ABSTRACT

Title of Thesis: EFFECTS OF TYPICAL CONCENTRATIONS OF NITROGEN AND PHOSPHORUS IN AGRICULTURAL TREATMENT WETLANDS ON POLYCULTURES OF *TYPHA LATIFOLIA* L. AND *JUNCUS EFFUSUS* L. AND A TEST OF THE N:P RATIO AS A PREDICTIVE TOOL.

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Nitrogen removal in agricultural treatment wetlands is determined by the health of macrophytes. Nitrogen is removed by microbes dependant on an oxic-anoxic boundary layer created by oxygen flow through macrophyte culms and by nitrogen sequestration in plant biomass. Phytotoxic ammonia concentrations can limit plant growth. Orthophosphate fertilization to balance the nitrogen to phosphorus tissue ratio may increase biomass. One test of this hypothesis investigated *Juncus effusus* and *Typha latifolia* ammonia phytotoxicity: ammonia concentrations above 150 $\mathrm{mgL^{-1}}$ suppress *Typha* growth and above 300 $\mathrm{mgL^{-1}}$ do not affect *Juncus* growth. The second experiment altered mesocosm N:P ratios. Some alleviation of ammonia toxicity was shown; highest biomass production at toxic levels occurred at a 15:1experimental N:P ratio. The two species used N and P differently: the average N:P ratio was 12:1 in *Juncus* and 9.1:1 in *Typha*. More effective waste removal may be based upon more precise control of N:P ratios.

EFFECTS OF TYPICAL CONCENTRATIONS OF NITROGEN AND PHOSPHORUS IN AGRICULTURAL TREATMENT WETLANDS ON POLYCULTURES OF *TYPHA LATIFOLIA* L. AND *JUNCUS EFFUSUS* L. AND A TEST OF THE N:P RATIO AS A PREDICTIVE TOOL.

by

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CHAPTER ONE:
INTRODUCTION AND BACKGROUND

Of paramount concern in agricultural areas, in particular those in close proximity to natural watersheds of ecological and economic importance, is the control of anthropogenically derived nitrogenous wastes entering those watersheds (Bobbink *et al.*. 1998, Aerts and Bobbink 1999). In addition to nitrogenous compounds, wastewater produced by dairy farms, feedlots, and hog farms also tends to have high carbon content, high total suspended solids, and often high phosphorus content as well (Cronk 1996); the sum effect of addition of excess amounts of these nutrients to natural waters may contribute to eutrophication (Cronk 1996, Knight *et al.* 2000). Concentrations of 100-300 mgL^{-1} of ammonia and 75-200 mgL^{-1} of total phosphorus are fairly common for agricultural wastewater. Hammer (1992) reports concentrations of up to 500 mgL^{-1} in raw livestock wastewater.

Production of wastewater in agricultural systems typically stems from cleaning practices (Tanner *et al.* 1995a), which flush excretions, mud, feed, waste milk, and detergents from barn or dairy parlor floors with high-pressure hoses (Cronk 1996, Tanner *et al.* 1995a). Flush water volumes tend to be quite high: Soil Conservation Service estimates suggest a total of 100 gallons per cow per day (SCS 1991), and independent researchers suggest almost an order of magnitude higher (Surrency 1993). Raw flush water is normally treated, though not always, prior to addition to natural waters, but tends to retain some portion of its high nutrient load (Clarke 1999, Cronk 1996).

