of high salinity. When grown in the presence of too much salt – any saline condition for non-halophytes or excessive salinity for halophytes – the stimulation of growth at elevated CO_2 was greater than when optimal salt conditions were maintained. Under moderate salt stress, enhancement of growth by CO_2 was the greatest. When stressed, the potential for growth to be compensated at elevated CO_2 may be greater than when not stressed, and plants therefore cannot be assumed to increase their ranges of salt tolerance as atmospheric CO_2 concentrations increase (Ball and Munns 1992).

Compared to plants controlled for salt, salt-stressed plants had much lower whole-plant biomass. However, in some species, salt tolerance has been seen to increase when grown in elevated CO_2 conditions, resulting in more plant growth at elevated CO_2 (Ball and Munns 1992, Nicolas et al. 1993, Garcia-Sanchez and Syvertsen 2006, Takagi et al. 2009, Perez-Lopez et al. 2010).

Despite the extensive lines of research focused on plant responses to CO_2 or salinity, there is still a lot more to be understood. This is especially true in the case of potential interactive effects of these two factors on plant biomass (Bray and Reid 2002). Therefore, I conducted experiments which fixed various levels of CO_2 and salinity simultaneously. With this design, I was able to describe the effects of each factor as well as interactive effects which are not yet well-documented. The grasses were given time to grow and mature, the aboveground and belowground biomasses were determined at harvesting. A subsequent statistical analysis of variance was performed to help describe any significant effects on biomass of elevated CO_2, salinity or any interaction between the two.

The objective of these experiments was to examine the growth responses of three common grass species to multiple CO_2 and salinity levels. My study was performed in order to improve the understanding of how plants will respond to environmental stress in the context of rising CO_2 in the atmosphere.

CHAPTER 2. MATERIALS AND METHODS

The three species chosen for study were grasses commonly used in lawns in the Midwestern United States. Kentucky bluegrass (*Poa pratensis* L., cv. Midnight, Hancock Farm and Seed Co, Inc., Dade City, Florida, USA) is recommended as the most popular and appropriate species for lawns in Indiana and other Midwestern states. Red fescue (*Festuca rubra* L., cv. Boreal, Athens Seed Co, Watkinsville, Georgia, USA) is noted for its shade tolerance and is another popular lawn grass species, most often packaged as part of a seed mix. Buffalo grass (*Buchloe dactyloides* (Nutt.) Engelm., cv. Bowie, Everwilde Farms Inc., Sand Creek, Wisconsin, USA) is native to the Plains states in the United States and considered a co-dominant species with blue grama (*Bouteloua gracilis* (Willd. Ex Kunth) Lag. Ex Griffiths) in the shortgrass prairie. Increasingly popular and marketed as a lawn grass, the short stature of *B. dactyloides* and its tendency to "fall over" gives an attractive lawn appearance without the need to mow more than once or twice a season. The C_3 species *P. pratensis* and *F. rubra* are cool season grasses, with longer growing seasons than the C_4 warm season *B. dactyloides*. Though *B. dactyloides* can thrive with less water than the other two species, it becomes active later in the spring as temperatures warm and goes dormant earlier in the fall, unable to endure frost conditions.

To administer distinct and constant CO_2 levels, two environmentally controlled growth chambers (Model E-15 Conviron growth chambers, Winnipeg, Manitoba,

Canada) were used. A concentration of 400 μmol mol^{-1} CO_2 was set for the ambient chamber, while a concentration of 760 μmol mol^{-1} was set for the elevated chamber. In all experiments, both chambers were set to the same 10-hour light period, 60% humidity, and 25/15 °C day/night temperatures.

2.1 Germination Experiment

A germination experiment was conducted to compare the performance of seeds to advertised rates, which were 85% for *P. pratensis*, 80% for *F. rubra*, and 65% for *B. dactyloides*. This was also an opportunity to better understand the effects of elevated CO_2 and salt exposure on the plants at an earlier stage. While the emergences observed in growth experiments hint at the success of seeds under stress, true germination rates could not be derived from the emergence parameters.

Table 1. Species, CO_2 levels, and salinity levels in the Germination Experiment.

Grass Species	CO_2 Levels (μmol mol^{-1})	Salinity Levels (mM)	
		NaCl	$CaCl_2$
Poa pratensis	400 (ambient)	0	0
Festuca rubra	760 (elevated)	25	0.25
Buchloe dactyloides		50	0.50
		75	0.75
		100	1.00
		250	2.50
3 Species x 2 CO_2 Levels x 6 Salinity Levels x 50 Seeds x 2 Replicates = 3600 Seeds			

For a total of 3600 seeds (Table 1), germination rates were determined on daily and overall bases. In an approximate 2x50 design, 102 seeds of each treatment (species x

CO_2 x salt) were tested. Germination was defined as the observable emergence of the radicle from the seed. The subsequent emergence of green plant matter from each seed was also recorded.

Seeds were placed on paper towels centered in 23 cm clear, round plant saucers (Bond Manufacturing, Antioch, California, USA). A total of 51 seeds of a species were placed on a Scott® paper towel and then covered with another towel. The towels were wetted with solutions of the prescribed salinities and covered with 13.5 cm x 13.5 cm weighing boats (Sigma-Aldrich, St. Louis, Missouri, USA) placed upside-down within the saucer to help contain moisture in the paper towels.

All seeds were checked daily for the germination parameters. 20-40 mL of the prescribed saline solution was added daily to each saucer. The amount of solution added depended on the observed moisture level of the paper towels, but the same volume of solution was added to each replicate on a given day. Every three days, the CO_2 level of each growth chamber was reassigned and the contents were moved to the appropriate chamber. On these days, the towels in each replicate were replaced before the solutions were added.

The germination experiment was run for 30 days. Statistical analysis was conducted using SPSS (IBM, Armonk, New York, USA) to determine whether CO_2, salinity, day of recording or the interaction of these factors affected germination parameters of the three test species. Tests were performed at 5% significance, so p-values less than 0.05 as determined by ANOVA indicated significant effects.

2.2 Growth Experiment 1

Plants were grown for 47 days in separate Conviron growth chambers set 400 or 700 μmol mol^{-1} CO_2 levels representing ambient and elevated conditions, respectively. The salt solution contained a 100:1 ratio of NaCl to $CaCl_2$, by molarity. This ratio is similar to that used in winter road applications in municipalities of this region, including the city of Indianapolis. Excess road salt is one potential source of salinity experienced by roadside and residential grasses. The addition of $CaCl_2$ was minor enough that the results remain well-suited to a comparison with experiments which use NaCl exclusively.

Seeds of a single species were sown evenly at a recommended density of 14.6 kg per 1000 m^2 over the 0.792 m^2 planting surfaces (0.159 g per pot) of individual, square Dillen plastic pots (approximately 600 cm^3 each). Separate pots, each planted with one of these three species, were distributed evenly between the two growth chambers and four salinity treatments (0, 50, 100, and 250 mM). A total of 144 pots were planted, and each treatment (species x CO_2 level x salinity level) had 6 replicates as shown in Table 2.

Table 2. Species, CO2 levels, and salinity levels in Growth Experiment 1.

Grass Species	CO_2 Levels (μmol mol^{-1})	Salinity Levels (mM)	
		NaCl	$CaCl_2$
Poa pratensis	400 (ambient)	0	0
Festuca rubra	760 (elevated)	50	0.50
Buchloe dactyloides		100	1.00
		250	2.50
3 Species x 4 Salinity Levels x 2 CO_2 Levels x 6 Replicates = 144 Total Pots			

Each pot contained only one species, while clear, round saucers from larger pots each held 3-4 of these small pots. Each saucer had only one particular salt treatment to ensure that pots would not inadvertently receive the wrong solution from below in the case of a temporary excess of water collecting in the saucer. The close proximity of adjacent pots located in each saucer reflected the dense planting of grass seed in a lawn, and pots were randomly reordered within their respective trays to account for advantages in the competition for light experienced by pots along the outside edge of a given tray. The contents of each Conviron growth chamber were exchanged weekly to eliminate any effect of the chambers, with the settings on the chambers adjusted to target the CO_2 levels prescribed for the plants they contained.

Stock saline solution was made as needed, typically every 2 applications. The stock solution was 250 mM NaCl and 2.5mM $CaCl_2$ in deionized (DI) water, and this solution was diluted with additional DI water as necessary to achieve the 50 mM and 100 mM NaCl concentrations. The 0 mM NaCl control treatment was DI water. On a given day, the volume of saline solution added ranged from 0-50 mL per pot, based on the observed need as determined by the appearance of soil and its dryness to the touch. Solution was added based upon the needs of the driest pots in an effort to prevent introducing any prolonged drought stress to the experiment. Regardless of treatment, however, every pot received an equal volume of solution on a given day.

Emergence data was collected in terms of days after planting (DAP) for the first seedling emergence for each pot. A harvest of all biomass was performed at 47 DAP, when many plants had grown and subsequently shown signs of salt stress or died. Biomass collected from the aboveground and belowground portions in each pot were

separated. After drying for 48 hours in a 70 °C oven, the aboveground and belowground dry biomass from each pot was determined. Statistical analysis was conducted using SPSS as in the Germination Experiment.

2.3 Growth Experiment 2

The need for a new experiment with more salt treatments over a narrower range was identified at the end of Growth Experiment 1. A second trial was performed using the same size pots and seeding densities, as well as the same ambient and elevated CO_2 levels of 400 and 760 μmol mol^{-1}, respectively. The 100:1 NaCl:$CaCl_2$ molar ratio was again used when preparing the saline solutions. The number of salt treatments was increased to five, and the range of salt treatments was narrowed to 0 to 100 mM NaCl, with intermediate treatments of 25, 50, and 75 mM NaCl. A total of 150 pots were planted, and each treatment had 5 replicates. Grass species, CO_2 and salinity level were again the fixed factors (Table 3). The planting, chamber, and watering procedures were repeated from Growth Experiment 1.

Table 3. Species, CO_2 levels, and salinity levels in Growth Experiment 2.

Grass Species	CO_2 Levels (μmol mol^{-1})	Salinity Levels (mM)	
		NaCl	$CaCl_2$
Poa pratensis	400 (ambient)	0	0
Festuca rubra	760 (elevated)	25	0.25
Buchloe dactyloides		50	0.50
		75	0.75
		100	1.00
3 Species x 2 CO_2 Levels x 5 Salinity Levels x 5 Replicates = 150 Total Pots			

Emergence parameters were extended in the second experiment. First emergences, recorded in days after planting, were recorded for each pot in all three species. Due to rapid succession of emergences following the first, the time to 20 *P. pratensis* emergences in each pot was recorded as well. For *F. rubra*, days to first, third, fifth, and tenth emergences were recorded. Because *B. dactyloides* emergences tend to occur in clusters of 1 to 3 shoots from the same bur, an emerging cluster was recorded as a single emergence.

In order to further quantify emergences and plant deaths, plant number was counted starting at 30 DAP. The number of living plants per pot were recorded a total of 12 days during the growth period. A plant was considered to be living as long as it had at least partial green coloration. In pots of *P. pratensis*, which typically had more numerous and dense emergences, the exact number of plants was recorded only for pots containing 20 or fewer distinct shoots. All pots of *P. pratensis* contained 20 or more plants for some or all of the growing period, and these instances were noted to be greater than 20 plants but not specifically quantified due to the dense growth. All *F. rubra* plants were quantified. While one emergence per seed was observed for all species, the seeds of *B. dactyloides* were housed together in burs. For typical burs, clusters of 1-3 emergences were observed. Counts of *B. dactyloides* reflected the number of planting units (burs) from which emergences were observed. Therefore, the total number of distinct, emerging clusters was counted each time rather than the total number of shoots.

Because the plant numbers were counted to provide snapshots of the rate of establishment and decline of the plants, the growth period was extended from Growth Experiment 1. At 100 DAP, biomass collection, drying, and weighing procedures were

repeated from Growth Experiment 1. As before, all biomass measures were taken when the plant matter was completely dried. Statistical analysis was conducted using SPSS as in previous experiments.

