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Pathology and seedling nutrition of Spartina alterniflora (smooth cordgrass)

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PATHOLOGY AND SEEDLING NUTRITION OF *SPARTINA ALTERNIFLORA* (SMOOTH CORDGRASS)

A Thesis

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To my family: my mother and stepfather, Kathy and Dwight Hutchinson; grandmother, Leatrice Clark; sister and brother-in-law Peggy and Jack Varnado; and niece and nephew Madelynn and Jacob. I am forever grateful for the support and encouragement I received from you to pursue this degree.

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ABSTRACT

Transplants of *Spartina alterniflora* Loisel. (smooth cordgrass, *Poaceae*) are effective in stabilizing and protecting vulnerable coastal wetlands from erosion. However, the expense and labor associated with propagation and transplanting restrict the widespread use of *S. alterniflora* in coastal restoration and protection projects. As an alternative, seeding of *S. alterniflora* by aircraft has been proposed as a more useful and economical approach for revegetating denuded marsh sites. However, a period of stalled growth, which lasts for 12 or more weeks, has been observed in smooth cordgrass seedlings, and this increases their susceptibility to being washed away by inundating tides. This stalled growth period must be overcome before aerial seeding can be implemented.

As with any plant species, it is reasonable to assume that *S. alterniflora* has optimal nutritional requirements for seedling and mature plant growth. Previous nutritional research has involved only mature smooth cordgrass plants. These studies showed that applications of nitrogen and phosphorus fertilizers increased plant growth. The objectives of this study were to document the lag phase of seedling growth observed in field and greenhouse conditions; determine the possible role of soil microbes, including seed and seedling pathogens and mycorrhizal fungi, as a cause of delayed seedling growth; and investigate the role of nutrition in seedling growth.

This study confirmed the existence of stalled seedling growth in *S. alterniflora* and concluded that pathogens are not the cause of this lag period. Furthermore, supplemental N and P (240 kg N ha^{-1}, 49.5 kg P ha^{-1}) reduced the lag phase from over 100 days to less than 50 days under ideal greenhouse conditions. However, nutrient additions did not completely overcome

stalled seedling growth. Future research possibilities include investigating the effects of plant growth regulators (hormones) and seed preconditioning treatments on seedling growth.

1. INTRODUCTION

Louisiana's coastal marshes account for 41% of the total coastal marshes of the continental United States (DeLaune et al. 1990, Williams et al. 1997). This vast expanse of coastal land is an important habitat for commercial and recreational fisheries and waterfowl as well as the first line of defense against storm-induced tidal surges (Mitsch and Gosselink 1993). Unfortunately, this habitat is being lost at rates between 26 and 100 km^2 per year as a result of rising sea levels, subsidence, geologic processes, and anthropogenic causes, including river channelization (DeLaune et al. 1983, 1990; Mitsch and Gosselink 1993; Williams et al. 1997).

In order to maintain itself, a salt marsh must maintain its elevation above sea level within a specific range via accretion. Marshes become inundated when accretion rates do not equal or exceed loss rates relative to sea level, which is rising (DeLaune et al. 1983, Mitsch and Gosselink 1993). Louisiana's coastal marshes are being lost primarily as a result of deterioration from within rather than shoreline erosion. This change from emergent marsh to open water accounts for approximately 75% of the total land loss (DeLaune et al. 1990). Within a Louisiana marsh, DeLaune et al. (1983) estimated that accretion rates averaged 0.8 cm/yr, whereas coastal submergence was proceeding at about 1.2 cm/yr. In other southeastern U.S. marshes, the rate of sediment accretion is approximately 1.2 mm/yr, which is less than the average rate of sea-level rise for the region (1.9 mm/yr) (Hackney and Cleary 1987, Stevenson et al. 1986).

One effort to combat coastal erosion is the deposition of dredged material into marsh sites that are particularly critical with regard to barrier island preservation, coastal protection, and habitat restoration. The use of dredge spoils for this purpose has considerable merit because it offers a unique means by which to economically resolve spoil disposal problems while simultaneously restoring and enhancing marsh habitats. Several innovative ideas, including spray

dredging, sediment transport through abandoned oil and gas pipelines, as well as construction of siphons and diversions, have been investigated (Cahoon and Cowan 1988, Ford et al. 1999, Harrison et al. 2003). Regardless of the means by which marshes are restored, it is critical that a self-sustaining plant community be established as quickly as possible to prevent erosion of the spoil material. This is particularly critical in coastal marshes where elevation may be only a few inches above sea level and where the newly elevated marsh may be inundated by unusually high tides or storm surges.

Spartina alterniflora Loisel. (smooth cordgrass) is the predominant plant species that thrives in this habitat. It produces a dense stand of stems that is very effective in trapping sediment during periods of inundation, and its extensive fibrous root system prevents erosion of topsoil (Anon. 2000). There has been a sustained effort in Louisiana to reintroduce this plant into newly created marshes (Harrison et al. 2003). Unfortunately, native populations produce very few seeds, and these seeds require special handling to preserve viability and break dormancy (Broome and Seneca 1974, Harrison et al. 2003, Plyler and Carrick 1993, Plyler and Proseus 1996, Seneca and Broome 1972). Therefore, commercial restoration efforts have depended upon transplanting pot-grown, vegetatively propagated plants. This is a very expensive and laborious undertaking because the plants must be propagated and grown in 1-gallon containers in greenhouses; these potted plants must be transported to the sites by barge; and then the plants must be carried across the marsh and transplanted by hand (M. Materne, personal communication). This can be done only at sites that are accessible by barge, and it is not a cost effective means for marsh restoration. Obviously, this approach is of limited value and is reserved for critical restoration sites.

A more useful and economical approach would be to plant seeds of *S. alterniflora* from aircraft. Such an approach would allow many acres to be planted in a short time (seconds per acre), and there would be fewer problems with inaccessible sites. Aerial planting technology is well developed in Louisiana having been used in the rice industry for many years. However, certain biological prerequisites must be met before this technology can be adapted to planting *S. alterniflora*. First, there must be adequate seed production of locally adapted ecotypes. Second, seeds must be stored moist to retain viability, and, if subject to dormancy, seeds must be treated to break dormancy. Third, seeds must germinate and seedlings must become established quickly after planting in order to avoid being swept away by inundating tides.

The first two hurdles have been overcome in ecotypes that were originally collected from a Louisiana salt marsh (Harrison et al. 2001). Substantial progress has been made in this area because of a large multidisciplinary project that includes an agronomist/plant breeder, a molecular geneticist, a biotechnologist, a seed physiologist, a wetlands biologist and restoration technologist, and a plant pathologist (Harrison et al. 2001, 2003). In fact, aerial planting was successfully accomplished in that seeds were dispersed from an aircraft in a relatively uniform pattern in a marsh. Furthermore, the seeds were viable and began to germinate. However, germination and seedling establishment were so slow that seedlings were washed away by an inundating tide soon after planting (M. Materne, personal communication). In addition, this lag phase in seeding growth has been observed in seedlings grown in the greenhouse under flooded conditions. An investigation by Broome and Seneca (1974), regarding the propagation of *S. alterniflora* from seeds, also indicated very little growth during the first growing season when direct seeded onto dredge spoil in coastal North Carolina. Thus, the third prerequisite must be met before commercial interests and governmental agencies can adopt this technology. It is

encouraging to note that direct seeding has been attempted in North Carolina and Delaware, but there are no reports of its being adopted as a widespread practice or of the use of aircraft to plant seeds (Broome and Seneca 1974, Leslie 1987, Seneca et al. 1976).

As with any plant species, it is reasonable to assume that *S. alterniflora* has its optimum nutritional requirements for seedling and mature plant growth. The literature base is very restricted in this area, with only one report dealing with seedling nutrition (Broome et al. 1975b). Therefore, the objectives of this study were to document the lag phase of seedling growth observed in field and greenhouse conditions, determine the possible role of soil microbes, including seed and seedling pathogens and mycorrhizal fungi, as a cause of delayed seedling growth, and investigate the role of nutrition in seedling growth.

2. MATERIALS AND METHODS

2.1 Microbial Interactions, Including Seed and Seedling Diseases

Spartina alterniflora seeds were planted in both raw and steam-sterilized native marsh soil collected on Grand Terre Island, Louisiana. Each experimental unit consisted of a disposable, 1920-ml capacity food storage container (model 9291-64 FL OZ, Alcoa, Pittsburgh, PA) filled with soil to a depth of 60 ml and a soil surface area of 245 cm^2. Six containers each were filled with the raw and steam-sterilized native marsh soil. Distilled water was added to each container to saturate the soil. On June 7, 2001, twenty seeds were planted in each container by laying them on the soil surface. Seeds had been harvested in December 2000 from *S. alterniflora* plants located at the LSU Agricultural Center Ben Hur Research Farm (Baton Rouge, LA). After harvesting, the seeds were stored in sealable plastic bags with moist paper toweling and maintained at 4ºC. Prior to planting, the seeds were sorted using a light box to eliminate seeds without embryos. After planting, distilled water was added to each container to flood the soil to a depth of 1-2 mm. The containers were then placed in growth chambers set at 25ºC with 12 hours of light per day. Distilled water was added as needed.

Seedlings were removed from the soil after 14 days, by carefully pulling the seedlings and roots out of the soil. Once removed from the soil, seedlings for raw and sterile soil were placed in separate beakers covered with a piece of fiberglass screen, a layer of brown paper towel, and a final piece of screen. The seedlings were then washed in the covered beakers under cold running tap water for 1 hour.

After washing, 24 seedlings received a 5 second dip in 70% ethanol, followed by a 10 minute soak in sterile deionized water. Another 24 seedlings were placed in sterile deionized water without receiving an ethanol dip. Seedlings from both groups were then removed and

blotted dry with autoclaved paper toweling before discolored root sections were plated onto water agar and acidified potato dextrose agar. Culture dishes were incubated at 27ºC under fluorescent lights. Plates were observed for fungal growth on June 28, 2001.

This experiment, as described above, was repeated three times during the following time periods: July 6, 2001 through August 10, 2001, September 24, 2001 through November 1, 2001, and November 29, 2001 through January 22, 2002.

Roots of mature *S. alterniflora* plants collected from marsh sites were stained and viewed for the presence of mycorrhizal infection following the protocol described by Dhingra and Sinclair (1985). Plants with mycorrhizal relationships are known to benefit nutritionally from this association.

2.1 Initial Time Course Study

Spartina alterniflora seeds were planted in steam sterilized and raw mineral soil supplied by the Louisiana State University (LSU) Greenhouse Services. Results of soil analyses for both the steam sterilized and raw soil are listed in Appendix A. Four experimental treatments of nutrient enrichment were randomly assigned to four containers of steam-sterilized soil and four containers of raw soil in each sampling group (control, N, P, N plus P) for a total of eight containers per sampling group. Ten sampling groups were planted to establish a growth rate curve over time. The containers were placed on concrete benches in a glasshouse located in the main campus greenhouse complex at LSU. An evaporative cooling pad system was used in the greenhouse for cooling. No supplemental lighting was used.

Nutrient treatment levels were based on studies of nutrient enrichment along the Neuse River shoreline near Oriental, North Carolina (Broome et al. 1983) and near Beaufort, North Carolina (Broome et al. 1975a, 1975b). These sites had been investigated with regard to soil

nutrient interactions with *S. alterniflora*. Reagent grade ammonium sulfate was used (21.2% N) for the nitrogen treatments. Ammonium sulfate was used because ammonium is the primary nitrogen source for many *Spartina*-dominated salt marshes (Mendelssohn 1979). Phosphorus was supplied using equimolar amounts of reagent grade monobasic calcium phosphate ($Ca(H_2PO_4)_2H_2O$, 24.6% P and 15.87% Ca) and dibasic calcium phosphate ($CaHPO_4$, 21.2% P and 27.4% Ca). Fertilization rates within each container were 224; 99; and 224 plus 99 kg/ha for N; P; and N+P treatments, respectively. In addition, calcium sulfate (23.25% Ca) was added to the N only treatments to balance the amount of Ca present in the soil between the N and N + P treatments. Nutrients were incorporated in the soil prior to flooding and planting.

Each experimental unit consisted of a disposable, 720 ml capacity food storage container (Gladware, Oakland, CA) filled with soil to a depth of 50 millimeters and a soil surface area of 140 cm^2. Each container was placed in a plastic storage container (Rubbermaid, Fairlawn, OH) filled with 2 L of a 15 parts per thousand (ppt) salt solution. The salt solution was made by mixing 30 g artificial sea water mix (Crystal Sea / Forty Fathoms Marinemix, Baltimore, MD) with 2 L deionized tap water. The chemical assay for Crystal Sea Marinemix is listed in Appendix B. Tap water was deionized using two mixed bed deionization tanks (U.S. Filter, Metairie, LA). Deionized water was added as needed to maintain the water level above the soil surface. Salinity was measured periodically using a handheld refractometer (model S/Mill-E, Atago, Tokyo, Japan) to insure a constant salinity level of 15 ppt.