In such typically nitrogen-limited natural systems, additions of high nitrogen compounds such as ammonium (NH_3) and nitrate (NO_3^-) tend to alter species composition and biomass production (Shaver and Chapin 1995, Lammerts *et al.* 1999, Van Duren and Pegtel 2000, Camacho *et al.* 2003). Biomass production in natural systems tends to be enhanced by the addition of a limiting nutrient; the addition of non-limiting nutrients tends to have little or no effect on biomass production (Vitousek and Howarth 1991, Verhoeven *et al*. 1996, Gusewell *et al*. 2003). Species composition is affected by the addition of limiting or non-limiting nutrients, due to differential species responses to different nutrients (Dijk and Olff 1994, Mamolos *et al*. 1995, Shaver and Chapin 1995, Lammerts *et al*. 1999). Species responsiveness to nutrient availability differs, then, dependant on relative nutrient requirements and on their ability to take up and use the available nutrients (Kielland 1994, Perez-Corona *et al*. 1996, Ryser *et al*. 1997, Aerts and Chapin 2000). In watersheds, the most responsive species tend to be algae and phytoplankton (e.g. Rhee 1978, Downing and MacCauley 1992); responses are most frequently seen to increases in available nitrogen rather than increases in available carbon (Camacho *et al.* 2003) or in available phosphorus (Jansson *et al.* 2001). Fertilization increases are characterized by rapid biomass production of one or few species, in response to increases in previously limiting nutrients (Grime 1979, Camacho *et al.* 2003). This rapid biomass production is associated with decreases in species richness (Bedford *et al.* 1999, Kent *et al.* 2000, Green and Galatowitsch 2003, see Mittelbach *et al*. 2001 for an excellent synopsis), and increases in the presence of invasive species (Green and Galatowitsch 2002), which may end in decrease in economic and ecological function in the watershed or other system (Engelhardt *et al*. 2001, Ostertag and Verville 2002).

Methodologies to remove the nitrogen component of agricultural system wastes were historically limited to the creation or restoration of riparian buffer strips of naturalized vegetation (e.g. Brix 1989, Vought *et al*. 1993, Konya *et al*. 1995, Tanner *et al*. 1995a, Craft 1999). Stemming from the theories that such strips remove contaminants by translocating chemicals into the biomass and providing suitable habitat for microbial colonization (and subsequent redoxic removal mechanisms), the use of engineered wetlands as dedicated waste water purifiers has been investigated and implemented in a range of conditions and at a range of sites (e.g. Hammer 1992, Cronk 1996, Karpiscak *et al.*, 1999, Knight *et al.* 2000).

Current estimates indicate that 65% to 85% of nitrogenous contaminants are removed by vegetation and bacteria as water moves through an agricultural treatment wetland (ATW) (Cronk 1996, Knight *et al.* 2000). Of nitrogenous compounds (which can include organic forms such as urea, amino acids, amines, and nucleic bases, and inorganic forms such as ammonia, nitrite, nitrate, and nitrogen gases), ammonia is the most problematic to remove, and percentage removal rates are often significantly lower than the removal rates of other compounds, nitrogenous and otherwise (i.e. Mitsch and Gosselink 1993, Hammer and Knight 1994, Cronk 1996, Humenik *et al.* 1999, Clarke 1999, Knight *et al.* 2000, Clarke and Baldwin 2002). Ammonia, nitrite, and nitrate are recognized pollutants of natural waters, with toxicity limits of ammonia placed as low as 0.2 mgL^{-1} (Kadlec and Knight 1996). Maximum removal efficiency of ammonia tends to be less than 90%, with some estimates as low as 48% (Knight *et al.* 2000). On the other hand, under conditions of maximum efficiency, up to 97% of biological oxygen demand (a surrogate measure for total carbon) can be removed, as can 99% of total nitrogen, and

93% of total phosphorus (Cronk 1996, Clarke 1999, Schaafsma *et al.* 2000,Knight *et al.* 2000, DeRico 2000, Clarke and Baldwin 2002). As previously explained, poor removal of ammonia or other nitrogenous compounds from wastewater can lead to eutrophication in nitrogen-limited natural systems; nitrogen removal becomes, therefore, an important goal of ATWs (Cronk 1996, Clarke 1999, Knight *et al.* 2000). Of the portion removed from the wastewater, approximately 40% of this nitrogen is translocated into plant biomass and used for the production of proteins, nucleic bases, protonated hydrocarbons, etc (Reddy *et al.* 1989). The remaining 60% is removed through the redoxic nitrification-denitrification pathway (Howes *et al.*. 1981, Gumbricht 1993, Bachand and Horne 2000a), although percentage values as to the treatment effects of plants on nitrogenous wastes range from 90% (Rogers *et al.* 1991) to just 5% (Hammer 1992). In any event, the importance of the nitrogen-coupled bacterial reaction is not to be overlooked; through this pathway, ammonium (NH_4^+) is oxidized to nitrate, which is then reduced to nitrogen gas (N_2 or N_2O), which leaves solution. Figure 1.1 illustrates these direct effects as well as associated indirect effects.