CHAPTER 3. RESULTS

3.1 Germination

Overall, the first radicle emergences to occur were at Day 6 in *P. pratensis* (Figure 1), and the rate of emergence in all species had slowed by Day 30. Although fewer in number than radicle emergences, green emergences from seeds followed a similar pattern (Figure 2), but occurred at a lower rate. Measures of both parameters increased steadily to 15 or 20 days in *P. pratensis* exposed to 0, 25, or 50 mM NaCl before the percentages leveled. At higher salinity levels, radicle and green emergence rates were much lower overall. At each salinity level, *P. pratensis* had higher percentages of radicle and green emergences (Table 4) than the other species. The exception was at 250 mM NaCl, where no seeds germinated in any of the species. *P. pratensis* was significantly affected by CO_2 level with respect to green emergences, but radicle emergences were not affected by CO_2 (Tables 4 and 5). For both parameters, salinity levels and the number of days after planting were significant factors, while the interactions of salinity with CO_2 and with days after planting were also significant. Significantly different effects occurred between each successively higher salinity level from 0 to 250 mM NaCl.

In *F. rubra*, the rates of radicle and green emergences never exceeded 6.9% overall (Table 4). While the no salt controls had the most radicle emergences at ambient

and elevated CO_2 within this species, the relationship between salinity and radicle emergences cannot be clearly defined. A great deal of overlap was apparent (Figure 1) between rates of radicle emergence in seeds at different salinity levels. The same was true in the resulting green emergences (Figure 2), which varied from one salinity level to the next but did not occur for the ambient 75 mM NaCl group or for either CO_2 level at 100 mM NaCl (Table 4). Green emergences (Table 6) in *F. rubra* seeds were significantly affected by CO_2 and by the interaction of salinity and days after planting, while radicle emergences (Table 5) were not. In both parameters, salinity, days after planting, and the interaction of these two factors were all significant. Differences between salt levels (Table 7) were all significant, except between 25 and 50 mM NaCl and between 100 and 250 mM NaCl.

Some variation characterized the green and radicle emergences among salt levels in *B. dactyloides* as well (Table 4). The ambient no salt control group clearly exceeded the other groups in both radicle (Figure 1) and green emergences (Figure 2). Both radicle (Table 5) and green emergences (Table 6) were significantly affected by CO_2, salinity, days after planting, and the interaction of CO_2 with salinity. As in *F. rubra*, the differences between salt levels (Table 7) were all significant in *B. dactyloides*, except between 25 and 50 mM NaCl and between 100 and 250 mM NaCl.

Because green emergences were only observed in seeds which had previous or concurrent radicle emergences, the ratio of green to radicle emergences (Table 4) was calculated. In *P. pratensis*, these ratios generally decreased as salinity levels increased, with the only variation occurring at 25 mM NaCl. These ratios were variable among salinity levels in *F. rubra*, but when green emergences occurred they were little more

than half as frequent as radicle emergences, except at 50 mM NaCl where 80% of radicle emergences were followed by green emergences. Finally, radicle emergences in *B. dactyloides* were almost always followed by green emergences. The ratio of green to radicle emergences was 0.95 for the ambient 0 mM NaCl group, while all other radicle emergences were followed by green emergences. If radicle emergences occurred, green emergences often followed, but the overall germination rates in both *F. rubra* and *B. dactyloides* were low.

3.2 Plant Emergences

At both ambient and elevated CO_2, a strong positive correlation existed between the salinity treatment and the days to first emergence and days to twentieth emergence in *P. pratensis* (Figure 3; Table 8). Not every replicate is accounted for in Figure 3. While first (Tables 9 and 10) and twentieth emergences (Table 14) occurred in all pots of *P. pratensis*, results varied in the other species. Typically, as salt levels increased, the number of pots with first (Tables 9 and 10), third (Table 11), fifth (Table 12), and tenth (Table 13) emergences of *F. rubra* and *B. dactyloides* decreased.

The first, third, fifth, and tenth emergences of *F. rubra* varied in their occurrences relative to salinity. While the upward slopes of the dotted red lines suggest a correlation between salinity and days to emergences at ambient CO_2 (Figure 3), the coefficients of determination (Table 8) indicate that there is not a strong linear relationship between these variables for the first or third emergences. The fifth and tenth emergences at ambient CO_2, however, do have strong linear responses to salinity. At elevated CO_2, strong linear relationships were observed as first, third, fifth, and tenth emergences occurred later, on average, as salinity increased.

Although each emerging cluster of *B. dactyloides* was recorded, only the average days after planting to first, third, fifth, and tenth cluster emergences are plotted (Figure 3). This allowed for a clearer comparison to the other species while still providing an accurate representation of the inconsistent emergence responses to salinity. Though the ambient and elevated lines for the first emergences suggest that *B. dactyloides* took less time to emerge as salinity increased, the coefficients of determination (Table 8) suggest there is little to no true linear relationship. Where the lines for third emergences are more indicative of the expected delaying of emergences at high salinity, their coefficients of determination similarly suggest no linear relationship. Because fifth and tenth emergences of *B. dactyloides* at ambient and elevated CO_2 only occurred at 0 mM NaCl, there were not sufficient treatment averages for regression lines to be fitted.

3.3 Plant Number

In Growth Experiment 2, counts of the number of plants in each pot were performed regularly. The numbers of plants recorded for each pot were averaged, so that each point plotted (Table 15; Figure 4) represents the average of five replicate pots per treatment.

Near the halfway point of the 100-day growing period, observable differences in plant density existed between CO_2 treatments and among salt levels for *P. pratensis* (Figure 4). For both ambient and elevated CO_2, treatment average plants per pot at 0, 25, and 50 mM NaCl treatments exceeded 20 plants per pot for the duration of the growing period. However, quantifiable decreases in the average number of plants per pot occurred for both ambient and elevated CO_2 for 75 and 100 mM NaCl treatments.

Effects of CO_2, salinity, and their interaction upon plant numbers of *F. rubra* and *B dactyloides* were significant (Table 16). A fairly steady trend characterized the plant numbers of *F. rubra* (Figure 4), with no significant differences within treatments calculated between the first and last recordings at 30 and 100 days after planting, respectively. Across the range of salt levels, greater numbers of plants grew and survived at lower salinities. The no salt control groups had the most plants, followed by the 25 and 50 mM NaCl treatments, respectively. At the 75 and 100 mM NaCl treatments, averages did not exceed 2 plants per pot for the entirety of the experiment. Differences between salt levels were statistically significant in every case, except between 75 and 100 mM NaCl (Table 17).

Only in *B. dactyloides* did a marked increase in plant number occur beyond 30 days after planting (Figure 4). This upward trend occurred in the ambient and elevated CO_2 groups, with averages increasing by 3.6 and 5.4 clusters per pot before harvesting, respectively. At both ambient and elevated CO_2, the 25, 50, 75, and 100 mM NaCl groups did not exceed an average of 2 clusters per pot for the entirety of the growing period. The no salt control groups were significantly different than all other salt levels (Table 15). All other differences among salt levels were statistically significant except between 25 and 50 mM NaCl and between 75 and 100 mM NaCl.

3.4 Dry Biomass

For the salinity levels repeated between Growth Experiments 1 and 2, *P. pratensis* had less dry biomass when grown for 47 days (Figure 5) than when grown for 100 days (Figure 6). For plants harvested at 47 DAP and for plants harvested at 100 DAP, elevated aboveground (Table 18) and belowground (Table 19) dry biomasses exceeded ambient at

each salinity level, and biomass decreased at each increasing salinity level. Regardless of harvest time, salinity significantly affected aboveground and belowground biomass. The interaction of CO_2 and salinity significantly affected aboveground biomass for both harvests, but only significantly affected belowground biomass in the 47-day harvest. The same was true with respect to ambient aboveground biomass, but at elevated CO_2 the 25 mM salt group actually exceeded the 0 mM NaCl group. This difference, however, was not significant (Table 20). All other salt levels, except 75 and 100 mM NaCl, were significantly different with respect to aboveground and belowground biomasses.

Similar trends were apparent in *F. rubra* grown for 47 days (Figure 7) and 100 days (Figure 8). Again, the mean dry biomasses within salt levels were greater when grown at elevated versus ambient CO_2 and when harvested at 100 DAP versus 47 DAP. CO_2, salinity, and the interaction of these two factors significantly affected the aboveground dry biomass (Table 18) of *F. rubra* in both growth experiments. Belowground dry biomass (Table 19) was only significantly affected by salinity when harvested at 47 DAP, but both factors and their interactive effects were significant in *F. rubra* grown for 100 days. Aboveground biomass differed significantly among all salinities (Table 20) except for the highest levels, 75 and 100 mM NaCl. Belowground biomass was significantly affected at most levels, with higher levels again being the exception. Differences among 50, 75, and 100 mM NaCl groups were not significant.

The dry biomass for *B. dactyloides* harvested at 47 DAP (Figure 9) was less than for that harvested at 100 DAP (Figure 10). The effects of CO_2 were not significant on either aboveground (Table 18) or belowground (Table 19) dry biomasses at 47 DAP, and large error bars accompany the exceptional relative abundance of ambient biomass at the

0 mM salinity level. Biomass was significantly affected by CO_2 in *B. dactyloides* when harvested at 100 DAP, with greater mean biomasses at elevated CO_2. Salinity affected both biomass parameters regardless of harvest date. The differences, as evident from the relatively large biomass bars at 0 mM NaCl, were significant between 0 mM NaCl and all other salt levels (Table 20). None of the salt levels from 25 to 100 mM differed from one another at 100 DAP, however.

CHAPTER 4. DISCUSSION

4.1 Germination

While elevated CO_2 conditions did result in different radicle emergence rates in *B. dactyloides* and different green emergence rates in all species, these differences did not occur in any clear pattern. As evident from Figures 1 and 2, the radicle emergences and subsequent green emergences were similar at ambient and elevated CO_2 levels. Thus, despite some indication that elevated CO_2 affects germination, the results did not conclusively show any clear connection.

On the other hand, the adverse effects of salinity on radicle and green emergences generally resulted in a reduction of germination with increasing salinity. The exceptions to the rule of significant salinity effects were the same in *F. rubra* and *B. dactyloides*. Moderate salt stresses of 25 to 50 mM NaCl did not differ, but these levels were less inhibitive than the 100 mM NaCl treatments. Radicle and green emergences were so inhibited at 100 mM NaCl that the rates did not differ statistically from the 250 mM NaCl group, in which no germination occurred. The stress level induced by 100 mM NaCl suggests that 250 mM could far exceed the actual salinity level at which germination can still occur. Further, 1-2% seedling establishments have been reported in *B. dactyloides* grown at 100 mM NaCl (Wu and Lin 1994). At 100 mM NaCl, the observed 1% overall rates of green emergences at ambient and elevated CO_2 suggest that reasonable estimates

of the eventual rates of seedling establishment may be reflected at germination. While the germination parameters of individual seeds of *B. dactyloides* grown in all conditions were recorded, up to three radicle and three green emergences were observed in particular burs.

Neither germination parameter was consistently higher at one CO_2 level than the other, though, so while the inhibitive tendency of salinity is clear its interaction with CO_2 remains difficult to describe outside of specific comparisons. Similar to the independent effects of elevated CO_2, the interaction of elevated CO_2 and salinity did impact all species, but inconsistently and incompletely. While, within certain salinity levels, elevated conditions did increase the rates of radicle and green emergences, ambient germination rates were actually higher in even more cases. Therefore, germination of these three grass species cannot be said to respond to the interactive effects of CO_2 and salinity in any discernible pattern.

Grass species that are relatively tolerant to salinity during growth are not always salt tolerant during germination, and *B. dactyloides* notably fits into this category (Marcar 1987, Zhang et al. 2012). An important consideration for my results, then, is the fact that all growth parameters could be affected by the performance of each species at germination.

4.2 Plant Emergences

When seeds were planted in soil for the Growth Experiments, the first indication of a viable seed was a plant emergence and not necessarily any direct germination. Every pot of *P. pratensis* sown in Growth Experiment 2 had 20 or more emergences by 14 DAP. The trend, with increased salinity, was for both first and twentieth emergences to occur later on average at both CO_2 levels. In all cases these were strong, linear relationships.