Seeds had been harvested in December 2002 from *S. alterniflora* plants located at the LSU Agricultural Center Ben Hur Research Farm, and they were stored and sorted as described above. Thirty-six seeds were planted in each container by placing them on the soil surface on March 21, 2003. Sample group one was harvested 15 days after planting (April 6, 2003) when a

majority of seedlings had two true leaves. Harvesting of sampling groups continued at 15-day intervals through sample group 4 (May 21, 2003). Starting with sample group five (harvested May 28, 2003), harvesting was conducted at 7-day intervals until the last sample group (10) was harvested on July 2, 2003.

After harvesting, whole plants were washed twice in deionized water, and the number of plants per treatment container was recorded. Roots were separated from shoots and both weighed after oven drying at 65ºC for 48 hours as recommend by the LSU Agricultural Center Soil Testing and Plant Laboratory. Shoot and root dry weights for each treatment were divided by the number of plants per treatment for an average plant weight. In addition to dry weight analysis, plant heights were measured throughout the experiment.

2.2 Plant Nutrition Study I

Seeds of *S. alterniflora* and rice (*Oryza sativa* L. cultivar Cypress) were planted in steam sterilized mineral soil supplied by LSU Greenhouse Services. Rice was included for comparative purposes because it is a grass that grows in flooded soils and its response to soil nutrient treatments has been well documented (Anon. 1987, Fageria et al. 1997). Five replications with various nutrient treatment levels were planted for *S. alterniflora* and four replications were planted for rice. Eighteen experimental treatments of nutrient enrichment were randomly assigned to 18 containers of steam-sterilized soil in each replication (Table 2.1). The containers were placed on concrete benches in a glasshouse located in the main campus greenhouse complex at LSU. Details of the experimental set-up, including containers, sources of N and P, and artificial seawater are described above. Fertilization rates within each container for N and P are listed in Table 2.2.

Table 2.1: Nitrogen and Phosphorus Treatment Combinations Used in Nutrition Study I

	Nutrient addition treatments					
P Levels	N Control	N Level 1	N Level 2	N Level 3	N Level 4	N Level 5
P Control	N0P0	N20P0	N40P0	N80P0	N160P0	N240P0
P Level 1	N0P49.5	N20P49.5	N40P49.5	N80P49.5	N160P49.5	N240P49.5
P Level 2	N0P99	N20P99	N40P99	N80P99	N160P99	N240P99

Table 2.2: Fertilization Rates of Nitrogen and Phosphorus for Treatment Levels Described in Table 2.1

Treatment	Fertilizer (N or P) (kg/ha)	Amount of nutrient added per container (mg)
Nitrogen Control	0	0
Nitrogen Level 1	20	142
Nitrogen Level 2	40	283
Nitrogen Level 3	80	566
Nitrogen Level 4	160	1132
Nitrogen Level 5	240	1698
Phosphorus Control	0	0
Phosphorus Level 1	49.5	188.5 dibasic
		139.6 monobasic
Phosphorus Level 2	99	377.0 dibasic
		279.3 monobasic

Seeds of *S. alterniflora* were harvested and stored as described above. Rice seeds were supplied by Dr. M.C. Rush in the Department of Plant Pathology and Crop Physiology at LSU.

Thirty-six seeds were planted in each container by placing them on the soil surface on July 10, 2003. Seedlings were removed randomly from each container on July 18, 2003 to reduce the number of plants per container to 15. Plant heights for the tallest four plants per container were recorded on August 20, 2003, because many shoots had not yet emerged from the water. Harvesting of roots and shoots of *S. alterniflora* and rice plants began on August 21 and ended August 26, 2003. During harvesting, plants were removed from the containers by gently washing

away the soil from the roots with tap water. After harvesting, whole plants were washed twice in deionized water, and the number of plants per container was recorded. Then, the roots were separated from the shoots and both placed in separate labeled paper bags. Plant material was weighed after oven drying at 65ºC for 48 hours. Dry shoots were ground, and N analysis was performed by the LSU Agricultural Center Soil Testing and Plant Laboratory using dry combustion with a Leco N Analyzer (St. Joseph, MI).

2.3 Plant Nutrition Study II

The plant nutrition study was repeated as described above from November 17, 2003 through February 12, 2004. Differences in the experimental design were restricted to the number of replications (four replications each for S. alterniflora and rice) and the nutrient treatment levels used, which are listed in Table 2.3. Fertilization rates for each nutrient treatment level corresponded to those previously listed (Table 2.2).

Table 2.3: Nitrogen and Phosphorus Treatment Combinations Used in Nutrition Study II

	Nutrient addition treatments		
P Levels	N Level 3	N Level 4	N Level 5
P Control	N80P0	N160P0	N240P0
P Level 1	N80P49.5	N160P49.5	N240P49.5
P Level 2	N80P99	N160P99	N240P99

2.4 Time Course Study II

Each of two experimental treatments of nutrient enrichment (control and N plus P) was randomly assigned to four containers of steam-sterilized soil in each sampling group for a total of eight containers per sampling group. Nutrient treatment levels used in this study were the N level 5 plus P level 1 (N240P99) and a control (N0P0) (Table 2.2). These rates were chosen based upon findings from the initial time course study and the first nutrition study. This

experiment was expanded to four replications per treatment and reduced from ten sampling periods to four in order to establish a growth curve.

Seeds were harvested, stored, and sorted as described above. Thirty-six seeds were planted in each container by placing them on the soil surface on November 17, 2003. Seedlings were randomly removed from each container on December 2, 2003 to reduce the number of plants per container to 15. Sample group one was harvested 56 days after planting (January 12, 2004) when a majority of seedlings had four or five true leaves. Harvesting continued at 8-day intervals through sample group 4 (February 5, 2004). Plants were harvested and dried as described above. In addition, height measurements for each shoot were recorded at the time of harvest.

2.5 Temperature Study

The effect of temperature on seedling growth was examined using five diurnally lighted growth chambers. Each growth chamber was maintained at one of five temperatures (15, 20, 25, 30, and 35ºC), and each was set for a 12-hour photoperiod. Three thermometers were placed in each chamber, and a hand held photometer (Phytotronics Inc., St. Louis, MO) was used for light intensity readings taken at each shelf level.

The nutrient level used in this study was the N240P49.5 treatment (Table 2.2). This rate was chosen based upon findings from the initial time course study and the first nutrition study. Seeds were harvested, stored, and selected and nutrients were incorporated into the steam-sterilized soil as described above. Also, the same containers and protocols described above were used. Three experimental units were randomly assigned to four sampling groups for a total of 12 containers per growth chamber. Four sampling groups were planted to establish growth curves over time.

Thirty-six seeds were planted in each container by placing them on the soil surface on October 6, 2003. The seeds were allowed to germinate and grow until all containers were harvested on January 5, 2004. One harvest was conducted, instead of the originally intended four, because of a small number of plants per temperature treatment, and only shoots were harvested. Height at the time of harvest and shoot dry weights were recorded after oven drying at 65ºC for 48 hours.

2.6 Statistical Analysis

Plant dry weights from the initial time course nutrition study were assessed graphically for differences among nutrient treatments and between steam-sterilized soil and raw soil because there were no replications. All remaining analyses were conducted using Minitab Release 14, in which univariate analysis of variance (ANOVA) was used to compare treatment differences. Tukey's pairwise comparisons with a 95% simultaneous confidence interval also were used to determine significant differences ($P = 0.05$) among nutrient treatment levels and temperature treatments, respectively.

3. RESULTS

3.1 Microbial Interactions, Including Seed and Seedling Diseases

Discoloration of primary and secondary roots, and seed rot were not observed. Observations of cultures of fungi that grew from root sections plated onto water agar and acidified potato dextrose agar and identification of these fungi based on spore type, did not reveal the presence of seed or seedling pathogens. Fungi were primarily saprophytic species including *Penicillium, Trichoderma,* and *Rhizopus* spp. Typical seed and seedling pathogens, including *Pythium* spp. were rarely observed and not associated with seedlings displaying stalled growth. These findings indicated stalled seedling growth was not disease related, which prompted the hypothesis that the lag phase of seedling growth may be a result of nutritional deficiencies. This led to the examination of roots from plants collected in the marsh for mycorrhizal infection because of the known nutritional benefits resulting from this association. No mycorrhizal infection was observed.

3.2 Initial Time Course Nutritional Study

Differences in plant growth related to nutrient treatments were apparent 45 days after planting (40 days after germination). Visual observations revealed that the N + P treatment in steam-sterilized soil had the effect on plant height (data not shown). N + P in steam-sterilized soil produced the greatest individual shoot dry weights along with the tallest plants, followed by the N + P treatment in raw soil and N only in steam-sterilized soil treatments (Fig 3.1). No changes in shoot dry weights were observed throughout the experiment for the remaining nutrient treatments in either steam-sterilized or raw soil.

Graphical analysis of individual root dry weights indicated that the N + P in raw soil treatment had the greatest effect on increasing root growth (Fig 3.2). N + P and N only in steam

sterilized soil ranked second and third, respectively, in affecting dry root weight. The remaining treatments had minute to no effects on dry root weight. Unfortunately, because of extremely diminished growth of the seedlings, plants had to be combined for analysis, and replicates were not available for statistical analysis.

No visual observations were noted during the experiment that would indicate a pathogenic involvement inhibiting seedling growth or causing premature plant death. Likewise, root and shoot individual dry weights did not give reason to suspect pathogenic involvement inhibiting seedling growth. Visual inspection of roots showed no signs or symptoms of disease for all nutrient and both soil treatments.

3.3 Time Course Study II

3.3.1 Mean Shoot Height

There were significant differences in shoot height among treatments ($P<0.000$). Shoot heights for the control group showed no increase in growth throughout the 80-day experiment (Fig. 3.3). Mean shoot heights for the N240P49.5 treatment seedlings continually increased throughout the experiment (Fig. 3.3). Analysis of sampling periods using Tukey's pairwise comparisons with 95% simultaneous confidence intervals found significant increases in mean shoot heights for seedlings in the N240P49.5 treatment with sample periods. These increases in shoot heights occurred between the first (56 days) and the remaining three sample periods, the second (64 days) and the last sample period, and between the third (72 days) and the last sample period (80 days).

3.3.2 Mean Shoot Weight

Shoot dry weights for the N240P49.5 treatment increased significantly ($P < 0.000$) over time when compared to the control. The control group showed no increase in dry weight over time (Fig. 3.4). Seedlings in the N240P49.5 treatment showed a continual increase in shoot

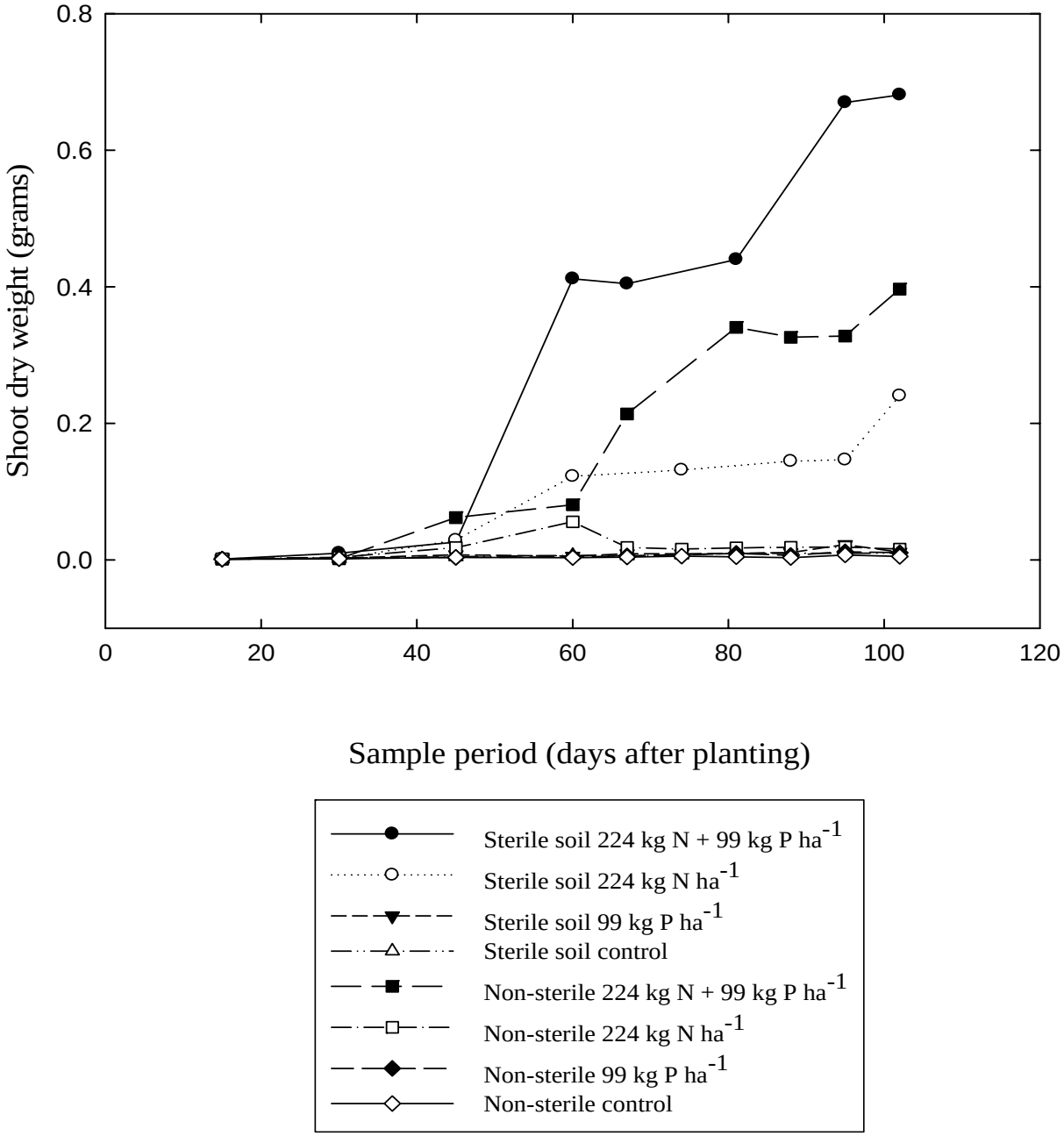

Figure 3.1. The effects of nitrogen and phosphorus nutrient treatments on *Spartina alterniflora* dry shoot weight over time for the initial time course study.