Clearly, then, this pathway requires an oxygen gradient; translocation in plants of oxygen from atmosphere to rhizosphere through aerenchymous tissues helps to provide an anoxic-oxygenated interface over which this reaction may occur (Brix 1989, Brix *et al*. 1992, Bendix *et al*. 1994, Tornbjerg *et al*. 1994, Brix *et al*. 1996, Brix and Sorrell 1996, Brix 1997, Jespersen *et al*. 1998, Smith *et al*. 2000). Tanner and Kadlec (2003) consider rhizosphere oxygenation to be the rate-limiting step in nitrogen removal in treatment wetlands. Greater plant biomass results in greater oxygen flow into the rhizosphere, thus increasing the degree of development of this boundary layer. This gas

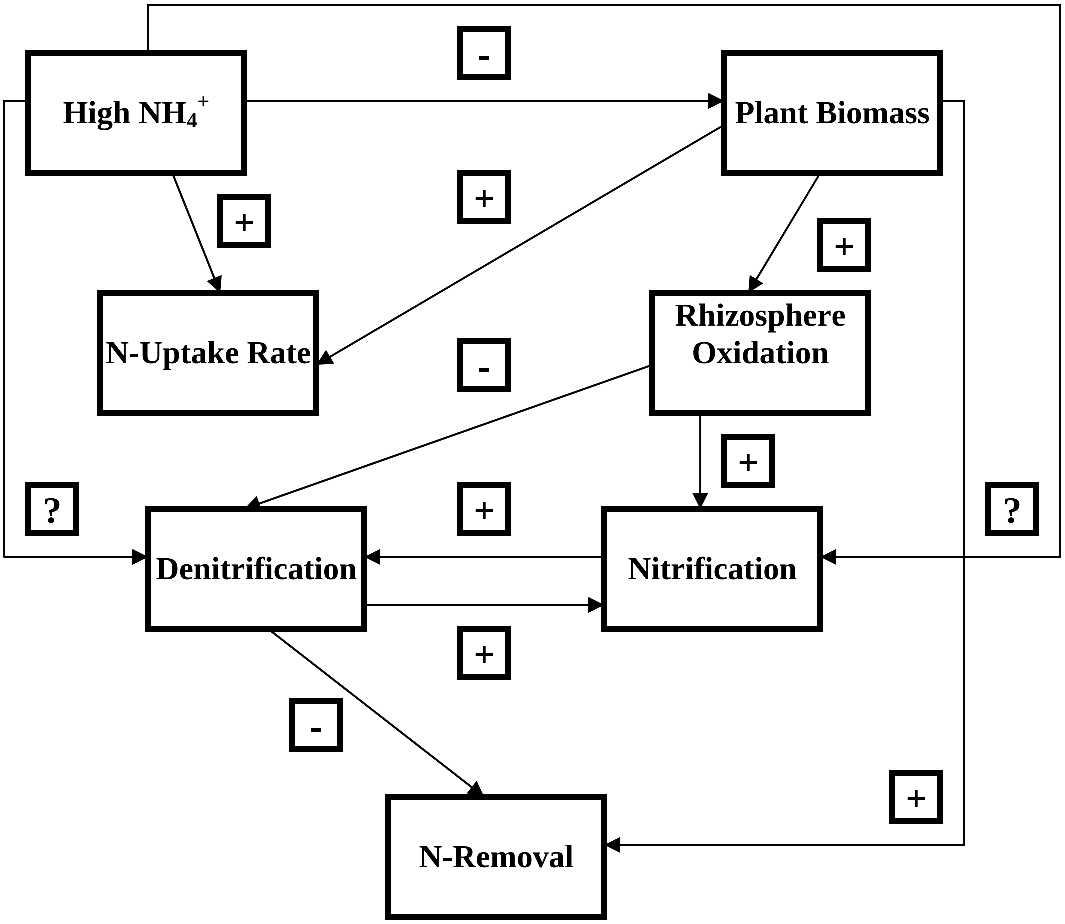

Figure 1.1. Conceptual framework of experiments summarizing direct and indirect effects of ammonia addition to ATWs. Signs associated with effects indicate feedback within the system. Question marks may indicate where further research could add to the precision with which ATWs are modelled.