Plant emergences occurred relatively rapidly in *P. pratensis*, with the average time between first and twentieth emergences ranging from 1 to 3 days in each treatment. From these results, it was clear that plant emergences were increasingly delayed by each successive salinity level. Given its apparent relative salt tolerance and sowing density, *P. pratensis* emergences occurred and became too numerous to count very quickly. The twentieth emergences of *P. pratensis* occurred within days of the first emergences, and on average these twentieth emergences occurred earlier than first emergences in the other two species.

Both speed and number of plant emergences were reduced in *F. rubra* and *B. dactyloides*, relative to *P. pratensis*. In *F. rubra,* the first emergences were later with each successive salt level from 0 to 75 mM NaCl. The trend did not hold for 100 mM NaCl, however. At ambient CO_2, the first emergences occurred earlier at 100 mM than 75 mM NaCl. At elevated CO_2, the first emergences occurred later at 100 mM than 75 mM NaCl. In each of the 75 and 100 mM NaCl levels, however, emergences were observed in only 3 or 4 (out of 5) pots. The average time to first emergence in the 100 mM NaCl group of *F. rubra* grown at elevated CO_2, which was the longest of all among that species, was driven up by the occurrence of a first emergence in one pot at 34 DAP. This was by far the latest initial emergence, coming at least 17 days later than all others in *F. rubra*. Subsequent emergences did not occur for every salinity treatment, but where emergence benchmarks were realized at multiple salt levels the tendency for increased salinity to lead to later emergences persisted. Third emergences were attained in at least one pot for all treatments except 75 mM NaCl at ambient CO_2 and 100 mM NaCl at elevated CO_2. Regardless of CO_2 level, fifth emergences were only observed in some pots grown at 0,

25, or 50 mM NaCl. Tenth emergences were uncommon, but when they occurred they were restricted to 0 or 25 mM NaCl levels at either ambient or elevated CO_2. Ambient and elevated R^2 values indicate linear relationships for fifth and tenth emergences. However, the fact that these lines are fit to just two or three points makes their equations less reliably predictive than the linear relationships for first and third emergences at elevated CO_2, which were fit to five points each.

The results for *B. dactyloides* were less consistent with respect to emergence times. Trends were not evident for this species, due in part to the low rate of seedling emergence. Of the 50 pots planted with *B. dactyloides*, 16 had no emergences for the duration of the growth period. Failures to emerge occurred evenly between CO_2 levels, 8 pots each at ambient and elevated CO_2, but they were particularly prevalent at the 75 and 100 mM NaCl levels. A third emergence only occurred in one pot (75 mM NaCl at ambient CO_2) of twenty grown at these highest salt levels, while they occurred at least once in every treatment group between 0 and 50 mM NaCl. Fifth and tenth emergences occurred only in some pots watered with DI water (0 mM NaCl), but the very few data points contributing to these plots in Figure 3 can be confusing to read. Tenth emergences occurred in only one pot in each CO_2 treatment at 0 mM NaCl, with the one ambient replicate (97 DAP) reaching its tenth emergence much later than the one elevated replicate (30 DAP). Overall, most *B. dactyloides* emergences seemed delayed relative to the other species, in part because the temperatures would likely favor the cool-season species. Thus, while the filled black diamond represents just one replicate of *B. dactyloides* at 100 mM NaCl and elevated CO_2, the filled black star (with higher average days to emergence) actually represents a full set of 5 replicates at 75 mM NaCl. An

important distinction to recall when viewing *B. dactyloides* plant emergence data is the fact that it reflects cluster emergences, which are more closely tied to the number of burs than the number of seeds. As observed in the germination study, a given bur had up to three radicle and three green emergences, and similarly each cluster of plant emergences included one to three seedlings.

No effect was significant for all species, but plant emergences of each species were affected by at least one factor. The only significant CO_2 effects were in *B. dactyloides*, the C_4 species, with elevated emergences occurring earlier on average than ambient emergences ($P < 0.001$). This result resembled those of the radicle emergences in the Germination Experiment, where CO_2 affected only *B. dactyloides* radicle emergences before significantly affecting the green emergences of all species. CO_2 did interact with salinity to affect *F. rubra* ($P = 0.045$), though, with the specific difference occurring at 0 mM NaCl. At this level, elevated emergences (25.9 DAP) appeared an average of 20 days earlier than ambient emergences (45.5 DAP). Overall, increasing salinity delayed plant emergence times in both C_3 species, *P. pratensis* ($P < 0.001$) and *F. rubra* ($P < 0.001$). The differences among salt treatments for these species may have actually been indicative of the significant salt effects as seen in the Germination Experiment, in which radicle and green emergences were also delayed with increasing salinity in the C_3 species.

Seedling emergences may provide more useful information about the effects of increasing CO_2 and salinity on some species more than others. Species like *P. pratensis*, which is typically sown and then emerges in a rapid, dense pattern, may not be well-suited for demonstrating differential rates of plant emergences beyond a certain limit. In my design, plant emergences greater than twenty were difficult to quantify. Similar

results and timing could be expected from green emergences from seed, and these could prove considerably easier to manage and quantify. Species which have lower sowing densities or germination rates may be very well-suited to measures days to plant emergences, however. This was true in my experiment, where emergences were reasonably recorded more frequently in *F. rubra* and *B. dactyloides*. Overall, days to plant emergences – even just first emergences – could be useful observations for experiments relating germination to growth but which begin treatment prior to germination of seeds planted in soil.

4.3 Plant Number

Near the halfway point of the 100-day growing period, observable differences in plant density of *P. pratensis* existed between CO_2 treatments and among salt levels. The plant numbers at 0, 25, and 50 mM NaCl remained greater than 20 plants per pot for the duration of the growing period. The higher salt levels demonstrated quantifiable declines in plant number. The first treatment to fall below 20 plants per pot was the 100 mM NaCl group at ambient CO_2, followed by the 100 mM elevated, 75 mM elevated, and 75 mM ambient treatment groups. While the combination of categorical and numerical data could not be statistically analyzed, these instances of decline to less than 20 living plants per pot do not appear to follow a pattern relative to the CO_2 treatments. The declines of the 100 mM NaCl groups occurred earlier than those of the 75 mM NaCl groups, though, and the pots in which plants were not quantified were nonetheless observed to have lower apparent plant densities as salt levels increased.

A fairly steady trend characterized the plant numbers of *F. rubra*, with no substantial differences calculated between the first and last recordings at 30 and 100 days

after planting, respectively. Generally speaking, the plots are arranged according to salt level, with lower salinities allowing for more plants to grow. The salt control groups had more plants than the salt-exposed groups. Plants at the 25 and 50 mM NaCl treatments appear to show moderate salt stress, with plots through the center part of Figure 4. The 75 and 100 mM NaCl treatments appeared to have substantial inhibitory effects, with average plant counts never exceeding 2 plants per pot for the entirety of the experiment.

Adding any salt to *B. dactyloides* appears to majorly limit the number of plants that can grow. None of the salt-exposed treatments yielded an average greater than 2 plants per pot any time the plant counts were conducted. For ambient and elevated 0 mM NaCl treatments, the average number of plants per pot increased during the growing period to exceed 7 and 10 plants per pot, respectively. The increase experienced by this no salt control group reflects the general tendency for *B. dactyloides* to proceed through germination and growth slowly, while the C_3 cool season species in this experiment experienced no sustained, quantifiable increase in plant numbers after 30 days. The temperature settings could have also played a role in this result, as the temperature range may have favored the quick establishment of the C_3 species while further delaying the C_4 *B. dactyloides*. Interestingly, the moderate salt stresses induced at 25 and 50 mM NaCl were, like in the germination parameters, not significantly different in terms of plant number. Aside from these as well as the most inhibited 75 and 100 mM groups, all salt levels differed from one another. One possible connection may be partially explained by the calculated ratios of green to radicle emergences for *B. dactyloides*. As each plant counted could tie back to a particular seed or bur, the relationship between germination rate and later plant numbers seems obvious. The connection may be even stronger in *B.*

dactyloides, though, as radicle emergence almost always resulted in green emergence. These green emergences from seeds would be counted as subsequent plant emergences and then as individual plants. With such a similar distribution of between-level differences, it may be that the differences as observed by plant numbers were really settled at the germination stage.

The interaction of CO_2 and salinity impacted plant number in *F. rubra* and *B. dactyloides*, but these effects did not occur consistently. In *F. rubra*, the only significant difference between ambient and elevated CO_2 treatments was at the 50 mM NaCl level, where plant numbers at elevated CO_2 were higher on average than those at ambient CO_2, as evident from Figure 4. This interaction only affected *B. dactyloides* at the 0 mM NaCl level, where the elevated plant numbers were also higher than ambient plant numbers. Despite the overall interactive effects indicated by statistical analysis, the fact that this did not occur consistently makes it difficult to predict any future trends or results.

Plant numbers, like plant emergences, appear to be useful parameters for demonstrating elevated CO_2 and salinity effects. In my experiment, the growth density of *P. pratensis* could not be reasonably quantified until plant numbers averaged less than 20 plants per pot on the declines at high salinity. Many species, however, could likely be quantified regularly as I was able to do with *F. rubra* and *B. dactyloides*. My plant counting method was straightforward and produced data that could easily be graphed to show trends. Quantitative plant number data, when statistically analyzed, helped explain the effects of increasing CO_2 and salinity on plants during the many weeks of time between germination and biomass harvest. In my experiment, plant number data provided a clear understanding of the differences between salt levels used in *F. rubra* and *B.*

dactyloides, and the dynamics of the plant count trends can add depth to the interpretation of biomass results. Regardless of other parameters used, plant number seems to be useful for species and protocols which would allow for reasonable quantification of plants on a regular basis during growth.

4.4 Dry Biomass

All cases in which CO_2 had significant effects on dry biomass were those in which elevated CO_2 had a stimulating effect. This is consistent with numerous results in the past, in which increased plant biomass has generally been associated with elevated CO_2 levels, with the magnitude of this enhancement varying by species or functional group (Curtis and Wang 1998, Wand et al. 1999). When grown for 100 days, elevated CO_2 correlated with increased aboveground and belowground dry biomass in all three grass species. However, when grown for 47 days, aboveground biomass increased at elevated CO_2 in the C_3 species, *P. pratensis* and *F. rubra*. In this shorter growing period, belowground biomass was only stimulated by elevated CO_2 in *P. pratensis.* This result suggests that time may be a factor in the nature or detection of CO_2 effects, especially for species like *B. dactyloides* which take longer to germinate, emerge, and grow. The stimulating effects of elevated CO_2 on aboveground and belowground biomass of the Midnight cultivar of *P. pratensis* at both 47 and 100 DAP, however, are consistent with a previous study with harvesting this and other *P. pratensis* cultivars at 70 DAP (Koch et al. 2011). Other species in the genus *Festuca* have similarly responded to elevated CO_2 with increased biomass (Barbehenn et al. 2004). The stimulating effect of elevated CO_2 appeared to develop over time in *B. dactyloides*. While not affected at 47 DAP, at the 100-day harvest the biomass of the C_4 species was greater at elevated CO_2. If time were

to have an effect on other species' responses to elevated CO_2, it might help explain why previous conclusions about C_4 responses are varied. While the 47-day results agree with previous results suggesting no effects of elevated CO_2 upon *B. dactyloides* biomass, the 100-day results provide evidence consistent with the suggestion that many C_4 species are stimulated by elevated CO_2 similar to, if less than, the vast majority of C_3 species (Poorter 1993, Barbehenn et al. 2004). For instance, aboveground and total biomass increases in C_4 graminoids grown at elevated CO_2 have been reported, and the stimulation of *B. dactyloides* biomass is consistent with that finding (Wang et al. 2008).

Despite the general agreement in the literature that C_3 plants are stimulated more by increasing CO_2 than C_4 plants, the amount by which these functional groups are thought to differ is not consistent (Poorter 1993, Wand et al. 1999, Reich et al. 2001, Ainsworth and Long 2005). The effects of CO_2 on the C_4 species *B. dactyloides* were not evident at the 47-day harvest, but the harvest at 100 DAP yielded stimulatory effects of elevated CO_2 on both aboveground and belowground biomass in all species. As the plant number data suggest, *B. dactyloides* plants were still becoming established in the latter half of the 100-day experiment. Interestingly, when allowed to grow without salt stress for 100 days, *B. dactyloides* had much greater increases in aboveground and belowground dry biomass at elevated CO_2 (161.7% and 159.3%, respectively) than the C_3 species *P. pratensis* (37.0% and 14.8%, respectively) and *F. rubra* (54.6% and 58.4%, respectively). This result does not agree with most previous findings, as the biomass stimulation in my experiment is most pronounced for the C_4 species. Physiological characteristics of C_4 species suggest that *B. dactyloides* should have become saturated with CO_2 at a lower

concentration than the C_3 species, causing the stimulatory effects of elevated CO_2 to be low in *B. dactyloides* relative to the other species studied.