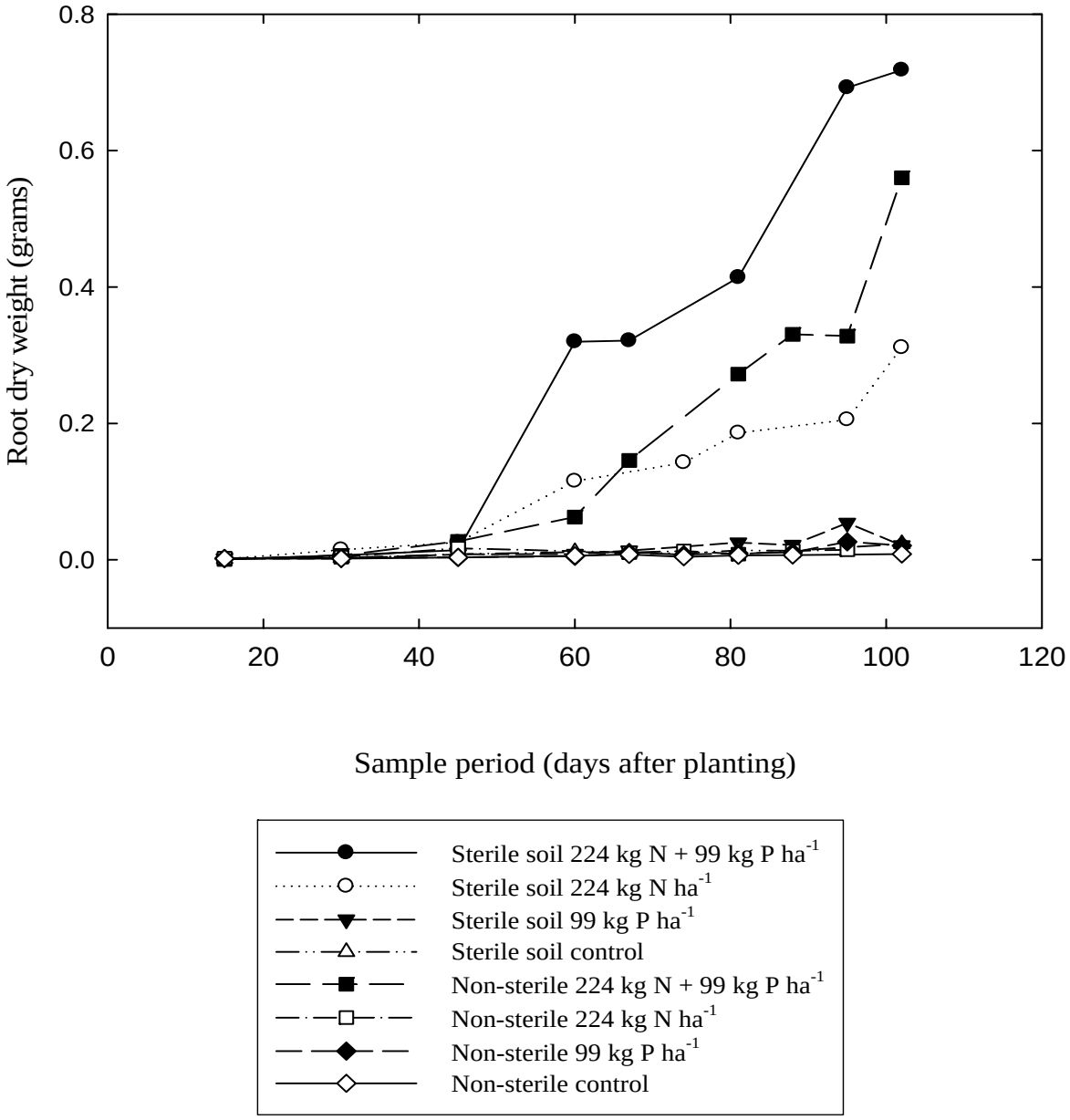

Figure 3.2. The effects of nitrogen and phosphorus nutrient treatments on *Spartina alterniflora* dry root weight over time for the initial time course study.

weight at each sampling period (Fig. 3.4). Analysis of sampling periods using Tukey's pairwise comparisons with 95% simultaneous confidence intervals found significant differences in mean shoot weights among sample periods for the N240P49.5 treatment. These occurred between the first (56 days) and the remaining three sample periods, the second (64 days) and the last sample period, and between the third (72 days) and the last sample period (80 days).

3.3.3 Mean Root Weight

Sample period and the N240P49.5 treatment had a significant ($P < 0.000$) effect on root dry weight. The control group showed no increase in root weight over time (Fig. 3.5). Root weights in the N240P49.5 plants increased continuously at each sampling period (Fig. 3.5). Analysis of sampling periods using Tukey's pairwise comparisons with 95% simultaneous confidence intervals found significant differences in mean root weights among sample periods for the N240P49.5 treatment. These occurred between the first (56 days) and the remaining three sample periods, the second (64 days) and third (72 days) and fourth (80 days) sampling periods, and between the third (72 days) and the last sample period (80 days). Analysis of sampling periods using Tukey's pairwise comparisons with 95% simultaneous confidence intervals did not detect significant differences between the control first and second sampling periods and the corresponding N240P49.5 sampling periods.

3.3.4 Root to Shoot Ratios

Root to shoot ratios (R:S ratios) were significantly higher ($P= 0.033$) for plants in the control group throughout the experiment (Fig. 3.6).

3.4 Plant Nutrition Study I

3.4.1 Mean Shoot Height

Height of *S. alterniflora* shoots was significantly ($P<0.000$) effected by nutrient treatment (Fig. 3.7). Two-way analysis of variance also indicated a significant interaction effect

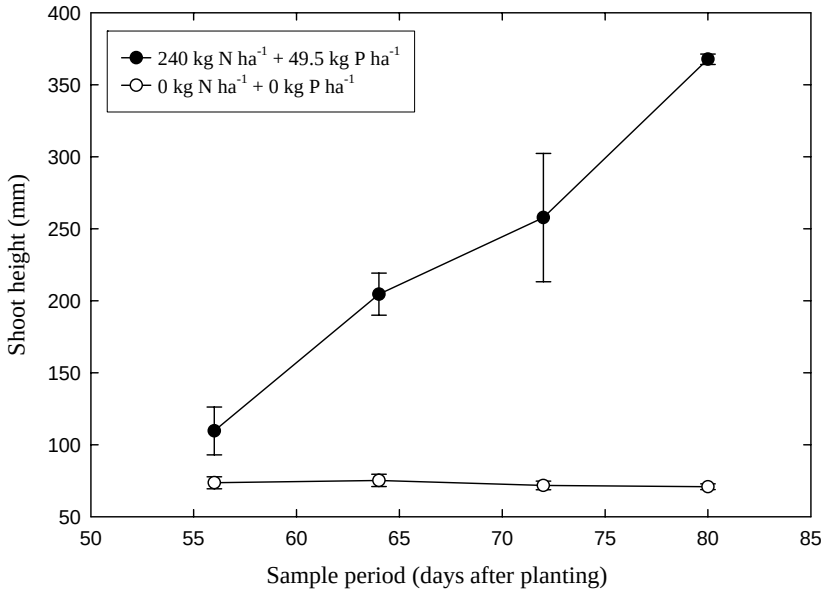

Figure 3.3. The effects of nitrogen and phosphorus nutrient treatments on *Spartina alterniflora* shoot height over time for time course study II. Bars indicate standard errors, where larger than the symbols.

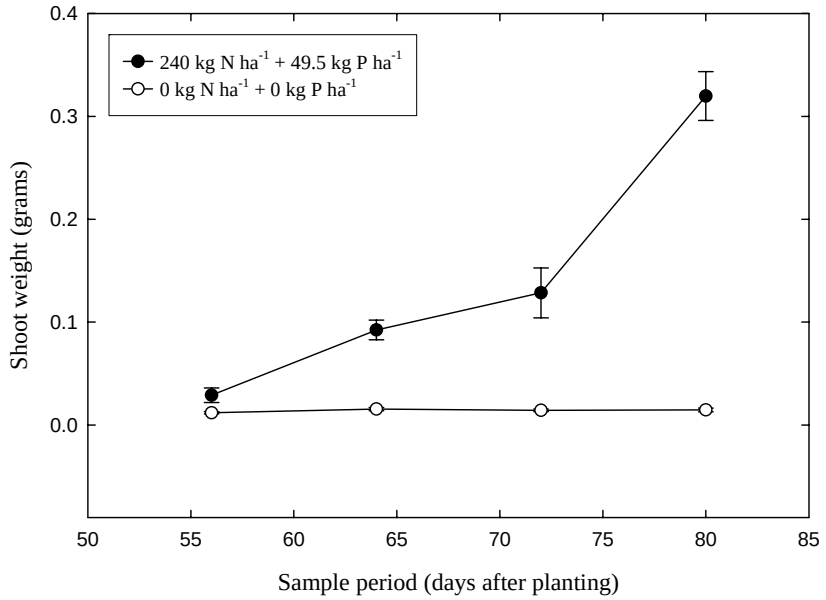

Figure 3.4. The effects of nitrogen and phosphorus nutrient treatments on *Spartina alterniflora* dry shoot weight over time for time course study II. Bars indicate standard errors, where larger than the symbols.

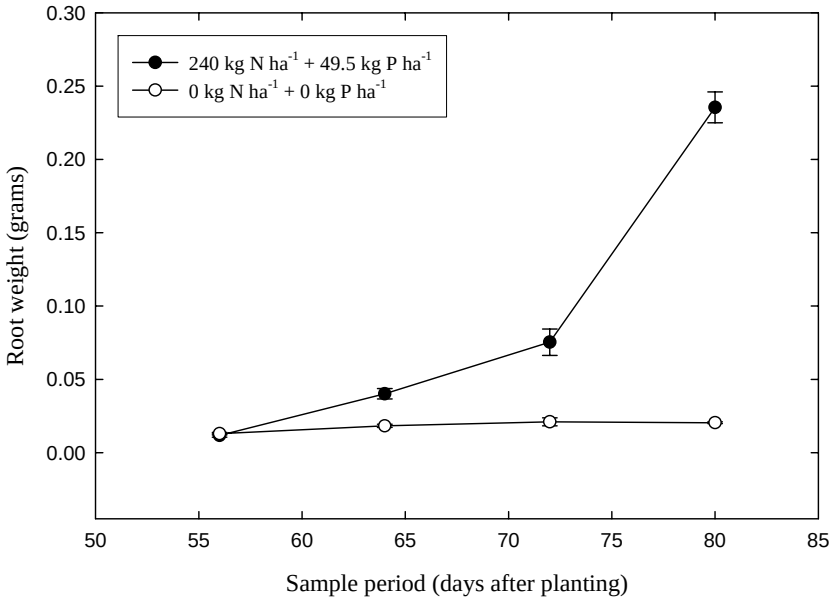

Figure 3.5. The effects of nitrogen and phosphorus nutrient treatments on *Spartina alterniflora* dry root weight over time for time course study II. Bars indicate standard errors, where larger than the symbols.