flow rate differs based on plant species, but in each case increases as biomass increases (Brix 1989, Brix *et al*. 1996, Romero *et al*. 1999). Thus, biomass may be seen as a rough surrogate for degree of development of oxygenation in the root zone, the resolution of which depends upon the gas flux constant of the species used. Root zone oxygenation is likewise proportional to degree of nitrification given that the bacteria necessary for such are in abundance in an oxygenated system. Decreases in biomass in treatment wetlands are therefore problematic in terms of nitrogen removal on two levels: (1) lower biomass production equates to lower translocation rate of nitrogen into plant tissue; (2) lower biomass equates to lower oxygen flow into the rhizosphere, lower degree of development of the redoxic boundary, and lower rate of nitrification. Additionally, (3) lower biomass equates to less surface area in the water column itself upon which nitrifying bacteria may be supported (a potentially overlooked but experimentally valid assumption; see Bastviken *et al.* 2003) (Figure 1.1).

Difficulties arise in designing ATWs for effective removal of nitrogenous wastes due to a lack of standardization in flow hydrology and chemical load into the system (Hammer and Knight 1994). The reactions which remove nitrogen tend to have a low rate of reaction; slower flows will allow greater amounts of denitrification. In the case of some treatment wetlands, wastes are pretreated to varying degrees by screening, settling basins, oxygenation, etc.; these measures tend to emphasize physical removal of solids more so than redoxic or translocational removal of chemical constituents (Gale *et al.* 1993, Hammer and Knight 1994, Knight *et al.* 2000). Wetlands receiving well-treated waste water tend to have a higher potential for removal of biochemicals due primarily to lower concentrations of those chemicals in the influent waste water. Conversely, wetlands receiving untreated or poorly treated wastewater tend to have lower potential for biochemical removal due to high concentrations of those chemicals in the influent water (Hammer and Knight 1994, Knight *et al.* 2000, Knight 2003), despite potentially higher total mass removal by the wetland.

High concentrations of waste water contaminants are problematic due to phytotoxic effects. Ammonium (NH_4^+) in untreated agricultural waste is known to be phytotoxic, although different species exhibit toxic effects at different concentrations of NH_4^+, though few tested concentrations approached observed ATW ammonia concentrations (Walker and Evans 1978, van der Eerden 1982, Wang 1990, Surrency 1993, Dijk and Eck 1995, Magalhaes *et al*. 1995, Tanner *et al*. 1995b, Clarke 1999, Clarke and Baldwin 2002). Surrency (1993) reports *Typha latifolia* L. growth was suppressed by ammonia concentrations between 160 and 170 mgL^{-1}. Hill *et al.* (1997) found that the growth of five wetland species, including both *Typha latifolia* L. and *Juncus roemerianus* L., was not affected by field ammonia concentrations of up to 82.4 mgL^{-1}. Humenik *et al.* (1999) found *Juncus effusus* tolerant of ammonia concentrations up to 350 mgL^{-1} over a two year period. Clarke (1999, Clarke and Baldwin 2002) reported growth inhibition of five species, including *Typha latifolia* and *Juncus effusus,* at NH_4^+ concentrations in excess of 200 mgL^{-1}, and discussed build-up of ammonia and time-dependant toxicity levels under continuous-loading treatment conditions.