The effects of salinity were very marked, with increasing salinity reducing both the aboveground and belowground dry biomass of all three grass species. This was true in both growth experiments. After 100 days, the effects of high salinity were clearly evident. Compared to the aboveground and belowground biomass for the no salt controls at ambient CO_2, biomass decreases of at least 98.7% and 99.5%, respectively, occurred at 100 mM NaCl in all species, regardless of CO_2 level. The inhibitive effect of salinity on *B. dactyloides*, which grew in relatively few pots watered with saline solutions, appears to contradict a previous study in which the species was observed to have "moderate salt tolerance" during growth and at maturity (Zhang et al. 2012). As previously discussed in the case of plant numbers, though, this apparent contradiction may trace to the watering with saline solution, which began before the fairly salt sensitive germination stage. Thus, only seeds that successfully germinated and established at their respective salinities could be included in the biomass measure.

While the more sensitive germinators may have residual inhibition measured by later parameters, the results of Growth Experiments 1 and 2 are quite meaningful. Naturally occurring *B. dactyloides* is often subject to arid, saline conditions (Wu and Lin 1994). The long-term success of a population of *B. dactyloides*, just like other species, would depend on the survival of more than just mature plants. A sustainable or growing population would require the production and dispersal of viable seeds, and those seeds would in turn need to withstand salt stress to germinate. Seedling emergence and plant growth in the wild would therefore depend upon successful germination. Where all of my

experiments accounted for salt exposure throughout the crucial germination stage, the methods could be modified to delay saline watering until seedlings emerge or reach some set level of growth. Such a modification could give results more directly comparable to studies focused on plant salt tolerance at maturity.

No significant differences between ambient and elevated paired groups occurred at high salinity. The strong interactive effect of CO_2 and salinity on aboveground dry biomass in *P.* pratensis grown for 100 days was specific to only the low to moderate salinity levels. As determined by 95% confidence intervals, elevated 0, 25, and 50 mM NaCl groups had greater aboveground dry biomass than the ambient 0 (37.0%), 25 (64.5%), and 50 (58.6%) mM NaCl groups respectively. In fact, the ambient aboveground dry biomass at 0 mM NaCl (1.90 g) exceeded the elevated mean at 50 mM NaCl (1.87 g) by just 1.6%. Although statistical analysis did not indicate an overall interactive effect upon belowground biomass grown for 100 days, there was one significant difference. The average 0.77 g of root matter harvested from the elevated 25 mM NaCl group was 95.5% greater than the 0.39 g harvested from the average ambient 25 mM NaCl replicate. While confidence intervals were very wide, the mean aboveground biomasses at high salinity and elevated CO_2 did far exceed those at ambient CO_2, with increases of 104.4% and 140.5% from ambient to elevated CO_2 within the 75 and 100 mM NaCl groups in *P. pratensis*, respectively. The interactive effects of CO_2 and salinity were significant in terms of aboveground and belowground dry biomass for both *F. rubra* and *B. dactyloides*, but the effects were most common in conditions free from salt stress. Increases in elevated versus ambient biomass within *F. rubra* treatments were localized to the 0 mM NaCl levels both aboveground (54.5% increase, ambient to

elevated) and belowground (58.4% increase, ambient to elevated) and the 25 mM NaCl level for aboveground (89.1% increase, ambient to elevated) biomass only. As in *P. pratensis*, some notable increases in biomass of *F. rubra* occurred at elevated CO_2 within salt levels, but replicates were too variable to indicate interactive effects. Notable, but not significant, increases in aboveground dry biomass occurred in *F. rubra* at elevated CO_2 within the 25 (89.1%), 50 (90.5%), and 75 (68.3%) mM NaCl levels. The same was true for belowground dry biomass as *F. rubra* grew more, but not significantly so, at elevated CO_2 within the 50 (87.4%), 75 (123.3%), and 100 (154.5%) mM NaCl levels versus ambient growth at those salinities. Interactive effects on *B. dactyloides* occurred strictly at the 0 mM NaCl level, as elevated means exceeded ambient means in terms of aboveground (0.30 versus 0.12 g, a 161.7% increase) and belowground (0.17 versus 0.06 g, a 159.3% increase). The differences between ambient and elevated CO_2 at higher salinity levels hint at potential mitigation of salt stress at these levels, but in this experiment the differences reflect individual cases and too much variability exists to conclude that interactive effects have occurred. Results from this experiment, where the grass species exposed to 75 and 100 mM NaCl salt levels were not affected by elevated CO_2, are consistent with a prior study in which CO_2 and salt interacted positively at low salinity but not at high salinity (Munns et al. 1999).

With the interactive effects occurring in the least salt-stressed conditions, carbon enrichment seems uncertain to mitigate the inhibitive effects of high salinity. While positive interactive effects of CO_2 and salinity often occur, they are not observed in all cases (Poorter and Perez-Soba 2001). Lower salt levels, like the 25 and 50 mM treatments, could indeed experience reduced growth because of salt stress, but some of

those negative effects could be overcome at elevated CO_2. At high salinity, my results appear to agree with previous suggestions that elevated CO_2 would likely have little to no effect when another factor limits plant growth (Ball and Munns 1992). In my experiment, the addition of NaCl at high concentrations produced a level of salinity stress that could not be overcome by elevated CO_2. On the other hand, where plants are not exposed to salinity, biomass increases correlated with elevated CO_2 seem very likely to occur. In all, CO_2 did not interact with salinity in such a way that ambient plants outgrew elevated plants at the same salt level. This suggests that elevated CO_2, while not always beneficial to plants under salt stress, should not be expected to exacerbate this stress as measured by plant biomass.

Some notes about the dry biomass data can be made when considering other parameters as well. The trends in plant number, recorded from days 30 to 100 of growth, suggest how biomass production might have been different at an earlier or later harvest. The decline of *P. pratensis* at high salinity and the increase of *B. dactyloides* in the no salt controls, for example, suggest that the 100-day biomasses were likely lesser or greater, respectively, than if harvesting had occurred earlier. While the literature reflects the popularity of biomass measurements, and with good reason, incorporation of germination and other growth parameters may provide a more complete understanding of the ultimate effects of environmental changes on plant biomass. Though CO_2 did not affect germination rates, it seems very likely that delays in plant emergences, decreases in plant numbers, and decreases in biomass as salinity increased can often be tied back to the germination stage. At salinity levels where radicle and green emergences were delayed or reduced, the parameters focused on plant growth reflected the same relative

inhibition. Taken together, plant emergence rates and regular counts of plant number help describe how plants respond to elevated CO_2, salinity, or their interactive effects over the long growing period between germination and biomass harvest. Altogether, considering germination rates, plant emergence rates, plant number, and biomass gave a more complete picture of how elevated CO_2 stimulates, salinity inhibits, and the two factors occasionally interact to affect the three grass species studied.

CHAPTER 5. SUMMARY

Results from my experiments demonstrated the contrasting nature of CO_2 enrichment and salinity stress in affecting the growth of three common grasses. The effects of CO_2 in stimulating grass growth often became more pronounced over time. The stimulating effect of CO_2 enrichment on germination and growth, however, was consistently modified by salinity level, producing significant differences among successive salinity levels. My results suggest that the growth of common grasses would be significantly greater in CO_2-enriched future environment, although the magnitude of growth stimulation would be affected by high salinity, a common environmental stress for plants in many habitats.

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TABLES

Table 4. Green and radicle emergence rates, as well as proportion of seeds with radicle emergences which subsequently had green emergences (Green ÷ Radicle) in *Poa pratensis*, *Festuca rubra*, and *Buchloe dacytloides* seeds allowed 30 days to germinate.

Species	Salt Level (mM NaCl)	Ambient CO_2			Elevated CO_2		
		Green (%)	Radicle (%)	Green ÷ Radicle	Green (%)	Radicle (%)	Green ÷ Radicle
Poa pratensis	0	89.2	94.1	0.95	79.4	94.1	0.84
	25	73.5	89.2	0.82	84.3	91.2	0.92
	50	71.5	85.3	0.84	67.6	86.3	0.78
	75	52.9	74.5	0.71	49.0	78.4	0.63
	100	9.8	37.3	0.26	12.7	48.0	0.26
	250	0	0	N/A	0	0	N/A
Festuca rubra	0	2.9	4.9	0.59	6.9	6.9	1.00
	25	2.0	3.9	0.51	2.9	4.9	0.59
	50	3.9	4.9	0.80	1.0	2.0	0.50
	75	0	2.9	0	2.0	3.9	0.51
	100	0	1.0	0	0	1.0	0
	250	0	0	N/A	0	0	N/A
Buchloe dactyloides	0	16.7	17.6	0.95	7.8	7.8	1.00
	25	6.9	6.9	1.00	8.8	8.8	1.00
	50	8.8	8.8	1.00	2.0	2.0	1.00
	75	3.9	3.9	1.00	2.0	2.0	1.00
	100	1.0	1.0	1.00	1.0	1.0	1.00
	250	0	0	N/A	0	0	N/A

Table 5. P-values for the effects of CO_2, salinity, days after planting (DAP), and interactions upon radicle emergences of *Poa pratensis, Festuca rubra,* and *Buchloe dactyloides*. Significant effects at 5% significance ($P < 0.05$) are in bold. Aside from the significant interactive effect of salinity x day on *B. dactyloides*, no significant effects of day after planting or interactive effects of day after planting with other factors were found.

Factor	P-values		
	Poa pratensis	*Festuca rubra*	*Buchloe dactyloides*
CO_2	0.552	0.812	**<0.001**
Salinity	**<0.001**	**<0.001**	**<0.001**
DAP	**<0.001**	**<0.001**	**<0.001**
CO_2 x Salinity	**<0.001**	**<0.001**	**<0.001**
CO_2 x DAP	0.155	1.000	0.616
Salinity x DAP	**<0.001**	0.153	0.826
CO_2 x Salinity x DAP	1.000	1.000	1.000

Table 6. P-values for the effects of CO_2, salinity, days after planting (DAP), and interactions upon green emergences of *Poa pratensis, Festuca rubra,* and *Buchloe dactyloides*. Significant effects at 5% significance ($P < 0.05$) are in bold. Aside from the significant interactive effect of salinity x day on *B. dactyloides*, no significant effects of day after planting or interactive effects of day after planting with other factors were found.

Factor	P-values		
	Poa pratensis	*Festuca rubra*	*Buchloe dactyloides*
CO_2	**<0.001**	**0.007**	**<0.001**
Salinity	**<0.001**	**<0.001**	**<0.001**
DAP	**<0.001**	**<0.001**	**<0.001**
CO_2 x Salinity	**<0.001**	**<0.001**	**<0.001**
CO_2 x DAP	0.860	0.916	0.274
Salinity x DAP	**<0.001**	**<0.001**	0.405
CO_2 x Salinity x DAP	1.000	0.291	1.000

Table 7. P-values for the effects of salinity on mean daily radicle and green emergences among salt levels in *Poa pratensis, Festuca rubra,* and *Buchloe dactyloides* allowed 30 days to germinate. Significant effects at 5% significance ($P < 0.05$) in bold.