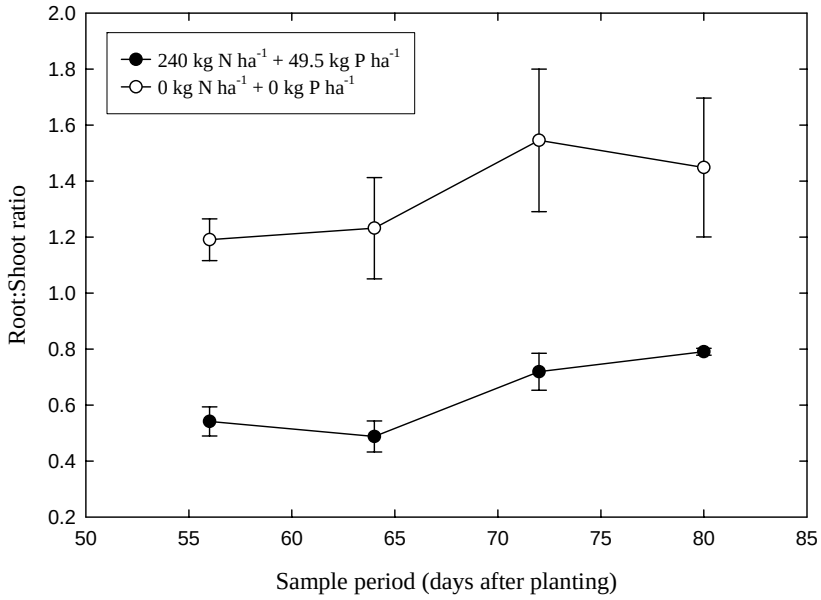

Figure 3.6. The effects of nitrogen and phosphorus nutrient treatments on weight based *Spartina alterniflora* root:shoot ratios over time for time course study II. Bars indicate standard errors, where larger than the symbols.

($P < 0.000$) between N and P levels on shoot height. Mean shoot height for the N240P49.5 treatment was significantly different ($P<0.000$) from all treatments except the N160P49.5 and N160P99 treatments. Average shoot height decreased with an increase in N level between the N160P99 treatment and the N240P99 treatment (Fig. 3.7). The increase in N level between the N160P49.5 and the N240P49.5 treatment did not result in a significant increase in mean shoot height (Fig. 3.7).

Two-way analysis of variance for mean heights of rice shoots indicated significant N treatment effects ($P < 0.000$), but did not indicate a significant P treatment effect ($P = 0.143$) or a significant interaction effect between N and P ($P = 0.716$) (Fig 3.8). While the increase in N level produced a significant increase in mean shoot height, mean shoot heights among P treatments, for each respective N treatment level, did not differ significantly (Fig 3.8).

3.4.2 Mean Shoot Dry Weight

Shoot dry weights of *S. alterniflora* increased considerably with added N and P. Graphical analysis indicated significant treatment effects for N and P. Two-way analysis of variance also indicated a significant interaction effect between N and P ($P <0.000$) on shoot dry weights (Fig. 3.9). The N4P1 treatment produced the greatest increase in shoot weight over the control and was significantly different from all treatments excluding N80P99, N240P49.5, N160P99, and N240P99. Shoot weights for plants receiving no P supplements remained constant among all N treatment levels, with no significant difference among the nitrogen treatments ($P=0.627$).

Two-way analysis of variance for mean shoot dry weights for rice plants emulates the findings of the rice shoot height analysis. Significant N treatment effects ($P < 0.000$) supported the graphical analysis (Fig. 3.10) in which increased N increased shoot weight. P had no effect

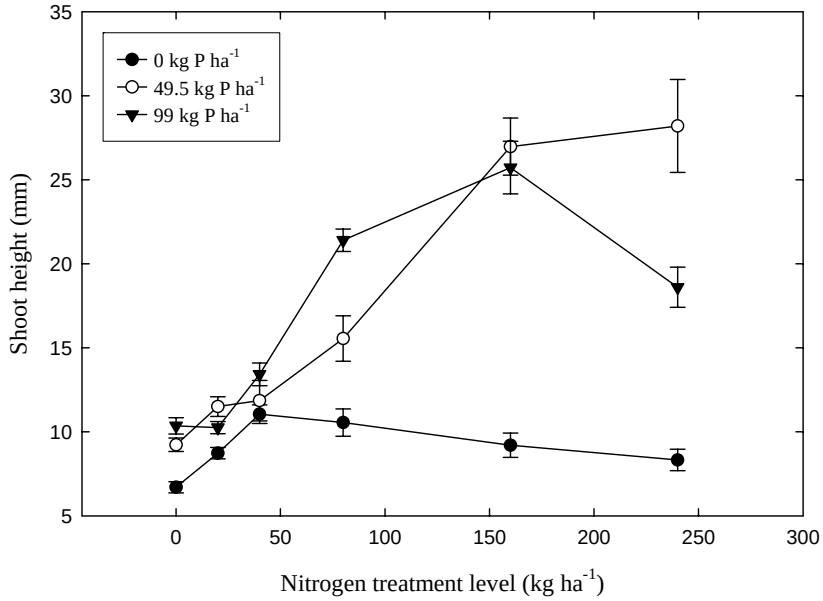

Figure 3.7. The effects of nitrogen and phosphorus nutrient treatments on *Spartina alterniflora* shoot height for plant nutrition study I. Bars indicate standard errors, where larger than the symbols.

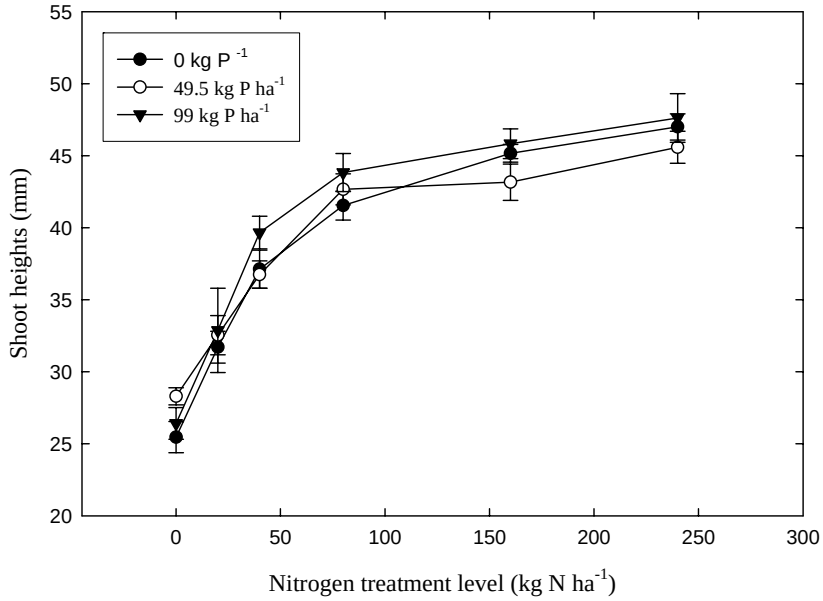

Figure 3.8. The effects of nitrogen and phosphorus nutrient treatments on rice shoot height for plant nutrition study I. Bars indicate standard errors, where larger than the symbols.

on treatment effects ($P = 0.103$). A significant interaction effect between N and P treatments ($P = 0.302$) was not evident. While the increase in N level produced a significant increase in mean shoot dry weights, dry weights among P treatments for each respective N treatment level did not differ significantly (Fig 3.10).

3.4.3 Mean Root Dry Weight

Root growth, as measured by root dry weight, of *S. alterniflora* responded to N and P supplements ($P = 0.001$ and <0.000, respectively). Two-way analysis of variance indicated a significant interaction effect ($P < 0.016$) between N and P levels on root dry weight. Graphical analysis of *S. alterniflora* mean root dry weights (Fig. 3.11) supported the findings, indicating significant interaction between combined N and P. High N levels increased root dry weight when P was added, but had no effect in the absence of P. Root weights for plants receiving no supplemental P nutrition showed no significant difference in growth among all N treatment levels ($P=0.568$).

As with rice dry shoot weights, two-way analysis of variance for rice dry root weights also indicated no significant P treatment effects ($P = 0.105$) or a significant interaction effect between N and P treatments ($P = 0.120$). Significant N treatment effects ($P < 0.000$) supported the graphical analysis (Fig. 3.12) that increased N resulted in increases in root weight. While the increase in N level produced a significant increase in mean root dry weights over the control, dry weights among P treatments for each respective N treatment level showed no significant differences (Fig 3.12).

3.4.4 Root to Shoot Ratios

Graphical analysis showed a general decrease in weight based R:S ratios for *S. alterniflora* as N levels increased (Fig. 3.13). Statistically, there was a significant N treatment

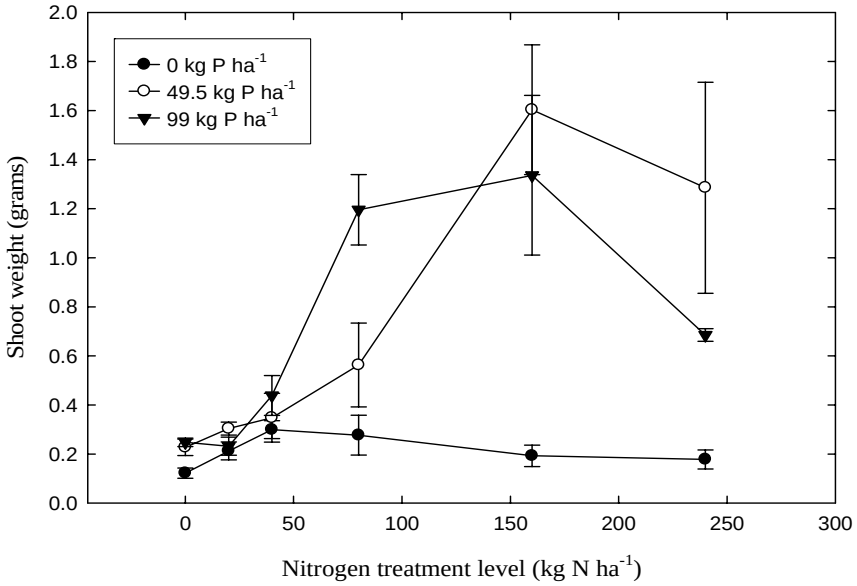

Figure 3.9. The effects of nitrogen and phosphorus nutrient treatments on *Spartina alterniflora* dry shoot weight for plant nutrition study I. Bars indicate standard errors, where larger than the symbols.

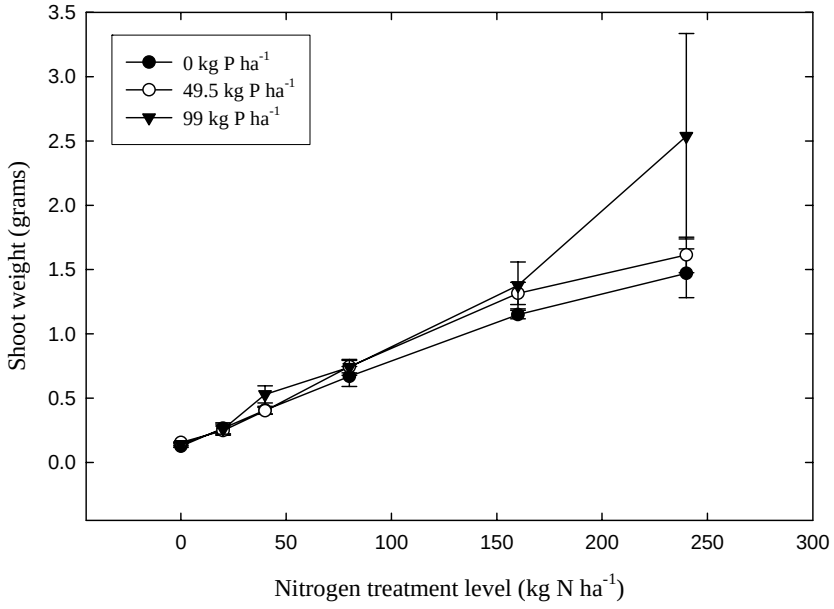

Figure 3.10. The effects of nitrogen and phosphorus nutrient treatments on rice dry shoot weight for plant nutrition study I. Bars indicate standard errors, where larger than the symbols.

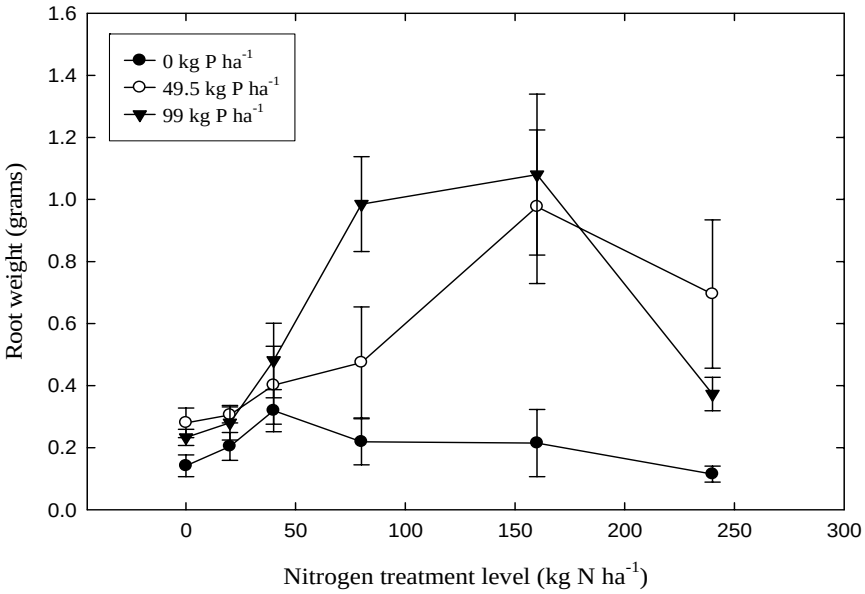

Figure 3.11. The effects of nitrogen and phosphorus nutrient treatments on *Spartina alterniflora* dry root weight for plant nutrition study I. Bars indicate standard errors, where larger than the symbols.