The exact mechanisms behind ammonium toxicity are not known; hypothetical links exists between NH_4^+ toxicity and alterations in differential ion selective uptake (such are the cases for other cation toxicities, such as Ca^{+2}, Mg^{+3}, Li^+, etc.(Hageman 1984, Britto and Kronzucker 2002). Ammonium ion toxicity may be mediated by incorporation of low concentrations of selectively transported cations such as potassium, Rb^+, or Cs^+, but not by Ca^{+2} (Cao *et al.* 1993), but deficiencies in tissue K^+ at high ammonium levels have been reported as a potential cause for low growth rates and poor biomass production (Abbes *et al.* 1995). Ammonia fertilization may also cause soil acidification due in increased H^+ ion concentration from uptake of ammonia; acidification further complicates uptake mechanisms and may result in biomass decline due to inability to take up other nutrients (Hageman 1984, Dijk and Eck 1995). Toxicity appears to function in some species at the enzyme or mRNA levels rather than by reducing pH or cation uptake. In mustard (*Sinapsis alba* L.), interference with the transformation of fats to carbohydrates via reduction of RuBPCase, NAD-GDP and NADP-GDP enzymes was found to be the cause of decreased biomass production; pH changes and osmotic effects were found to not interfere with biomass production (Mehrer and Mohr 1989). Ammonia toxicity has been implicated in reduced hormonally-mediated transporter functions (Glass *et al.* 2002), and auxin-deficient mutants in *Arabidopsis* display some resistance to ammonia toxicity (Cao *et al.* 1993). Developments in the field of plant microbiology and discovery of the genetic mechanisms controlling ammonia uptake and its relation to the uptake of other cations or maintenance of cellular pH or cation equilibrium, while outside the scope of this paper, may further elucidate toxic properties of excessive amounts of ammonia (Kronzucker *et al.* 2001, Glass *et al.* 2002). In any event, high ammonium levels are one of the factors responsible for decreases in plant biomass in ATWs (Surrency 1993, Hill *et al*. 1997, Clarke 1999), and macrophyte biomass in ATWs is essential to system health and proper functioning (Kadlec and Knight 1996, Brix 1997, Hunter *et al.* 2000, Clarke and Baldwin 2002).

On the other hand, a moderate concentration of ammonia, where “moderate” is again defined by the selected plant species, is likewise necessary for optimal system health and functioning. Due to adaptations to flooded soil and the reduced condition found therein (Mitsch and Gosselink 1993), most wetland plants preferentially uptake nitrogen in the ammoniacal form (Hammer and Knight 1994). Plant growth in natural wetland systems is often limited by a lack of abundance of reduced nitrogen in the system; ammonia concentrations below 2 mgL^{-1} are typical for a number of types of natural wetland (Shaver and Chapin 1995, Kadlec and Knight 1996, Lammerts *et al*. 1999, Van Duren and Pegtel 2000), with variations in NH_4^+ concentration inversely proportional to standing biomass.

Phosphate concentrations (PO_4^{-3}) in ATWs are not as high as NH_4^+ levels; toxicity of phosphates has not been ascertained (Kadlec and Knight 1996), and researchers have failed to find significant growth inhibition at high phosphate levels (Romero *et al* 1999). The mechanism by which phosphate uptake occurs is a series of proton pumps in the root epidermis with high $H_2PO_4^-$ affinity, indicating the necessity of available H^+ ions to oxidize PO_4^{-3} (Smith 2002, Smith *et al.* 2003). Generally, phosphates tend not to be as mobile in the environment as nitrogenous compounds; in the presence of cations they tend to form precipitates, in the presence of clays they tend to adsorb (Mitsch and Gosselink 1993). Having been removed from solution, these anions are not prone to removal by flow-through, and can accumulate at the redoxic boundary, maintaining availability for plants equipped with ATPase-linked membrane proteins with sufficient affinity for phosphates (Smith 2002).

The use of nitrogen to phosphorus ratios in plant tissues for predicting fertilization effects is controversial. Koerselman and Meuleman (1996) reviewed 40 field fertilization experiments in wetlands and concluded that biomass production of the vegetation community is typically limited by nitrogen if the aboveground N:P ratio is low (<14) and by phosphorus if the N:P ratio is high (>16). This result did not coincide well with the by-weight N:P ratio of 7.2:1 proposed by Redfield (1958), nearly 40 years earlier (although Redfield's prediction was based upon N:P ratios found in marine phytoplankton). They also hypothesized that individual plant population response to fertilization could be predicted using the same ratios due to the fact that over half of the sites included in the review were dominated by a single species making up 80% or more of the aboveground biomass for the site.