Species	Level A (mM NaCl)	Level B (mM NaCl)	P-values	
			Radicle Emergence	Green Emergence
Poa pratensis	0	25	**<0.001**	**<0.001**
	0	50	**<0.001**	**<0.001**
	0	75	**<0.001**	**<0.001**
	0	100	**<0.001**	**<0.001**
	0	250	**<0.001**	**<0.001**
	25	50	**<0.001**	**<0.001**
	25	75	**<0.001**	**<0.001**
	25	100	**<0.001**	**<0.001**
	25	250	**<0.001**	**<0.001**
	50	75	**<0.001**	**<0.001**
	50	100	**<0.001**	**<0.001**
	50	250	**<0.001**	**<0.001**
	75	100	**<0.001**	**<0.001**
	75	250	**<0.001**	**<0.001**
	100	250	**<0.001**	**0.001**
Festuca rubra	0	25	**<0.001**	**<0.001**
	0	50	**<0.001**	**<0.001**
	0	75	**<0.001**	**<0.001**
	0	100	**<0.001**	**<0.001**
	0	250	**<0.001**	**<0.001**
	25	50	0.725	0.656
	25	75	**<0.001**	**<0.001**
	25	100	**<0.001**	**<0.001**
	25	250	**<0.001**	**<0.001**
	50	75	**<0.001**	**<0.001**
	50	100	**<0.001**	**<0.001**
	50	250	**<0.001**	**<0.001**
	75	100	**0.014**	**0.005**
	75	250	**<0.001**	**0.005**
	100	250	0.079	1.000

Table 7. continued

Species	Level A (mM NaCl)	Level B (mM NaCl)	P-values	
			Radicle Emergence	Green Emergence
Buchloe dactyloides	0	25	**<0.001**	**0.027**
	0	50	**<0.001**	**0.002**
	0	75	**<0.001**	**<0.001**
	0	100	**<0.001**	**<0.001**
	0	250	**<0.001**	**<0.001**
	25	50	0.238	0.358
	25	75	**<0.001**	**<0.001**
	25	100	**<0.001**	**<0.001**
	25	250	**<0.001**	**<0.001**
	50	75	**0.001**	**<0.001**
	50	100	**<0.001**	**<0.001**
	50	250	**<0.001**	**<0.001**
	75	100	**0.019**	**0.013**
	75	250	**0.001**	**<0.001**
	100	250	0.299	0.235

Table 8. Linear regression lines for days to plant emergences of *Poa pratensis*, *Festuca rubra*, and *Buchloe dactyloides* grown at various salt levels and either ambient or elevated CO_2. Strong linear relationships ($R^2 \geq 0.8$) in bold, superscripts indicate number of averages to which line was fitted, if less than 5.

Species	Emergence	Ambient CO_2		Elevated CO_2	
		Equation f(x) (plants per pot)	R^2	Equation f(x) (plants per pot)	R^2
Poa pratensis	1	**0.035x + 5.950**	**0.955**	**0.039x + 5.842**	**0.956**
	20	**0.052x + 7.144**	**0.990**	**0.057x + 6.566**	**0.978**
Festuca rubra	1	0.039x + 8.825	0.461	**0.090x + 7.050**	**0.991**
	3	0.022x + 11.524	0.134(4)	**0.098x +8.749**	**0.921(4)**
	5	**0.260x + 11.153**	**0.991(3)**	**0.220x + 9.860**	**0.926(3)**
	10	**0.553x + 14.773**	**1.000(2)**	**0.124x + 19.152**	**1.000(2)**
Buchloe dactyloides	1	-0.074x + 22.802	0.575	-0.001x + 19.465	0.000
	3	0.063x + 28.926	0.000(4)	0.024x + 23.952	0.158(3)
	5	N/A		N/A	
	10	N/A		N/A	

Table 9. Number of pots with first emergences in Growth Experiment 1 for *Poa pratensis*, *Festuca rubra*, and *Buchloe dactyloides*.

Species	Salt Level (mM NaCl)	Number of Pots	
		Ambient CO_2	Elevated CO_2
Poa pratensis	0	6	6
	50	6	6
	100	6	6
	250	0	0
Festuca rubra	0	6	6
	50	6	6
	100	5	6
	250	0	0
Buchloe dactyloides	0	6	6
	50	3	4
	100	1	1
	250	0	0

Table 10. Number of pots with first emergences in Growth Experiment 2 for *Poa pratensis*, *Festuca rubra*, and *Buchloe dactyloides*.

Species	Salt Level (mM NaCl)	n	
		Ambient CO_2	Elevated CO_2
Poa pratensis	0	5	5
	25	5	5
	50	5	5
	75	5	5
	100	5	5
Festuca rubra	0	5	5
	25	5	5
	50	5	5
	75	4	4
	100	3	4
Buchloe dactyloides	0	5	5
	25	4	4
	50	4	4
	75	2	1
	100	2	3

Table 11. Number of pots with third emergences in Growth Experiment 2 for *Festuca rubra*, and *Buchloe dactyloides*.

Species	Salt Level (mM NaCl)	n	
		Ambient CO_2	Elevated CO_2
Festuca rubra	0	5	5
	25	5	5
	50	4	4
	75	0	1
	100	1	0
Buchloe dactyloides	0	5	5
	25	2	1
	50	1	1
	75	1	0
	100	0	0

Table 12. Number of pots with fifth emergences in Growth Experiment 2 for *Festuca rubra* and *Buchloe dactyloides*.

Species	Salt Level (mM NaCl)	n	
		Ambient CO_2	Elevated CO_2
Festuca rubra	0	4	5
	25	5	5
	50	1	4
	75	0	1
	100	0	0
Buchloe dactyloides	0	5	5
	25	0	0
	50	0	0
	75	0	0
	100	0	0

Table 13. Number of pots with tenth emergences in Growth Experiment 2 for *Festuca rubra* and *Buchloe dactyloides*.

Species	Salt Level (mM NaCl)	n	
		Ambient CO_2	Elevated CO_2
Festuca rubra	0	3	5
	25	2	2
	50	0	0
	75	0	0
	100	0	0
Buchloe dactyloides	0	1	1
	25	0	0
	50	0	0
	75	0	0
	100	0	0

Table 14. Number of pots with twentieth emergences in Growth Experiment 2 for *Poa pratensis*.

Species	Salt Level (mM NaCl)	n	
		Ambient CO_2	Elevated CO_2
Poa pratensis	0	5	5
	25	5	5
	50	5	5
	75	5	5
	100	5	5

Table 15. Linear regression lines for plant number per pot of *Poa pratensis* (quantifiable decline), *Festuca rubra*, and *Buchloe dactyloides* grown at various salt levels and either ambient or elevated CO_2. Strong linear relationships ($R^2 \geq 0.8$) in bold.

Species	Salt Level (mM NaCl)	Ambient CO_2		Elevated CO_2	
		Equation f(x) (plants per pot)	R^2	Equation f(x) (plants per pot)	R^2
Poa pratensis	75	**-0.467x + 56.569**	**0.968**	**-0.250x + 35.879**	**0.994**
	100	**-0.343x + 34.097**	**0.916**	**-0.309x + 35.231**	**0.986**
Festuca rubra	0	-0.006x + 10.616	0.115	**-0.018x + 12.275**	**0.904**
	25	**-0.020x + 8.544**	**0.974**	**-0.037x + 8.920**	**0.978**
	50	-0.005x + 3.392	0.277	**-0.024x + 7.221**	**0.848**
	75	-0.007x + 1.148	0.490	**-0.005x + 1.579**	**0.810**
	100	**-0.001x + 1.348**	**0.871**	-0.003x + 0.875	0.390
Buchloe dactyloides	0	**0.047x + 3.084**	**0.848**	**0.067x + 3.928**	**0.839**
	25	0.001x + 0.847	0.062	-0.002x + 1.538	0.250
	50	-0.005x + 1.534	0.433	-0.011x + 1.671	0.641
	75	**-0.009x + 1.096**	**0.863**	-0.003x + 0.275	0.026
	100	**-0.007x + 0.621**	**0.819**	**-0.011x + 1.167**	**0.830**

Table 16. P-values for the effects of CO_2, salinity, and interactions upon plant number per pot of *Poa pratensis, Festuca rubra,* and *Buchloe dactyloides*. Significant effects at 5% significance ($P < 0.05$) are in bold. Aside from the significant interactive effect of salinity x day on *B. dactyloides*, no significant effects of day after planting or interactive effects of day after planting with other factors were found.

Factor	P-values		
	Poa pratensis	*Festuca rubra*	*Buchloe dactyloides*
CO_2	N/A	**0.001**	**0.001**
Salinity	N/A	**<0.001**	**<0.001**
CO_2 x Salinity	N/A	**<0.001**	**<0.001**
Salinity x Day	N/A	1.000	**0.003**

Table 17. P-values for the effects of salinity upon plant number per pot among salt levels in *Festuca rubra* and *Buchloe dactyloides* grown for 100 days. Significant effects at 5% significance ($P < 0.05$) are in bold.

Species	Level A (mM NaCl)	Level B (mM NaCl)	P-value
Festuca rubra	0	25	**<0.001**
	0	50	**<0.001**
	0	75	**<0.001**
	0	100	**<0.001**
	25	50	**<0.001**
	25	75	**<0.001**
	25	100	**<0.001**
	50	75	**<0.001**
	50	100	**<0.001**
	75	100	0.540
Buchloe dactyloides	0	25	**<0.001**
	0	50	**<0.001**
	0	75	**<0.001**
	0	100	**<0.001**
	25	50	0.628
	25	75	**<0.001**
	25	100	**<0.001**
	50	75	**<0.001**
	50	100	**0.001**
	75	100	0.911

Table 18. P-values for the effects of CO_2, salinity, and interactions upon aboveground dry biomass of *Poa pratensis, Festuca rubra,* and *Buchloe dactyloides* in Growth Experiments 1 and 2. Significant effects at 5% significance ($P < 0.05$) are in bold.

Factor	Aboveground Dry Biomass	Species P-values		
		Poa pratensis	*Festuca rubra*	*Buchloe dactyloides*
CO_2	Experiment 1	**<0.001**	**0.011**	0.353
	Experiment 2	**<0.001**	**<0.001**	**0.005**
Salinity	Experiment 1	**<0.001**	**<0.001**	**<0.001**
	Experiment 2	**<0.001**	**<0.001**	**<0.001**
CO_2 x Salinity	Experiment 1	**<0.001**	**0.018**	0.336
	Experiment 2	**<0.001**	**<0.001**	**0.005**

Table 19. P-values for the effects of CO_2, salinity, and interactions upon belowground dry biomass of *Poa pratensis, Festuca rubra,* and *Buchloe dactyloides* in Growth Experiments 1 and 2. Significant effects at 5% significance ($P < 0.05$) are in bold.

Factor	Belowground Dry Biomass	Species P-values		
		Poa pratensis	*Festuca rubra*	*Buchloe dactyloides*
CO_2	Experiment 1	**<0.001**	0.156	0.569
	Experiment 2	**0.003**	**0.025**	**0.001**
Salinity	Experiment 1	**<0.001**	**<0.001**	**<0.001**
	Experiment 2	**<0.001**	**<0.001**	**<0.001**
CO_2 x Salinity	Experiment 1	**<0.001**	0.147	0.679
	Experiment 2	0.060	**0.044**	**<0.001**

Table 20. P-values for the effects of salinity upon aboveground and belowground dry biomass among salt levels in *Poa pratensis, Festuca rubra,* and *Buchloe dactyloides* grown for 100 days. Significant effects at 5% significance ($P < 0.05$) are in bold.