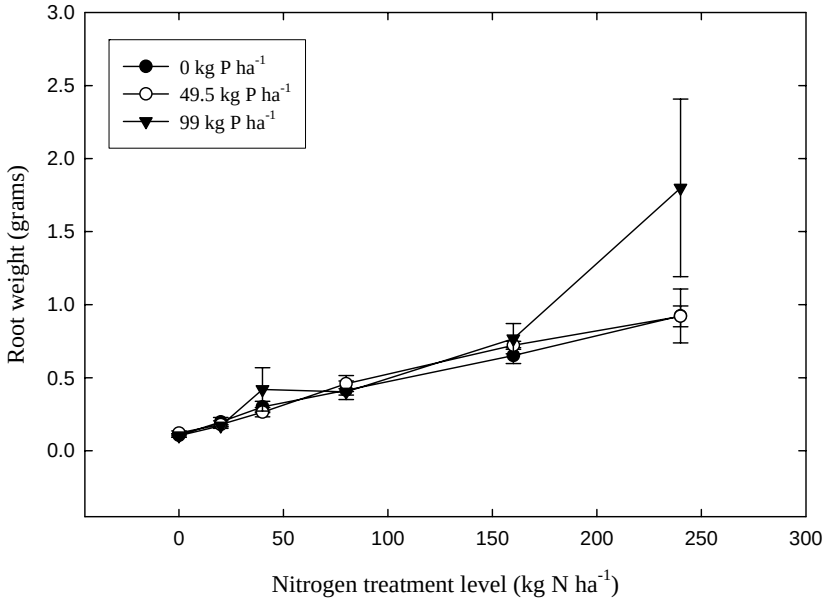

Figure 3.12. The effects of nitrogen and phosphorus nutrient treatments on rice dry root weights for plant nutrition study I. Bars indicate standard errors, where larger than the symbols.

level effect on R:S ratios (P = 0.005), but there was not a significant P treatment level effect (P= 0.788). Also, two-way analysis of variance failed to show an interaction effect among N and P treatment levels on *S. alterniflora* R:S ratios (P = 0.735).

Rice R:S ratios also showed a general decrease as N levels increased (Fig. 3.14). Rice plants were able to utilize added N without requiring supplemental P. Graphical analysis was confirmed by two-way analysis of variance findings for no significant P treatment level effect on R:S ratios (P = 0.689) and no interaction effects among N and P treatment levels on rice R:S ratios (P = 0.964). However, N treatment level had a significant effect on R:S ratios (P = 0.001).

3.4.5 Percent Leaf Nitrogen

Graphical analysis of the mean percent N content of *S. alterniflora* leaf tissue showed increases in N content as N treatment levels increased (Fig. 3.15). However, this increase in leaf tissue N did not begin until N treatment levels reached 80 kg N ha^{-1}, at which point differences among P treatment levels also can be observed. Two-way analysis of variance indicated significant N (P < 0.000) and P (P < 0.000) treatment level effects on percent leaf N. Statistical analysis also indicated a strong interaction effect among N and P treatment levels (P < 0.000) on the percent leaf N of *S. alterniflora*.

Increases in N treatment level resulted in substantial increases in the percent N found in rice leaf tissue (Fig. 3.16). There was no indication of a P treatment level effect on leaf tissue N content in the graphical analysis, which corroborated the findings of the two-way analysis of variance for no significant effect for P treatment level (P = 0.063). The effect of N treatment level on rice leaf tissue N content was significant (P < 0.000), but no significant interaction effect (P = 0.907) between N and P treatment levels was observed.

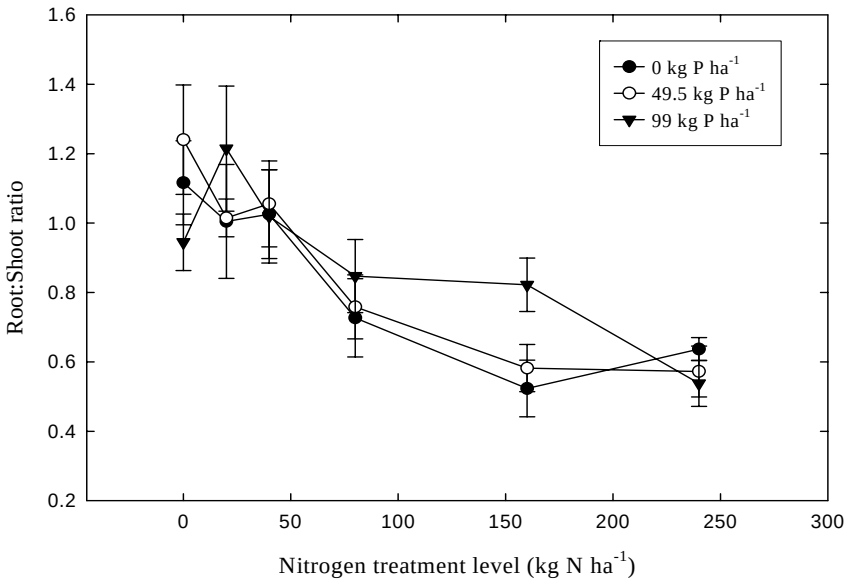

Figure 3.13. The effects of nitrogen and phosphorus nutrient treatments on weight based *Spartina alterniflora* root:shoot ratios for plant nutrition study I. Bars indicate standard errors.

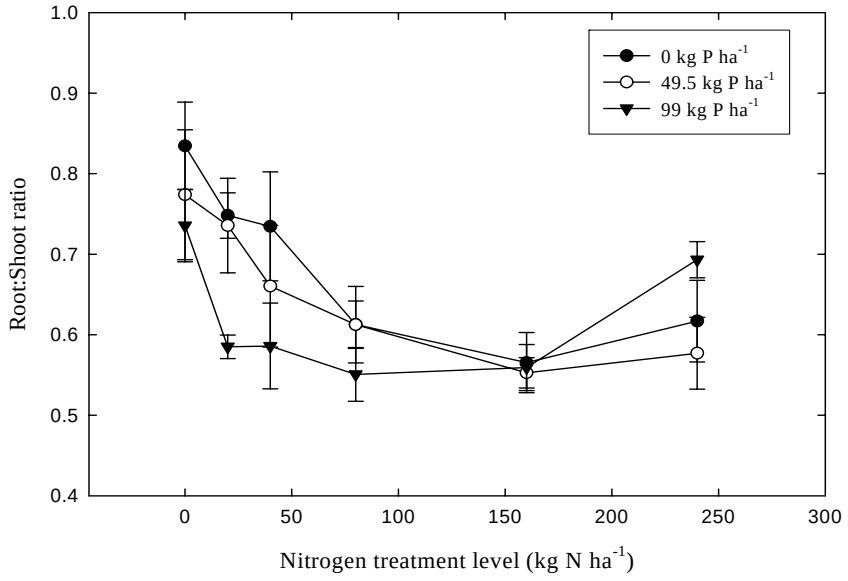

Figure 3.14. The effects of nitrogen and phosphorus nutrient treatments on weight based rice root:shoot ratios for plant nutrition study I. Bars indicate standard errors.

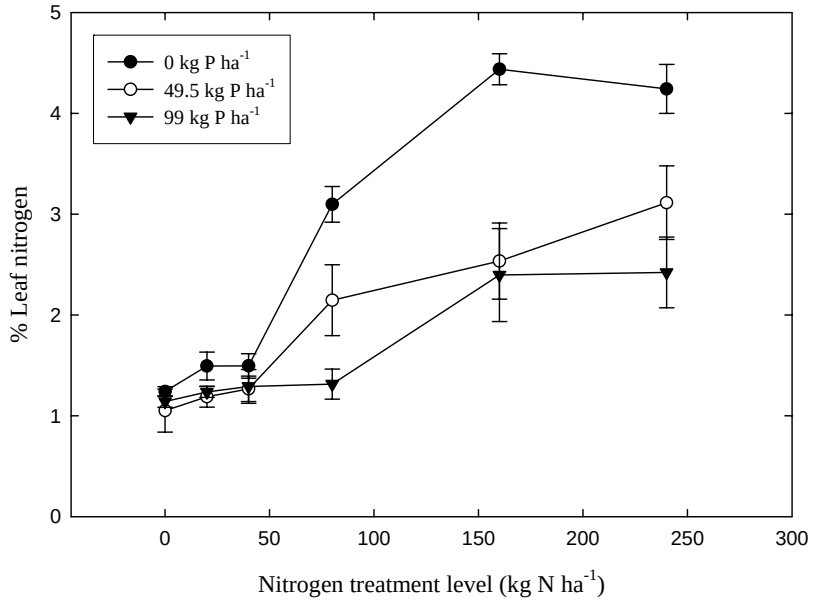

Figure 3.15. The effects of nitrogen and phosphorus nutrient treatments on *Spartina alterniflora* percent leaf nitrogen for plant nutrition study I. Bars indicate standard errors, where larger than the symbols.

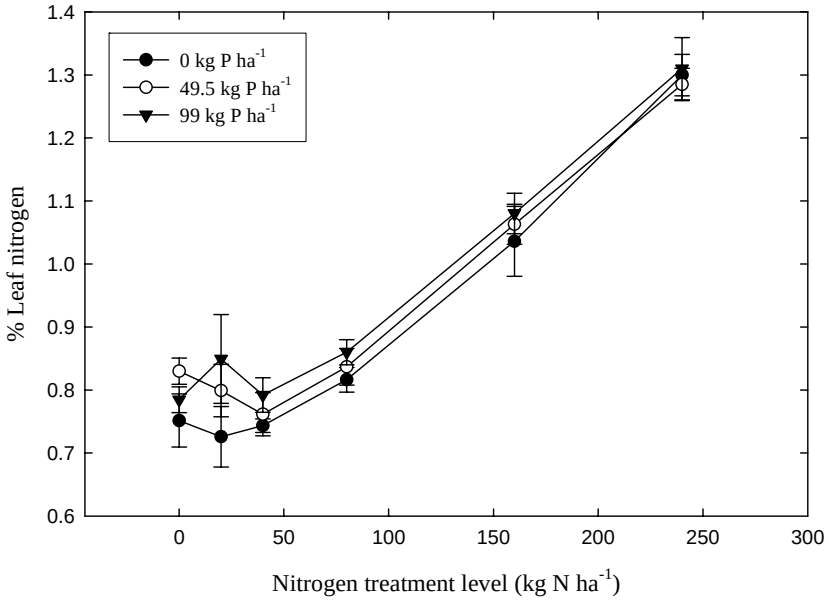

Figure 3.16. The effects of nitrogen and phosphorus nutrient treatments on rice percent leaf nitrogen for plant nutrition study I. Bars indicate standard errors, where larger than the symbols.

3.4.6 Nitrogen Uptake Efficiency Rates

Nitrogen uptake efficiency (NUE) rates were calculated for *S. alterniflora* and rice plants for the first nutrition study. These rates were calculated according to the method described by Lee et al. (2004) in which NUE = shoot dry weight divided by % leaf N. These values provide an indication for comparative purposes of relative nitrogen use efficiencies. Results are shown in Fig. 3.17.

S. alterniflora exhibited maximum NUE value range of 0.5 to 1.0 for the 49.5 and 99 kg P ha^{-1} amendments (Fig. 3.17). In contrast NUE for rice continued to increase with increasing levels of N up to 160 kg N ha^{-1}. At that point, NUE's values for 0 and 49.5 kg P ha^{-1} plateaued, and additional N was not efficiently used. However, at 99 kg P ha^{-1}, NUE continued to increase (Fig. 3.17).