Tilman (1982, 1985, 1987, 1997, *et al*. 1999), modeling nutrient limitations in wet meadow and prairie communities, described competition effects in response to fertilization in the "resource ratio model." This model predicts that if two nutrients (for example N and P) are limiting, fertilization with one nutrient will cause the other to become relatively scarcer, and will create differential selection or competitive pressure for those species that are strong competitors for the scarce nutrient. Fertilization with the opposite nutrient will create the opposite effect, and will select for a different group of species. Furthermore, the model predicts the competitive ability of plant species for certain resources based on the tissue concentration of a nutrient: strong competitors will have a low concentration, weak competitors a high concentration. Thus, if N and P are limiting, the tissue N:P ratio should indicate which species will be promoted by N enrichment (a high N:P ratio) or by P enrichment (a low N:P ratio). Likewise the addition or subtraction of a necessary limiting factor can enhance or diminish the presence of a certain species (Tilman *et al.* 1999). These results were corroborated by several authors (Mamolos *et al*. 1995, Mamolos and Veresoglou 2000).

Other authors have investigated whether N:P ratios suggest nutrient limitation patterns at all, although in studies of shorter duration. Wassen *et al*. (1995), Pegtel *et al*. (1996), and Gusewell *et al*. (2003) found that N:P ratios could indicate accumulation of the non-limiting nutrient rather than paucity of the limiting one. McJannet *et al*. (1995) found different N:P ratios in 41 species of wetland plants grown under the same nutrient concentrations. Lechowicz and Shaver (1982) suggested different N:P ratios reflected differences in growth habit. Gusewell *et al*. (2003) indicated biomass N:P ratios in plant communities are not the result of species-specific or growth habit-specific uptake rates but the result of differences in the relative availability of N and P. A number of authors (Braakhekke and Hooftman 1999, Roem and Berendse 2000, Gusewell *et al.* 2002) found that N and P ratios tended to differ more within species between sites or treatments than within species within a given site or treatment, and that variations in N:P ratios within species tended to correlate well with variations in N:P ratios between sites.

Ammonium toxicity tends to differ between species (see above; see Tanner *et al* 1995 for an excellent synopsis). Provided that N and P usage is not significantly different between species as suggested above, use of species specially selected for ammonium concentration tolerance may be beneficial to increasing treatment efficiency. However, since different species exhibit different gas flux constants, highest treatment efficiency may be achieved through a mix of species with graded ammonia tolerances and additive culm oxygen transmittal. It has been suggested that highest biomass production and stability of ecosystem processes occur in wetlands with a high degree of species richness (Tilman 1996, Engelhardt *et al*. 2001). Mixtures of species, then, may yield elevated treatment efficiency over monocultures due to a suite of morphologies and resource acquisition mechanisms more able to fully capture available light and nutrients. At the same time, increases in fertilization tend to favor the competitor with the best acquisition mechanisms for that nutrient (Tilman *et al.* 1999), but at toxic or semi-toxic nutrient concentrations, inhibitory effects may prove significant (Goldberg and Barton 1992, Mittelbach *et al.* 2001).