Species	Level A (mM NaCl)	Level B (mM NaCl)	Aboveground P-value	Belowground P-value
Poa pratensis	0	25	0.555	**<0.001**
	0	50	**<0.001**	**<0.001**
	0	75	**<0.001**	**<0.001**
	0	100	**<0.001**	**<0.001**
	25	50	**<0.001**	**<0.001**
	25	75	**<0.001**	**<0.001**
	25	100	**<0.001**	**<0.001**
	50	75	**<0.001**	**0.012**
	50	100	**<0.001**	**0.007**
	75	100	0.156	0.853
Festuca rubra	0	25	**<0.001**	**<0.001**
	0	50	**<0.001**	**<0.001**
	0	75	**<0.001**	**<0.001**
	0	100	**<0.001**	**<0.001**
	25	50	**<0.001**	**0.006**
	25	75	**<0.001**	**0.002**
	25	100	**<0.001**	**0.002**
	50	75	**0.043**	0.671
	50	100	**0.041**	0.664
	75	100	0.978	0.992
Buchloe dactyloides	0	25	**<0.001**	**<0.001**
	0	50	**<0.001**	**<0.001**
	0	75	**<0.001**	**<0.001**
	0	100	**<0.001**	**<0.001**
	25	50	0.544	0.855
	25	75	0.283	0.644
	25	100	0.288	0.645
	50	75	0.637	0.780
	50	100	0.645	0.780
	75	100	0.990	0.999

FIGURES

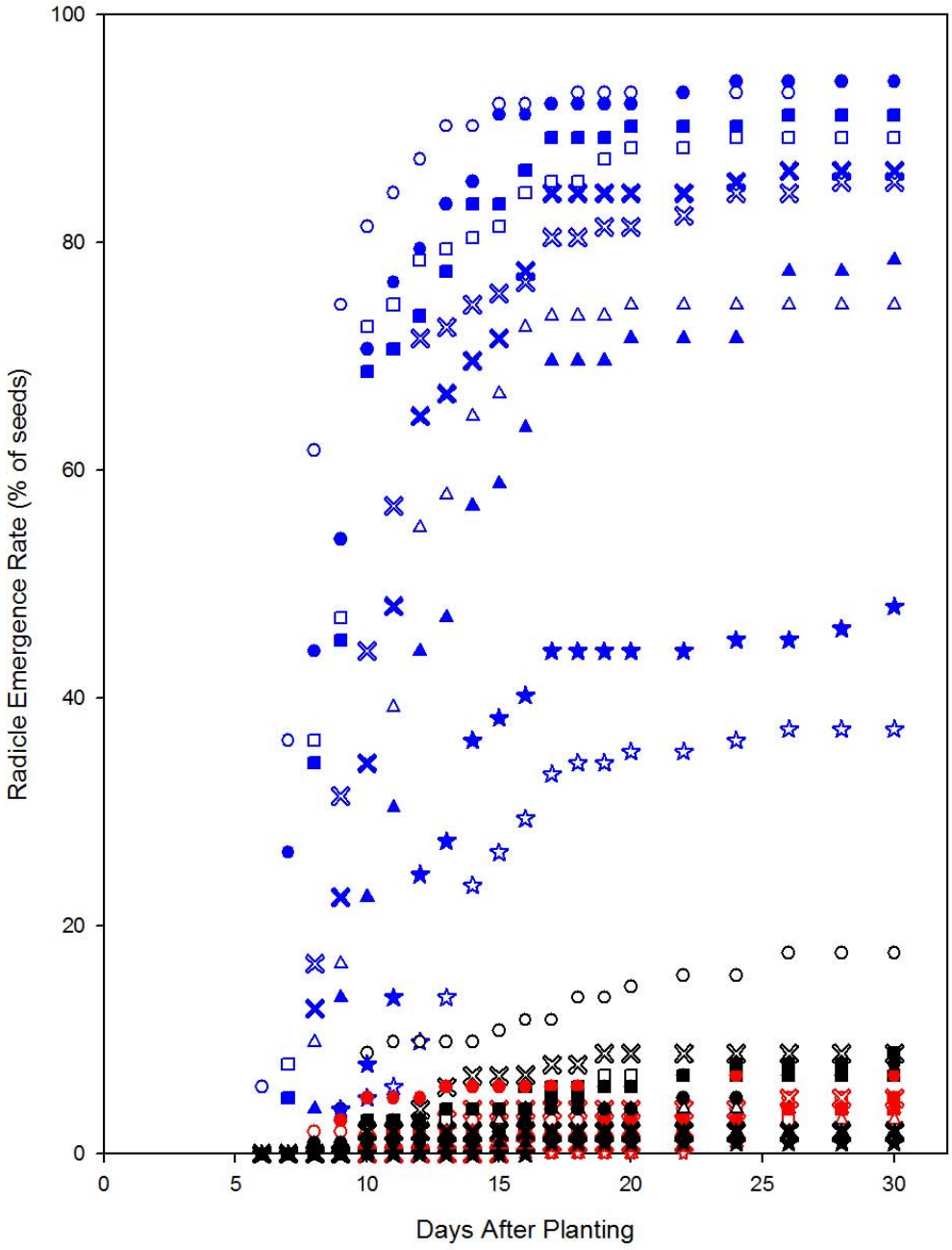

Figure 1. Cumulative percentage of radicle emergences over a 30 day period for all salinity levels in seeds of *Poa pratensis*(blue), *Festuca rubra* (red), and *Buchloe dactyloides* (black) at ambient (open) and elevated (closed) CO_2. Salinity levels were 0 (circle), 25 (square), 50 (X), 75 (triangles), and 100 (star) mM NaCl. n=5.

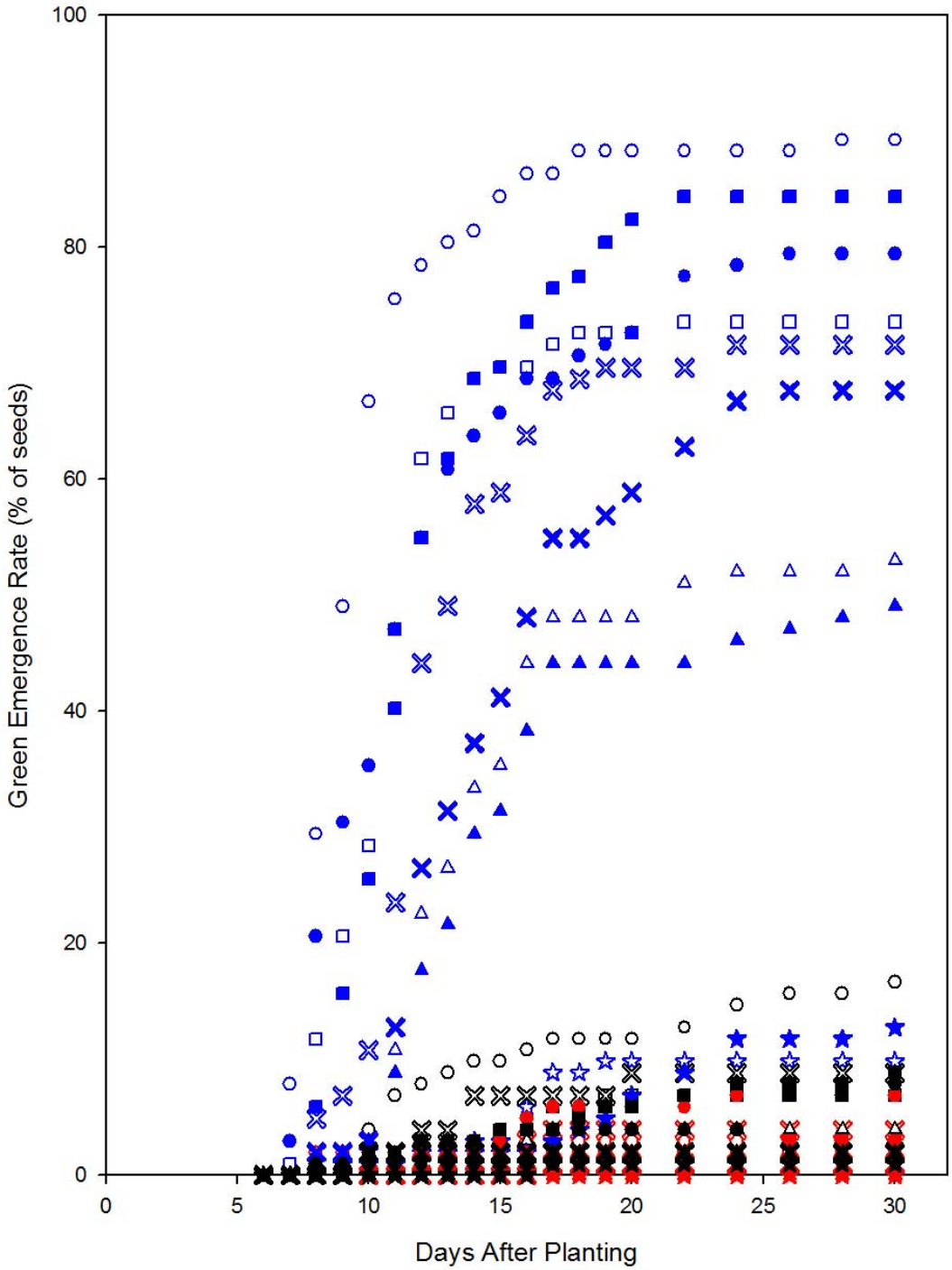

Figure 2. Cumulative percentage of green emergences over a 30 day period for all salinity levels in seeds of *Poa pratensis*(blue), *Festuca rubra* (red), and *Buchloe dactyloides* (black) at ambient (open) and elevated (closed) CO_2. Salinity levels were 0 (circle), 25 (square), 50 (X), 75 (triangles), and 100 (star) mM NaCl. n=5.

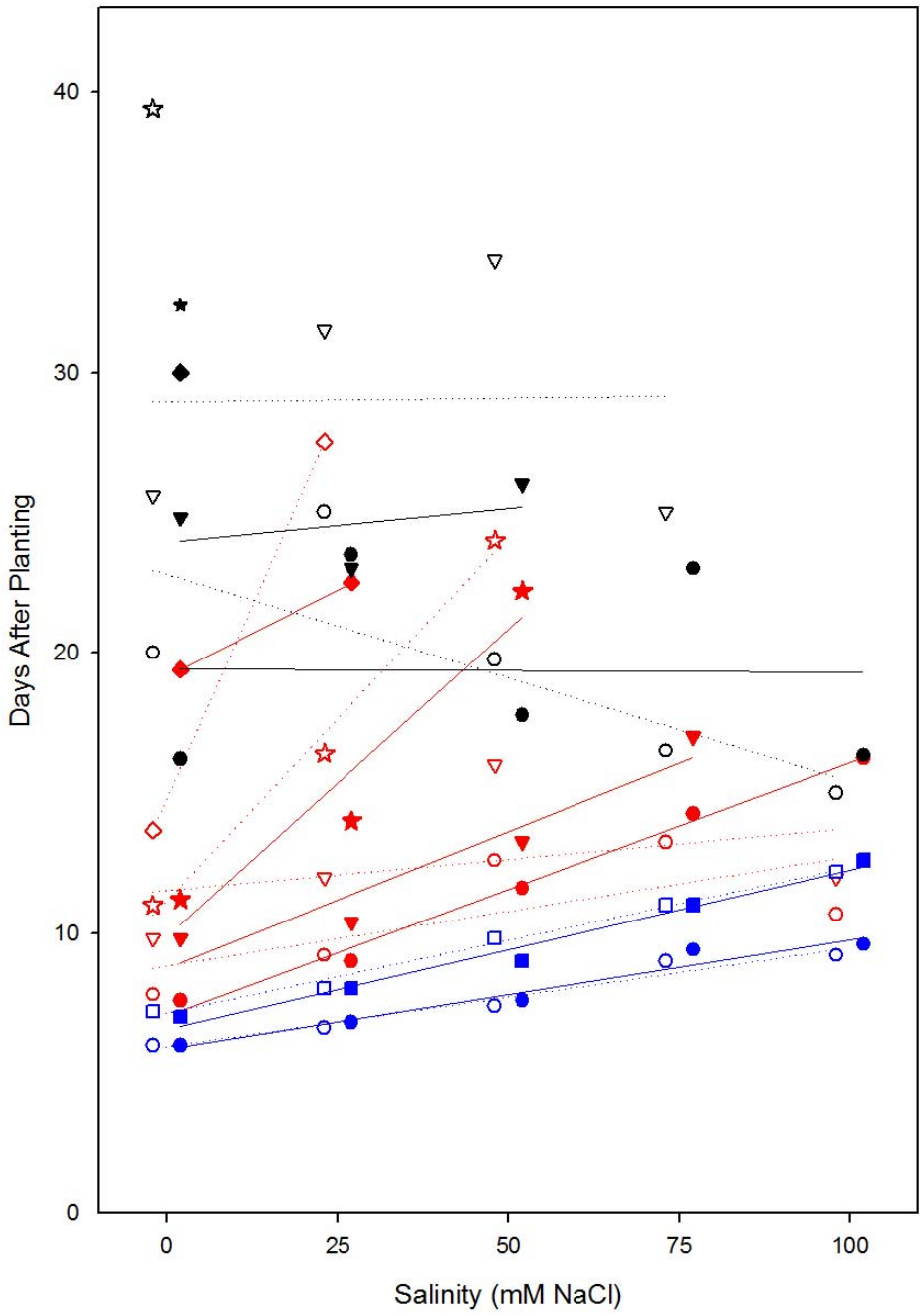

Figure 3. Average days to emergences of *Poa pratensis* (blue), *Festuca rubra* (red), and *Buchloe dactyloides* (black) at ambient (open) and elevated (closed) CO_2 and various salinity levels. First (circle), third (triangle), fifth (star), and tenth (diamond) emergences were recorded for *F. rubra* and *B. dactyloides*, while first and twentieth (square) emergences were recorded for *P. pratensis*. One pot of *B. dactyloides* had a tenth emergence at 96 DAP, not pictured. Best fit lines have been applied for each ambient (dotted line) and elevated (solid line) treatment. Individual seedlings were counted in *P. pratensis and F. rubra*, while seedling clusters were counted in *B. dactyloides*.