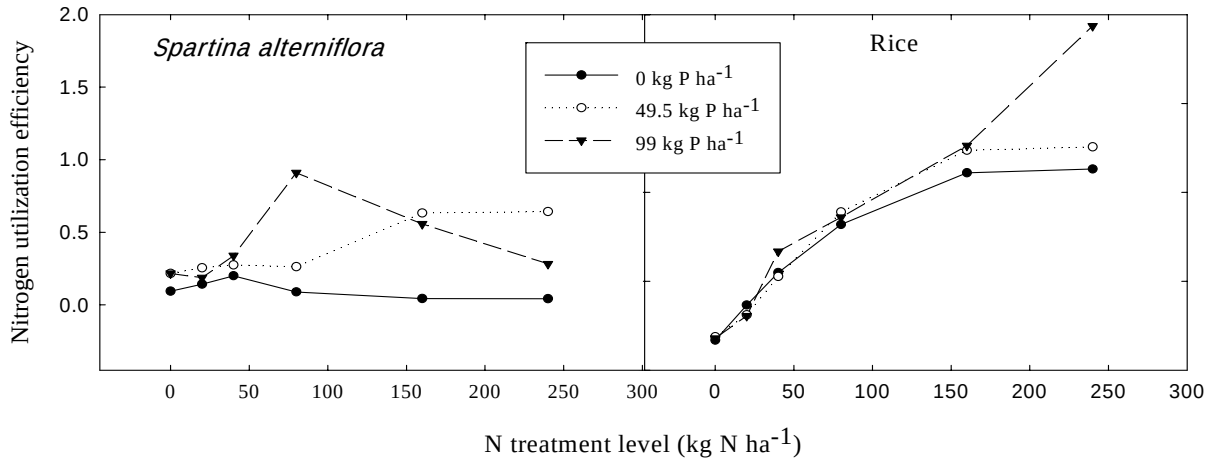

Figure 3.17. The effects of nitrogen and phosphorus nutrient treatments on nitrogen utilization efficiencies (shoot dry weight / % leaf N) for *Spartina alterniflora* and rice for plant nutrition study I.

Results from plant nutrition study II were similar to the first nutrition study. Graphical results are presented in Appendix C.

3.5 Temperature Study

3.5.1 Mean Shoot Height

S. alterniflora shoot heights increased with increasing temperatures until a maximum was reached at 25ºC. Further increases in temperature resulted in decreased mean shoot height (Fig. 3.18). No seedlings survived at the 35ºC treatment. One-way analysis of variance of shoot height versus temperature indicated a significant ($P = 0.005$) effect of temperature on shoot height.

3.5.2 Mean Shoot Weight

A graphical analysis of increasing temperature versus *S. alterniflora* shoot weights showed a maximum was attained at 25ºC followed by a decline in shoot weights (Fig. 3.19). Likewise, one-way analysis of variance for shoot weight versus temperature treatment showed temperature does significantly affecting shoot weight ($P = 0.039$).

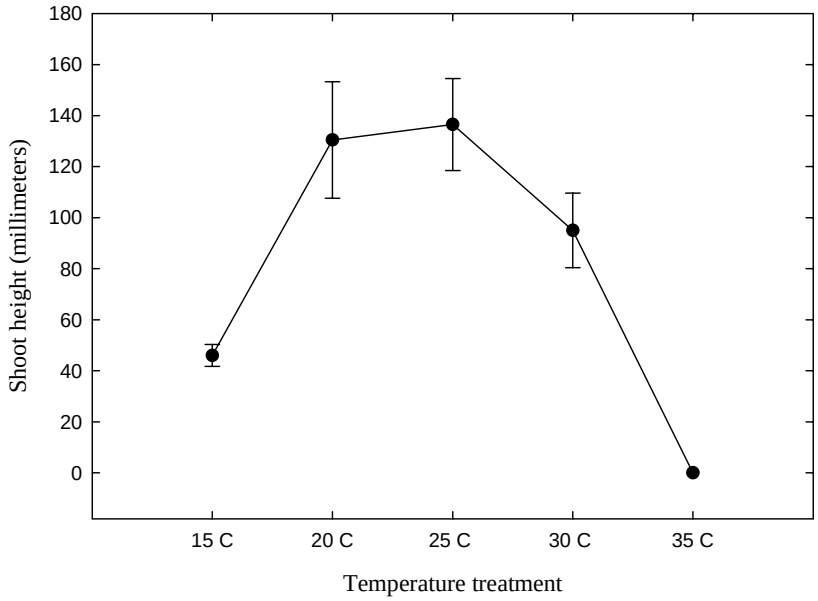

Figure 3.18. The effects of temperature on *Spartina alterniflora* shoot height. Bars indicate standard errors, where larger than the symbols.

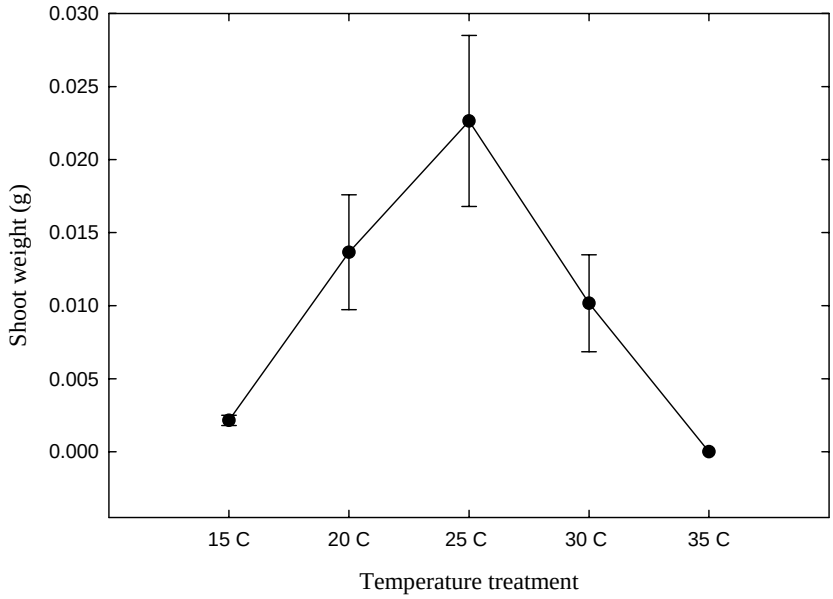

Figure 3.19. The effects of temperature on *Spartina alterniflora* dry shoot weight. Bars indicate standard errors, where larger than the symbols.

4. DISCUSSION

Successful colonization of barren mud flats and newly created coastal marshlands in Louisiana by *S. alterniflora* is an essential tool in combating coastal erosion and natural marsh subsidence. The potential for aerial seeding of *S. alterniflora* presents the possibility for substantial savings in labor and costs associated with growing transplants that must be hand transplanted in the marsh. Although the successful use of *S. alterniflora* seedlings as a means of propagation has been documented (Broome and Seneca 1974, Broome et al. 1975b), delayed or stalled seedling growth, which has so far been an inhibitory factor in aerial seeding, have not been previously described.

4.1 Microbial Interactions, Including Seed and Seedling Diseases

It was initially proposed that the stalled seedling growth may have been related to unknown seed or seedling diseases. There is limited information in the literature describing seedborne fungi for *S. alterniflora.* One survey conducted by Gessner (1978) mentions the presence of general decomposers of marine plant debris, but no pathogenic fungi were described. My experiments found that no fungal pathogens were present. These findings indicate stalled seedling growth probably is not disease related.

To further examine the role of microbial interactions affecting seedling growth, roots of mature *S. alterniflora* plants were assessed for the presence of mycorrhizal fungi. It is well documented that symbiotic relationships occur between many plant species and mycorrhizal fungi, which benefit the plant hosts through increased absorption of nutrients, especially P and minor elements (Abel-Fattah 2002). Investigations of other *Spartina* species, including *S. patens* (Burke et al. 2002) and *S. maritime* (Carvalho et al. 2001) found mycorrhizal associations.

However, no evidence of mycorrhizal fungi was found in the present study. This led to the hypothesis that the lag phase in seedling growth was a result of nutritional deficiencies.

4.2 Time Course and Nutritional Study

The initial time course nutritional study was conducted as a range finding experiment to determine the length and document the lag phase in seedling growth. Initially the test was designed so each plant within each treatment would serve as a replication. Unfortunately, because of the extremely small seedlings, plants had to be combined for analysis, and replicates were not available. However, it was observed, and then documented through replications during the second time course nutritional study, that additions of N and P shortened the lag phase from over 100 days to less than 56 days. Emergence from the stalled seedling growth phase was never observed in the absence of added nutrients.

The growth responses of *S. alterniflora* seedlings to N and P fertility treatments provided insight into difficulties associated with establishing *S. alterniflora* marshes from seed in coastal Louisiana. Results from this study indicated N and P nutrient additions are important for *S. alterniflora* seedling establishment. Soil P levels measured in this study were higher than P levels reported by Charbreck (1972) and Mendelssohn and Kuhn (2003) for coastal marsh soils of Louisiana (Appendix D). Despite higher P levels present, *S. alterniflora* seedlings required additional P for increased growth. N (224 kg ha^{-1}) and P (49 kg ha^{-1}) additions to dredge spoil were reported by Broome et al. (1975b) to have a substantial effect on increasing seedling dry weight during the growing season (3470 kg ha^{-1} to 10800 kg ha^{-1}), while a N (224 kg ha^{-1}) addition alone had a smaller effect on increasing seasonal growth (3470 kg ha^{-1} to 9340 kg ha^{-1}). Additional studies indicated P is second only to N in limiting growth in *S. alterniflora* seedlings (Broome and Seneca 1974) and established *S. alterniflora* stands (Broome et al. 1975a, Broome

et al. 1983, DeLaune and Pezeshki 1988, Foret 2001, Smart and Barko 1980). Other investigations of the role of N and P nutrition in established *S. alterniflora* stands concluded that availability of P does not limit plant growth (Buresh et al. 1980, DeLaune and Patrick 1980, Patrick and DeLaune 1976, Sullivan and Daiber 1974). However, none of these studies examined the effects of nutrients on seedling growth from germinating seeds, which would be required for aerial seeding of marsh sites.

Root and shoot growth responses from all nutrition experiments in this study indicated that *S. alterniflora* was very sensitive to N and P supplements in the single soil type used, and it may be necessary to add these nutrients to marsh soils if direct seeding is employed. Alternatively, it may be possible to soak seeds in nutrient solutions as a means of providing these limiting elements. Pelleting seeds with appropriate N and P formulations is probably not practical because seeds must be kept wet in order to preserve seed viability (Broome and Seneca 1974, Harrison et al. 2003, Plyer and Carrick 1993, Plyer and Proseus 1996, Seneca and Broome 1972).

Results from the initial time course experiment indicated the N + P treatment resulted in a greater increase in whole plant biomass. Although growth remained stalled during the initial 40 days after germination, growth during the remaining 62 days of the experiment was substantially greater than the nonamended control. The N only treatment in steam-sterilized soil showed increased growth over the remaining N only treatments. The plants receiving only N grew at a slower pace and did not attain the height or biomass of those in the N plus P treatments. These findings suggest that P is a limiting nutrient during the seedling phase of *S. alterniflora* growth. Results from this initial nutrition study were expanded upon in subsequent investigations to determine the specific N and P fertilizer rates required by *S. alterniflora* to overcome stalled

seedling growth. In particular, tissue concentrations reported in this study can be used to assess field grown plants, although it will be necessary to calibrate field and greenhouse results.

Two levels of P (49.5 kg P ha^{-1} and 99 kg P ha^{-1}) were used in the first nutrition study, along with a P control (0 kg P ha^{-1}), to determine the level of P that produced the greatest response in plant growth. All three P levels were used in combination with varying levels of N fertilizer amendments. The P levels were selected based on previous research on the effects of P fertilizer amendment levels on increases in growth of mature *S. alterniflora* plants (Broome et al. 1983) and on *S. alterniflora* seedlings (Broome et al. 1975b). Results from the first nutrition study showed that P applied at 49.5 kg ha^{-1} combined with N applied at a rate of 240 kg N ha^{-1} increased shoot height and shoot and root dry weights significantly more than the P control.

Established N and P nutritional requirements for rice, along with similarities between the growth habit and morphology of *S. alterniflora* and rice, led to the inclusion of rice as a standard for comparing *S. alterniflora* growth responses to N and P. P deficiency in rice is known to cause reduced seedling growth rate and emergence through water, reduced tillering, delayed maturity, reduced plant height, and reduced grain yield (Anon. 1987). These growth responses to P deficiency in rice were observed in *S. alterniflora*, particularly the delayed seedling growth. Likewise, rice P deficiency is known to occur in the coastal prairie soils of southwestern Louisiana, which are low in available soil P (Anon. 1987).

Fujiwara (1965) reported that decreased available P for rice suppresses early N uptake and prevents the synthesis of protein from nitrogenous materials, and he reported a plentiful supply of P promoted early growth in rice plants because of increases in the content of nucleic acid and phospholipids. Previous investigations failed to report whether or not the lack of available P prevents N uptake by *S. alterniflora* seedlings. My findings that an abundant

availability of P for *S. alterniflora* seedlings resulted in increased N uptake and consequently plant growth corroborated the findings of Broome et al. (1975b) who worked with seedling propagation on dredge spoil near Beaufort, North Carolina. Also, results from the present study suggested that *S. alterniflora* seedlings were not capable of adequate N uptake in the absence of higher P levels in the river silt soil used in these experiments.

These studies support the hypothesis that *S. alterniflora* seedlings experience the same suppressed growth response observed in rice seedlings grown in P deficient soils, although rice growth was not limited by P in the nonamended soils used in the present study. Rice appeared to grow to its maximum potential with regard to N availability in that tissue N concentrations were surprisingly similar across P treatments. Likewise, NUE values with increasing N levels under different P fertilization regimes provided a stark contrast between *S. alterniflora* and rice. The latter was able to utilize N far more efficiently then the former.

While P was not limiting for rice growth across N treatments, higher levels of tissue N in the lowest P treatment with *S. alterniflora* suggested that P was limiting when there was sufficient N available. *S. alterniflora* was not able to utilize higher levels of N, and N supplements above 80 kg N ha^{-1} were not efficiently used regardless of P status. Schulte and Kelling (1991) suggested plant growth declines when one nutrient supply is increased sufficiently as a result of an imbalance with other plant nutrients or because of toxic effects of the excessive nutrient. The marked repression in growth of *S. alterniflora* at the highest N and P treatment levels, as compared to rice, could be a result of an imbalance of plant nutrients or possibly a toxic effect of high P levels for *S. alterniflora* when sufficient N is not available. Phosphorus is rarely known to be toxic, but it is known to suppress the uptake of copper and zinc and be out of balance with respect to nitrogen or potassium when present at high levels (Schulte

and Kelling 1991). Furthermore, the present study showed that there is an optimum ratio of N and P in root and shoot growth and root:shoot ratios. NUE analyses clearly showed that fertilization of *S. alterniflora* in the marsh must be very carefully regulated with regard to excessive nitrogen applications.

It is likely that modern rice cultivars have been selected over many generations for fertilizer use efficiency. Given that *S. alterniflora* reproduces primarily via rhizomes, it does not rely upon the successful establishment of seedlings. Therefore, we would expect that there has been relatively little evolutionary selection for rapid seedling establishment and efficient nutrient uptake.

The levels of N and P used in these nutrition studies are within current fertilizer application rate recommendations for rice (Salton et al. 2002, Wilson et al. 1998). Recent analysis of P nutrition requirements for rice growth indicate that P applications made at preemergance, preflood, and post flood have significant effects on increasing yields compared to applications made at midseason (Salton et al. 2002). Salton et al. (2002) reported that it is critical to have sufficient P availability during the first 5 weeks, and especially during the first 2 weeks after flooding, to ensure high yield potential for direct-seeded, delayed flood, rice culture. Likewise, Wilson et al. (1998) concluded that increased preflood N applications results in increased total dry matter accumulation, grain yields, and a significantly greater ability for total N uptake.

4.3 Temperature Study

Temperature had a significant effect on *S. alterniflora* seedling growth. Seedlings produced the greatest shoot heights and biomass at 25ºC, and growth was suppressed at temperatures above and below the optimum. Other studies on the effects of temperature of *S.*

alterniflora seedling growth found taller plants and greater total plant dry weight when daytime temperatures ranged from 26 to 30ºC (Seneca and Blum 1984, Seneca and Broome 1972). This is consistent with the effect of temperature on seed germination observed by M.A. Cohn, seed physiologist, LSU Department of Plant Pathology and Crop Physiology (personal communication). These data suggest that breeding should select for seedling vigor at greater temperatures, since temperature in the field would be greater than optimal for plant growth.

5. CONCLUSION

There were significant differences in responses to supplemental N and P between rice and *S. alterniflora*. Rice shoots were efficient in utilizing the highest level of added N, whereas *S. alterniflora* displayed a pronounced decrease in growth at the highest N and P levels. Shoot weights were similar between the two species, but rice root growth responded readily to higher levels of N and P. Again, it is likely that fertilizer N and P supplements for direct seeding of *S. alterniflora* will have to be governed by soil analysis and extensive field investigations. Also, it may be possible to select for and breed *S. alterniflora* cultivars that are more responsive to fertilizer supplements. This strategy will have to be assessed critically because we would not want to develop cultivars that are dependent upon supplemental fertilizer additions other than those required to establish seedlings.

Through this study we were able to document the lag phase of *S. alterniflora* seedlings and conclude there is no evidence of pathogen involvement. Furthermore, it was observed that supplemental N and P aid in reducing the lag phase from over 100 days to less than 50 days for the soil type used in this study. However, nutrient additions are not able to completely overcome stalled seedling growth. Future research possibilities could include nutritional studies in native marsh soils and include other native *Spartina* species for a comparative analysis of seedling growth and response to N and P nutrient additions. Additional research possibilities include investigating the effects of plant growth regulators (hormones) and seed preconditioning treatments on seedling growth. Furthermore, the possibility of epicotyl dormancy in *S. alterniflora* should be investigated. Epicotyl dormancy is known to cause similar stalled growth periods in other plant species, and may be overcome through seedling stratification (M.A. Cohn, personal communication).

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APPENDIX A

RESULTS OF SOIL ANALYSES FOR STEAM STERILIZED AND RAW SOIL

Table A.1: Soil test results for steam sterilized and raw soil used in greenhouse experiments.

Soil component	Steam sterilized soil	Raw soil
NH_4^+	203 kg ha^{-1}	697 kg ha^{-1}
P	437 kg ha^{-1}	520 kg ha^{-1}
K	137 kg ha^{-1}	157 kg ha^{-1}
Ca	3107 kg ha^{-1}	3111 kg ha^{-1}
Mg	347 kg ha^{-1}	672 kg ha^{-1}
Na	56 kg ha^{-1}	54 kg ha^{-1}
% Organic matter	0.26	0.30
pH	8.3	8.2

APPENDIX B

ASSAY CHART FOR AVERAGE SOLUTION OF CRYSTAL SEA MARINEMIX HYDRATED TO A DENSITY OF 1.025 USING DISTILLED WATER. (SUPPLIED BY MARINE ENTERPIRSES INTERNATIONAL, BALTIMORE, MY.

Chemical/Element	Concentration (ppm)	Chemical/Element	Concentration (ppm)
Chloride	18,600	Scandium	trace
Sodium	10,400	Nickel	0.0002
Sulfate	2,600	Radium	trace
Magnesium	1,290	Thallium	0.00007
Calcium	410	Neodymium	trace
Potassium	380	Iron	0.01
Bicarbonate	149	Helium	trace
Strontium	12.5	Cobalt	0.0001
Carbonate	10	Palladium	trace
Bromide	6	Beryllium	trace
Boron	4.4	Neon	trace
Fluoride	1.5	Praseodymium	trace
Silicon	2.8	Mercury	0.0003
Barium	0.05	Yttrium	trace
Nitrogen	0.00	Ruthenium	trace
Zinc	0.014	Molybdenum	0.01
Lithium	0.11	Samarium	trace
Argon	trace	Tantalum	trace
Aluminum	0.17	Silver	0.003
Rubidium	0.19	Xenon	trace
Copper	0.001	Gold	trace
Tin	0.003	Antimony	0.0003
Bismuth	trace	Indium	trace
Niobium	trace	Arsenic	0.003
Vanadium	0.002	Zirconium	trace
Phosphorus	0.00	Tungsten	0.0001
Dysprosium	trace	Protactinium	trace
Erbium	trace	Lanthanum	trace
Cesium	0.002	Hafnium	trace
Manganese	0.001	Chromium	0.00005
Europium	trace	Radon	trace
Thorium	0.0002	Selenium	0.0039
Krypton	trace	Titanium	trace
Uranium	0.00005	Cerium	0.0007
Gadolinium	trace	Gallium	0.0003
Iodine	0.05	Cadmium	0.0001
Lead	0.004	Germanium	0.00007
Lutetium	Trace		

APPENDIX C

GRAPHICAL RESULTS FROM PLANT NUTRITION STUDY II

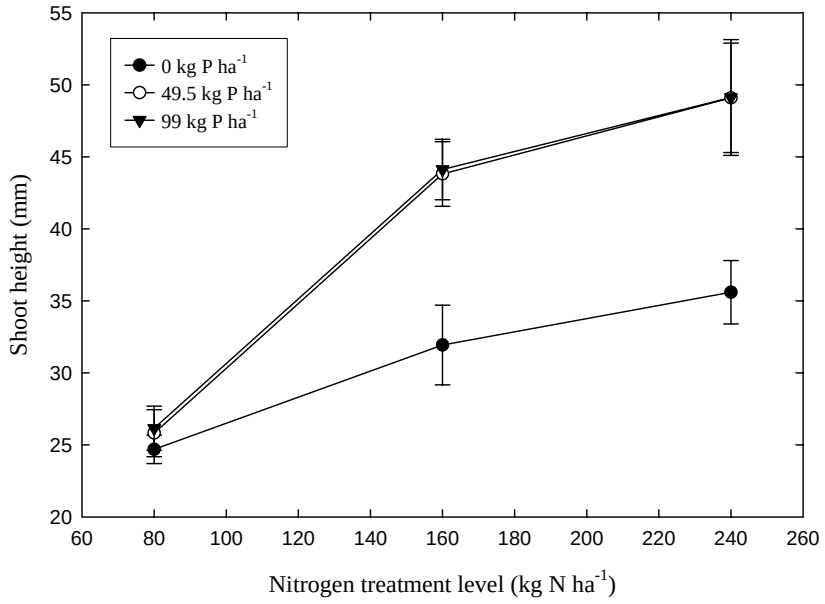

Figure C.1. The effects of nitrogen and phosphorus nutrient treatments on *Spartina alterniflora* shoot height for plant nutrition study II. Bars indicate standard errors.

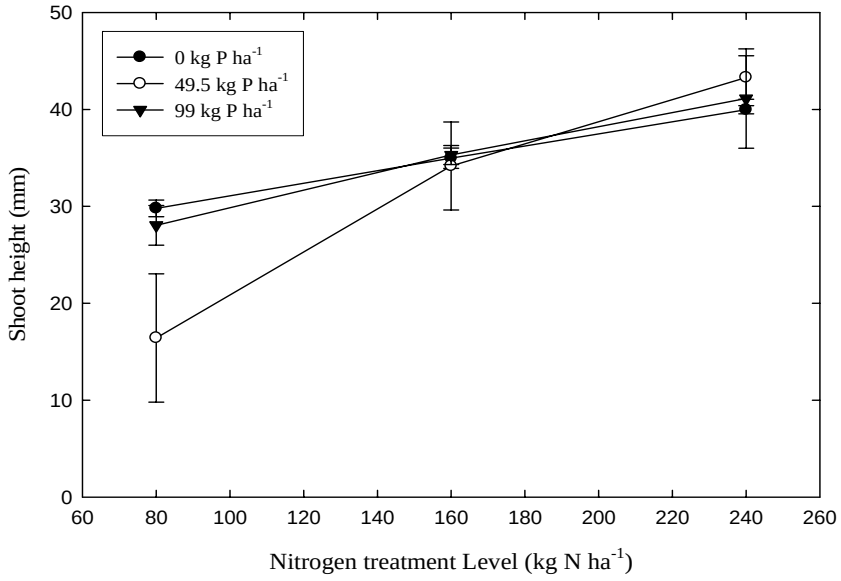

Figure C.2. The effects of nitrogen and phosphorus nutrient treatments on rice shoot height for plant nutrition study II. Bars indicate standard errors.

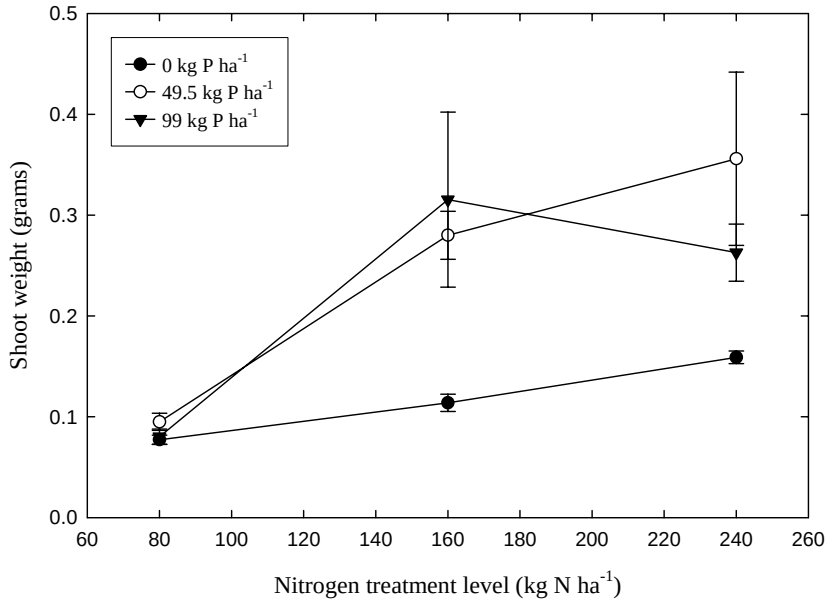

Figure C.3. The effects of nitrogen and phosphorus nutrient treatments on *Spartina alterniflora* dry shoot weight for plant nutrition study II. Bars indicate standard errors, where larger than the symbols.