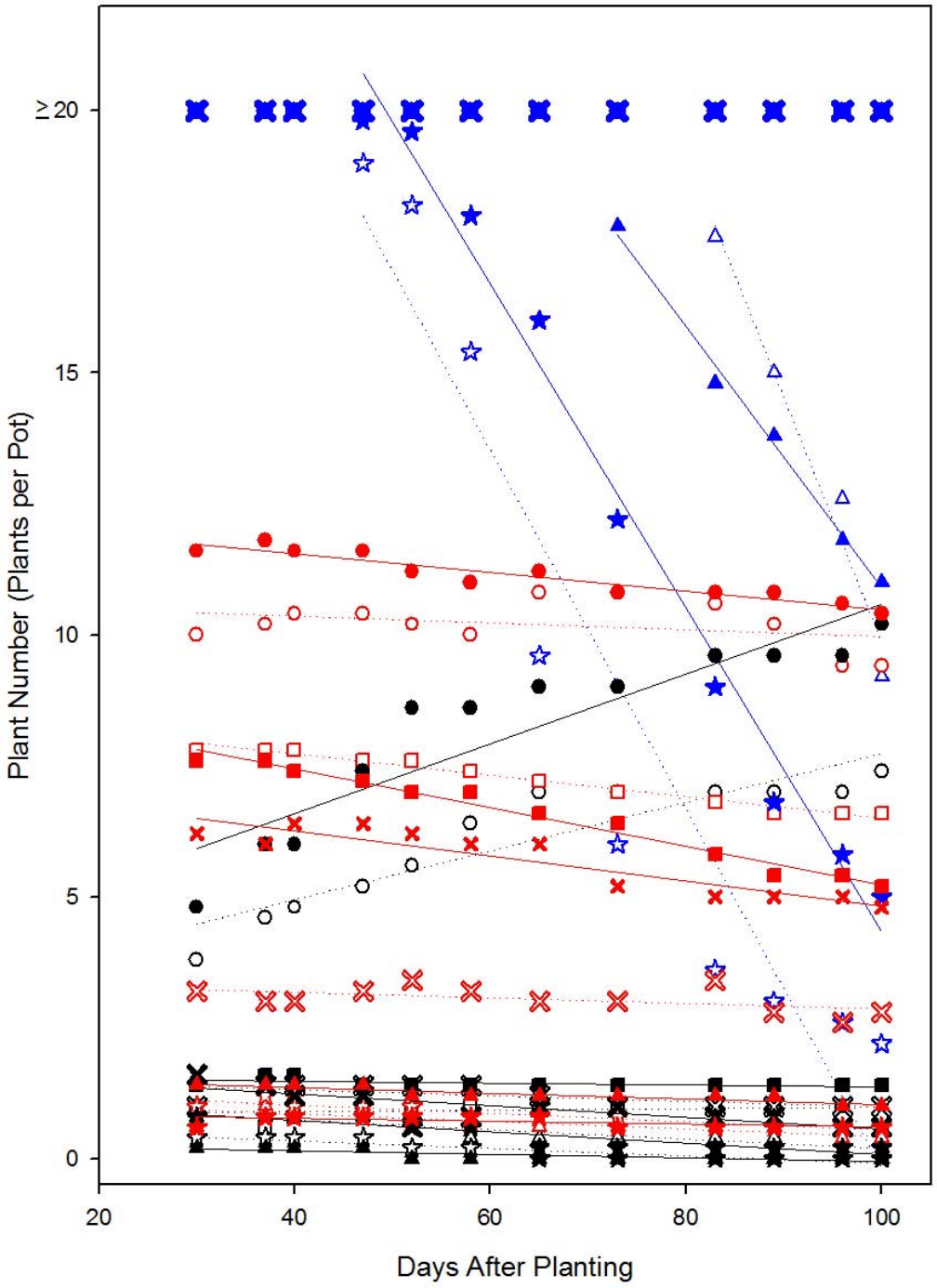

Figure 4. Average plant number per pot for all salinity levels in *Poa pratensis*(blue), *Festuca rubra* (red), and *Buchloe dactyloides* (black) at ambient (open) and elevated (closed) CO_2. Salinity levels were 0 (circle), 25 (square), 50 (X), 75 (triangles), and 100 (star) mM NaCl. n=5. Best fit lines have been applied for each ambient (dotted line) and elevated (solid line) treatment.

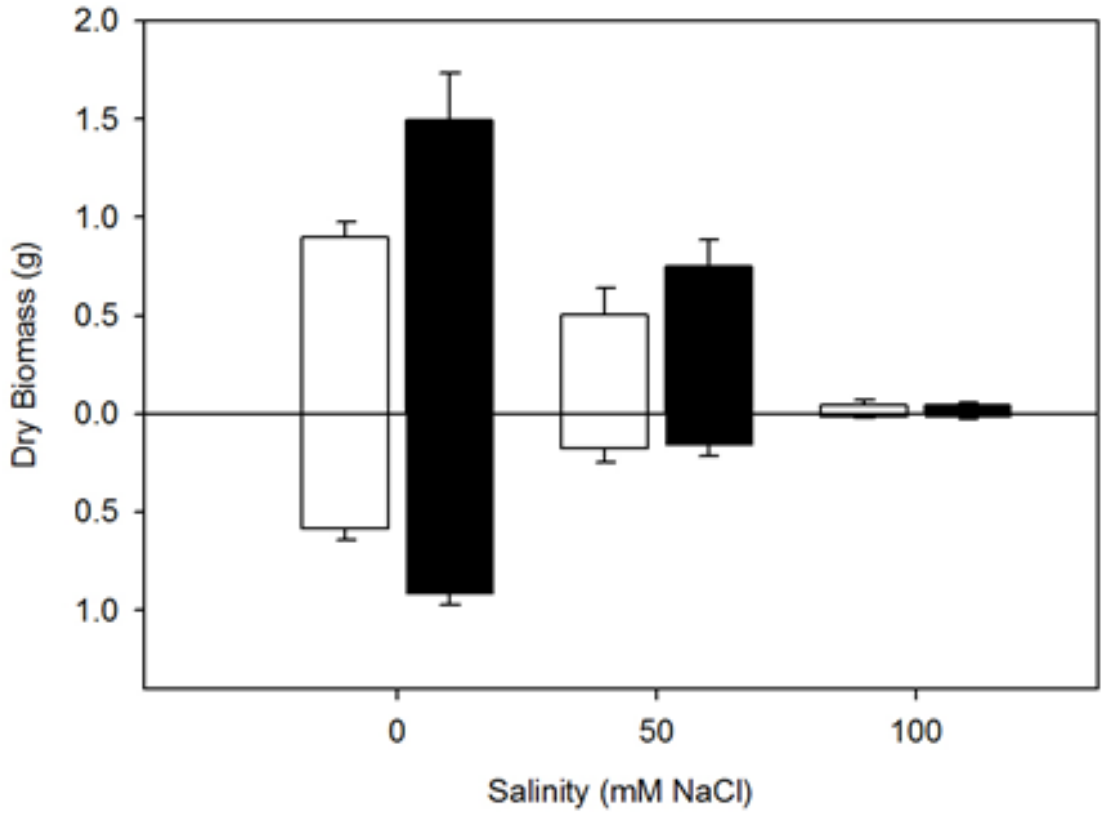

Figure 5. Dry biomass for *Poa pratensis* in Growth Experiment 1, grown at ambient (open bars) and elevated (closed bars) CO_2 and various levels of salt stress for 47 days. Aboveground and belowground biomasses appear above and below the baseline, respectively. No growth occurred at the 250 mM NaCl level. Error bars represent standard deviation.

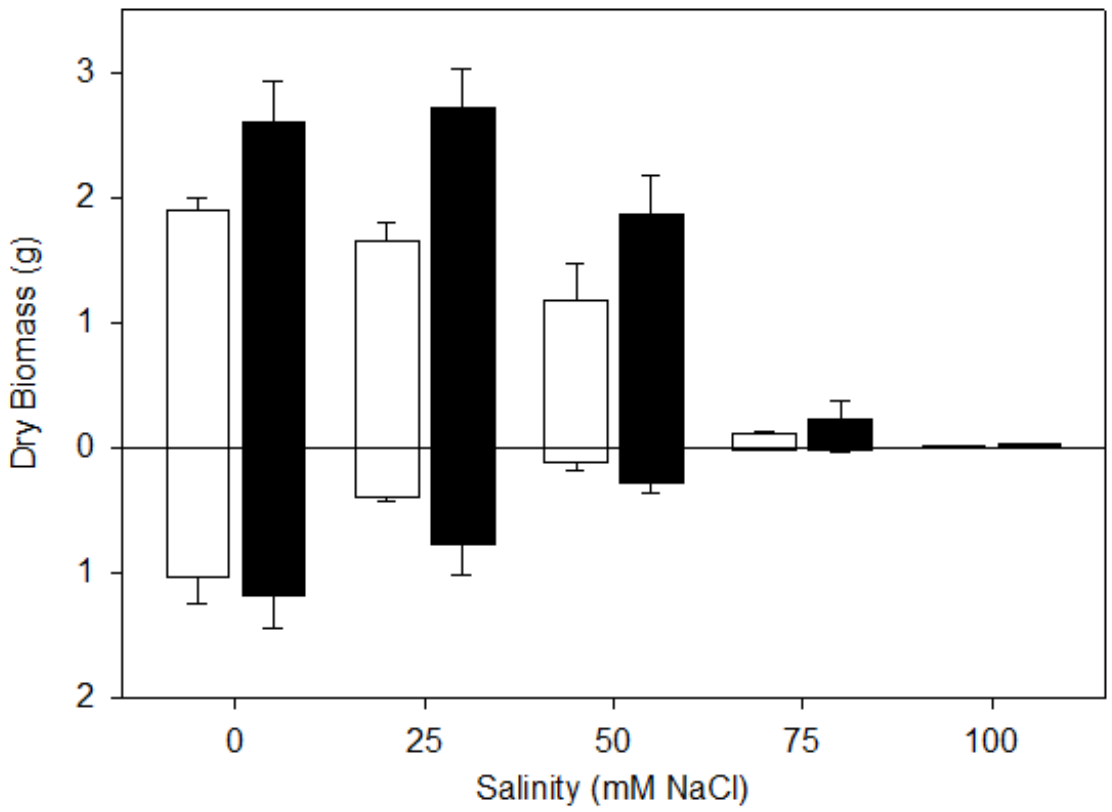

Figure 6. Dry biomass for *Poa pratensis* in Growth Experiment 2, grown at ambient (open bars) and elevated (closed bars) CO_2 and various levels of salt stress for 100 days. Aboveground and belowground biomasses appear above and below the baseline, respectively. Error bars represent standard deviation.

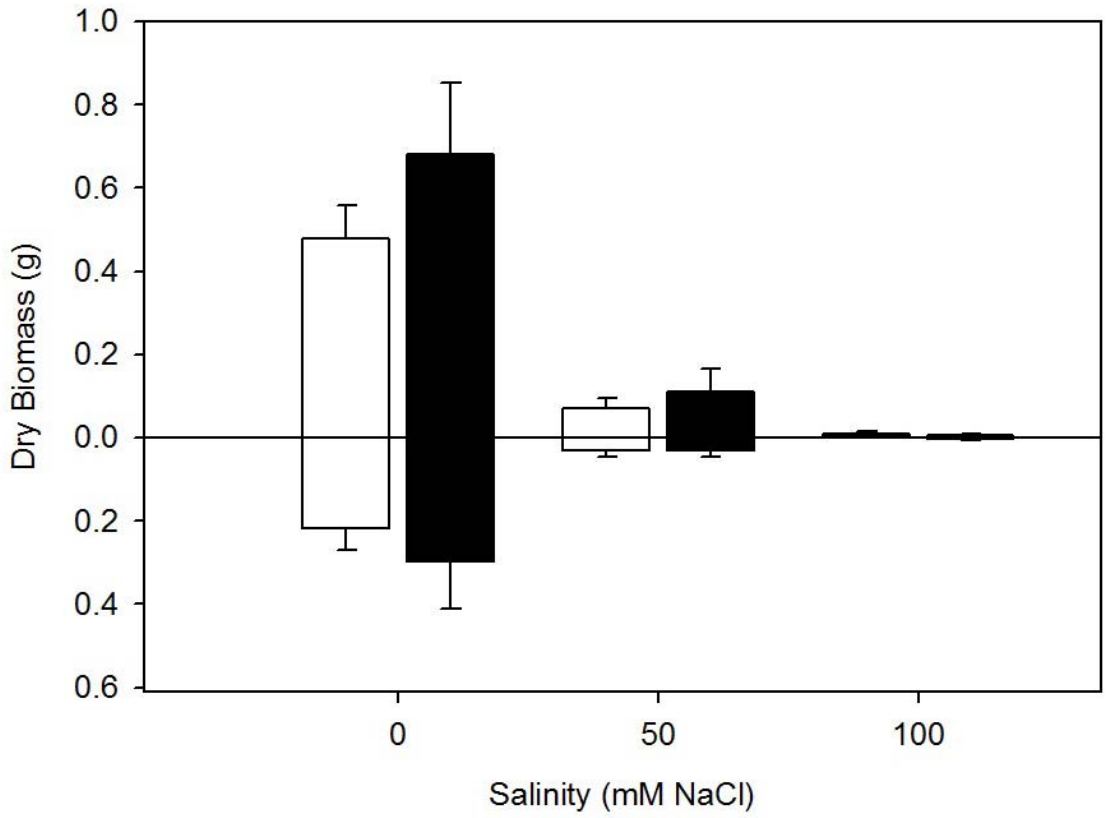

Figure 7. Dry biomass for *Festuca rubra* in Growth Experiment 1, grown at ambient (open bars) and elevated (closed bars) CO_2 and various levels of salt stress for 47 days. Aboveground and belowground biomasses appear above and below the baseline, respectively. No growth occurred at the 250 mM NaCl level. Error bars represent standard deviation.

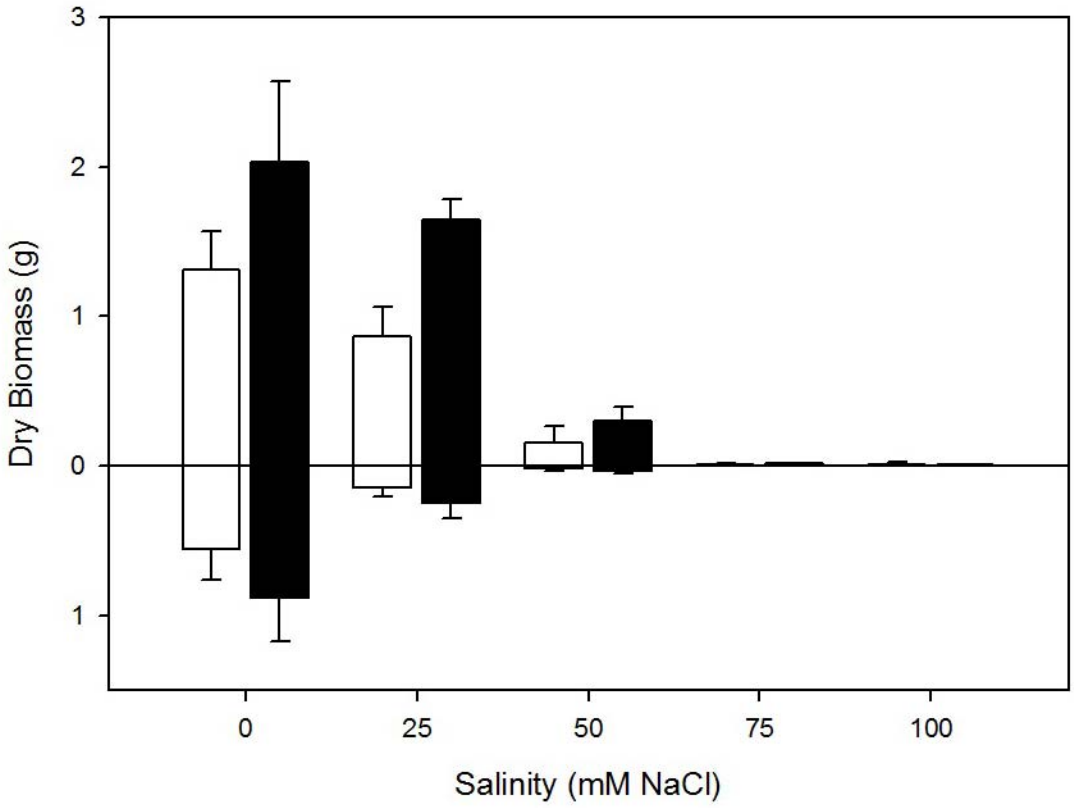

Figure 8. Dry biomass for *Festuca rubra* in Growth Experiment 2, grown at ambient (open bars) and elevated (closed bars) CO_2 and various levels of salt stress for 100 days. Aboveground and belowground biomasses appear above and below the baseline, respectively. Error bars represent standard deviation.

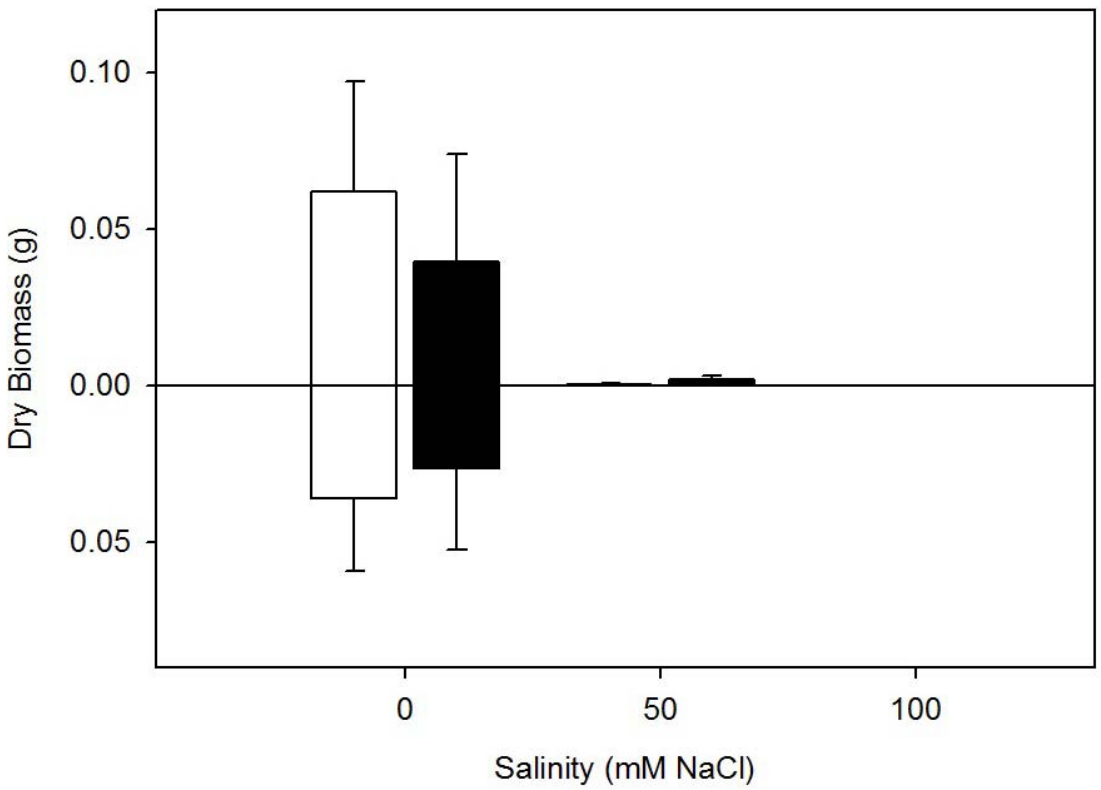

Figure 9. Dry biomass for *Buchloe dactyloides* in Growth Experiment 1, grown at ambient (open bars) and elevated (closed bars) CO_2 and various levels of salt stress for 47 days. Aboveground and belowground biomasses appear above and below the baseline, respectively. No growth occurred at the 250 mM NaCl level. Error bars represent standard deviation.

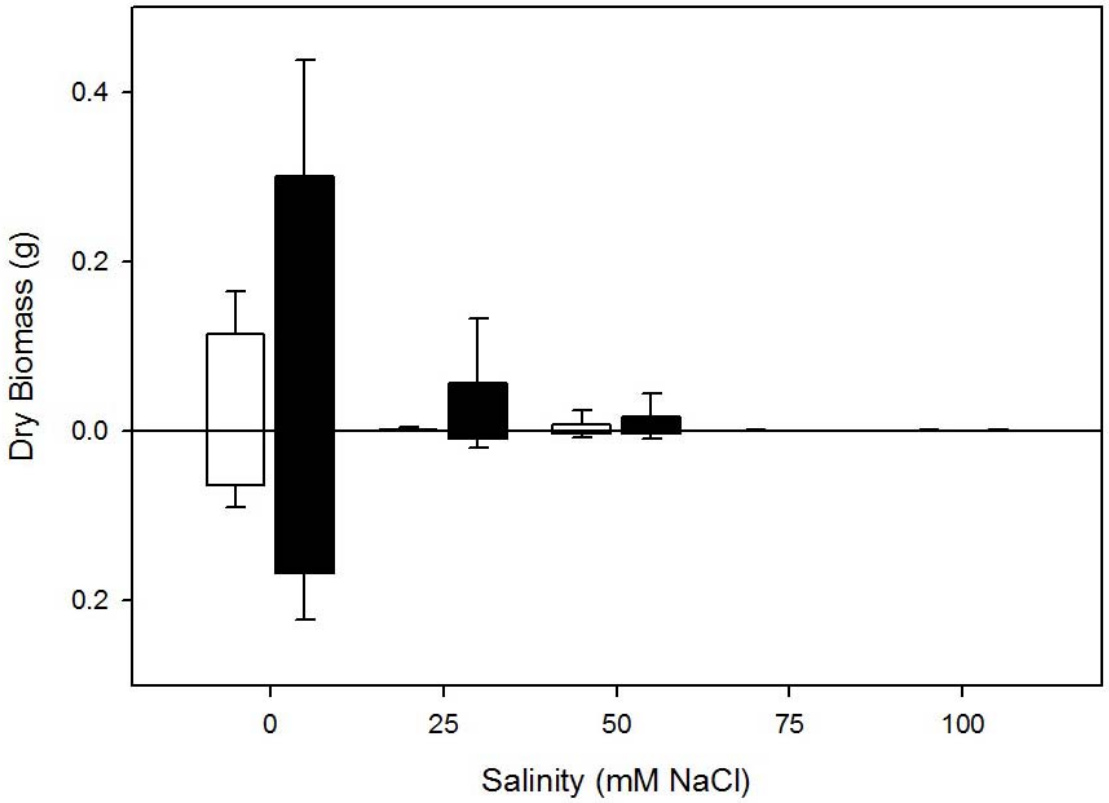

Figure 10. Dry biomass for *Buchloe dactyloides* in Growth Experiment 2, grown at ambient (open bars) and elevated (closed bars) CO_2 and various levels of salt stress for 100 days. Aboveground and belowground biomasses appear above and below the baseline, respectively. Error bars represent standard deviation.