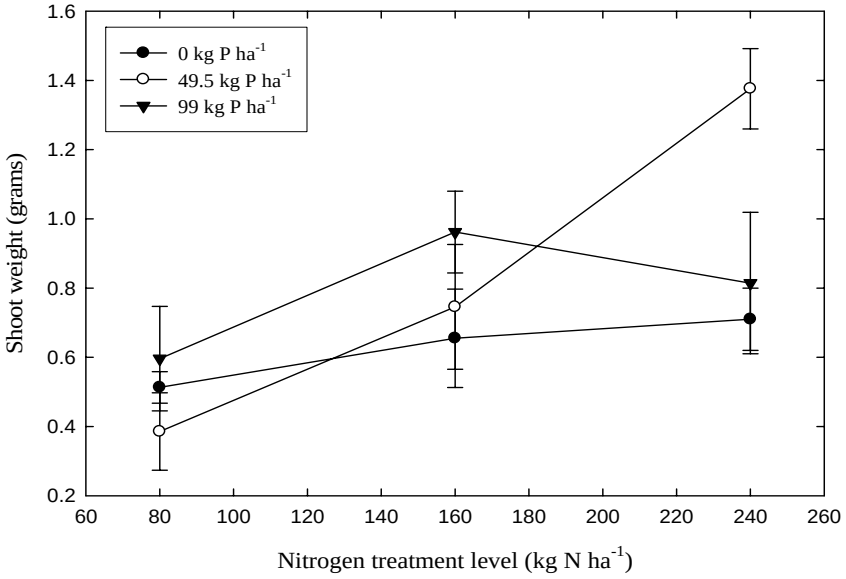

Figure C.4. The effects of nitrogen and phosphorus nutrient treatments on rice dry shoot weight for plant nutrition study II. Bars indicate standard errors.

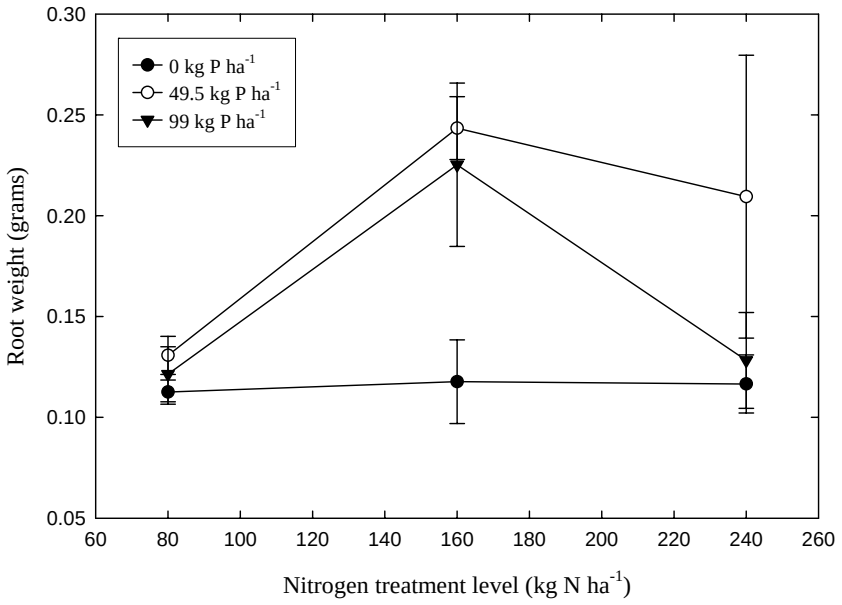

Figure C.5. The effects of nitrogen and phosphorus nutrient treatments on *Spartina alterniflora* dry root weight for plant nutrition study II. Bars indicate standard errors.

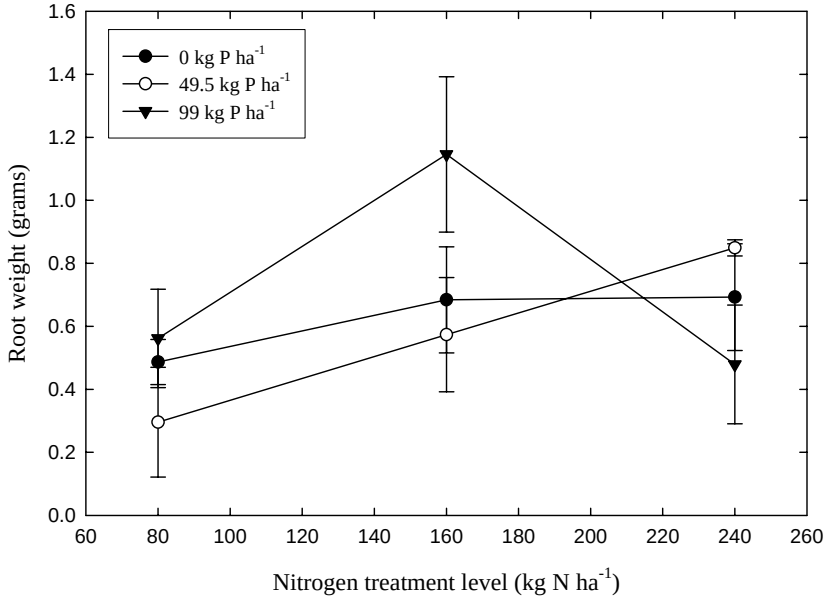

Figure C.6. The effects of nitrogen and phosphorus nutrient treatments on rice dry root weight for plant nutrition study II. Bars indicate standard errors.

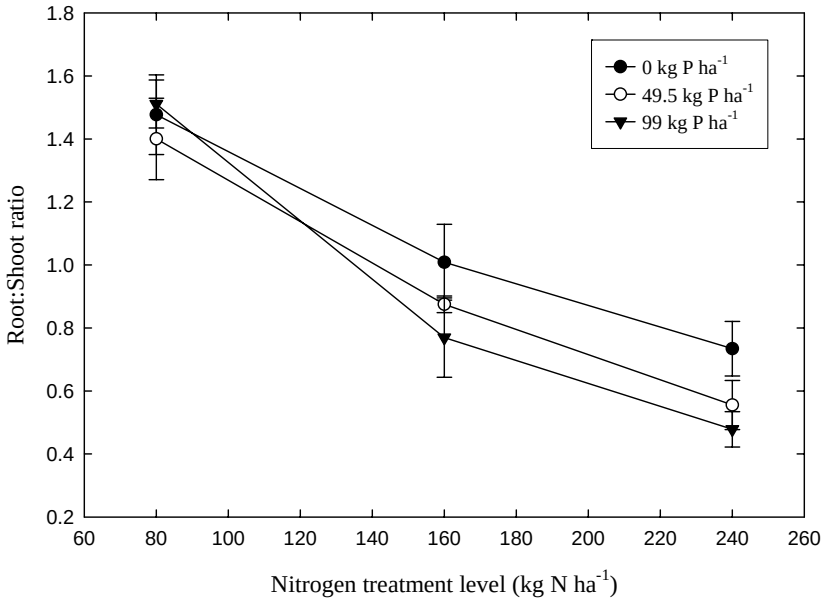

Figure C.7. The effects of nitrogen and phosphorus nutrient treatments on weight based *Spartina alterniflora* root:shoot ratios for plant nutrition study II. Bars indicate standard errors.

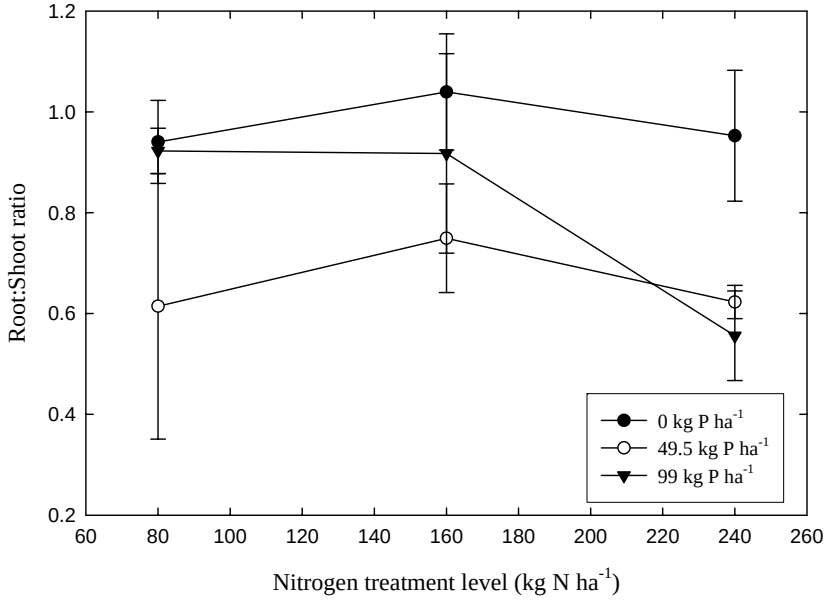

Figure C.8. The effects of nitrogen and phosphorus nutrient treatments on weight based rice root:shoot ratios for plant nutrition study II. Bars indicate standard errors.

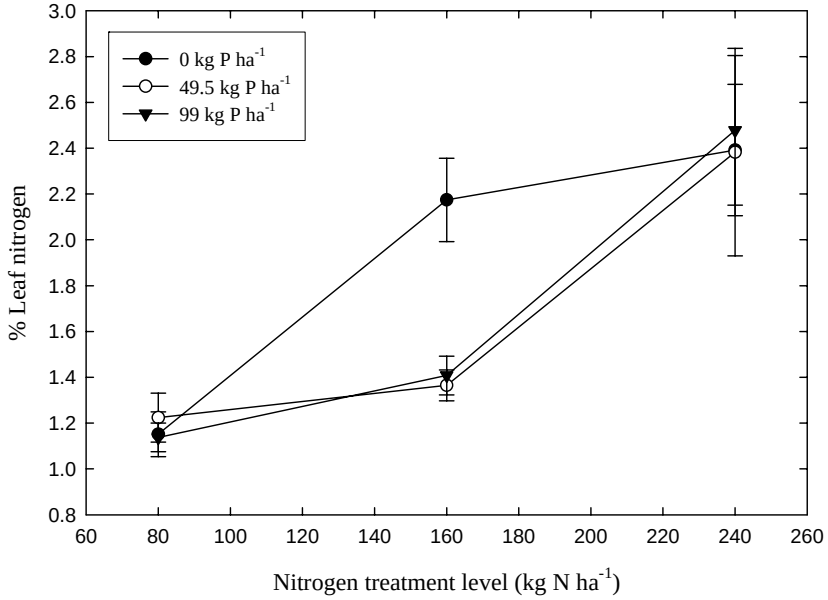

Figure C.9. The effects of nitrogen and phosphorus nutrient treatments on *Spartina alterniflora* percent leaf nitrogen for plant nutrition study II. Bars indicate standard errors.

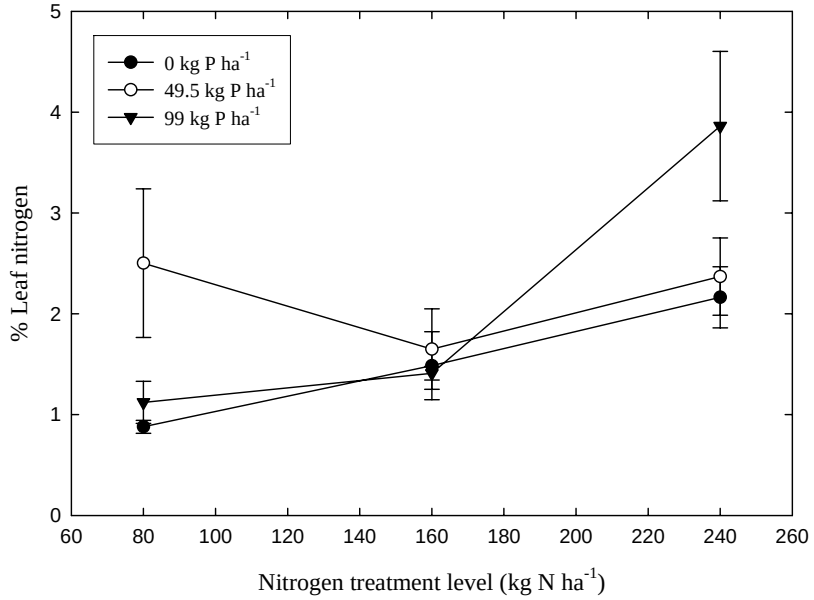

Figure C.10. The effects of nitrogen and phosphorus nutrient treatments on rice percent leaf nitrogen for plant nutrition study II. Bars indicate standard errors, where larger than the symbols.

APPENDIX D

NITROGEN AND PHOSPHORUS LEVELS OF NATIVE LOUISIANA MARSH SOILS

Table C.1: Total nitrogen and phosphorus level ranges present in three types of native marsh soils found in Louisiana (Charbreck 1972).

	Nutrient level ranges (kg ha^{-1})	
Marsh Type	Total Nitrogen	Phosphorus
Saline	1344 – 19,264	134 – 291
Brackish	6900 – 15,900	22 – 224
Fresh	5152 – 53,088	9 – 381

Table C.2: Phosphorus levels of Louisiana coastal marsh soil amended with sediment dredged from the Gulf of Mexico (Mendelssohn and Kuhn 2003).

Dredge Sediment Depth	Phosphorus Levels (kg ha^{-1})
No dredge sediment	134.3
Trace amounts of sediment	163.3
Not greater than 15 cm	274.8
15-30 cm	191.7
More than 30 cm	354.4

VITA

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