Taken together, the above suggest the possibility that N:P ratios can be used to predict biomass production, although the mechanisms by which this may be done are complex and often related to the species used, the community observed, or the time scale over which the experiment is run. In all, it appears that plant tissue concentrations may closely reflect concentrations of N and P in the surrounding environment; changes in N and P concentrations by fertilization may be reflected proportionally in the biomass, regardless of species-specific uptake mechanisms, or time duration. The predictive power of the N:P ratio has not been applied to agricultural wastewater treatment wetlands; the power and resolution of the tool may change when applied to systems of artificially elevated N and P levels. ATWs represent a unique case of circumstances for the application of a concept prior to this point used to determine end effects, given that treatment wetlands or other means by which to reduce nitrogenous output to the natural environment do not always function as planned with respect to nitrogen removal. Alterations in the environmental N:P ratio based on attempts to correct the vegetation N:P ratio may serve to increase treatment efficiency by removing nutrient limitations and allowing higher biomass production and higher tissue nutrient concentrations (Powell 1974, Rubio *et al.* 1997, Tu and Ma 2003, Vojtiskova *et al.* 2004). Environmental N:P ratios in ATWs indicate P-limitation (Clarke 1999, DeRico 2000). Likewise, lack of treatment efficiency, in terms of use by plants or bacteria, in removing nitrogen when compared to phosphorus would seem to indicate an overabundance of nitrogen (Cronk 1996, Knight *et al.* 2002, Tanner and Kadlec 2003). Leibig's Law of the Minimum states that biomass production may increase upon the removal of a limiting resource; in this case the limiting resource may be phosphorus. By increasing the available P, N usage in plant tissue may increase as well; increased use, in addition to conversion to N_2 or N_2O gas along the anoxic boundary, may result in lower cation concentration in solution, which may potentially alleviate ammonium toxicity issues. Additionally, use of a species assemblage over a gradient of specific ammonium toxicities and oxygen flux constants may result in more efficient use of nitrogen, likewise lowering the solution cation concentration and potentially alleviating ammonium toxicity.

In this thesis, I hypothesized that by altering N:P ratios in ATWs to better reflect naturally occurring N:P ratios, the phytotoxic level of NH_4^+ can be increased to exclude ammonium concentrations in high nitrogen ATWs. The following objectives were addressed in two experiments:

(1) Are environmental N:P ratios reflected in plant biomass under high concentrations of NH_4^+ and PO_4^{-3}?

(2) Is high productivity maintained in high $[NH_4^+]$ given appropriate $[PO_4^{-3}]$?

(3) How do responses of species grown in mixtures change over a gradient of ammonia and phosphate concentrations?

RESEARCH EXPERIMENTS

Planting guidelines for agricultural treatment wetlands available from the USDA (SCS 1991) advocate the use of polycultures as opposed to monocultures. Species were selected from these guidelines and based on the work of Clarke (1999 and Clarke and Baldwin 2002), Humenik *et al.* (1999), Hill *et al.* (1997), Surrency (1993), in nitrogen tolerances of wetland species. *Typha latifolia* L. and *Juncus effusus* L. (hereafter *Typha* and *Juncus*) were selected based on nitrogen tolerances; both species have high tolerances for NH_3 when grown in monoculture (Surrency 1993, Humenik *et al.* 1999, Clarke and Baldwin 2002). *Typha* is typically considered an excellent competitor, and monotypic stands are often associated with nutrient-rich conditions (Grace and Wetzel 1981, Tilman 1987, Surrency 1993, Clarke 1999, Svengsouk and Mitsch 2000). *Juncus* is not considered as vigorous a competitor, and ranked lower than *Typha* in competitive performance when measured against a common phytometer (Keddy *et al.* 2000). However, both species are clonal graminoids, and as such both should respond in similar fashion to additions of N and P in that additional culm growth should be the result of removal of P-limitation (Tilman 1982, DiTommaso and Aarssen 1989, Aerts *et al*. 1990, DeKroon and Bobbink 1997, Aerts *et al.* 1999).

Mesocosms were constructed at the greenhouses of University of Maryland (College Park, Maryland, USA) in May 2002. These greenhouses controlled precipitation inputs into the mesocosms, allowing for permanent and maintainable concentrations of solutes. *Typha* and *Juncus* were purchased from Environmental Concern, Inc. (St. Michaels, Maryland, USA) in quart pots. Two plants of each species were planted in sterile soil-less media (Metromix; Scotts, Marysville, Ohio, USA) into 6L pots. These pots were then placed in 10L pots lined with 4 mil polypropylene and filled with 6L of water, creating a pot-within-pot design. Nitrogen and phosphorus fertilizers were added as reagent-grade NH_4Cl and $Na_3(PO_4)_2$.

In two experiments, mesocosms arranged in this manner were subjected to two experiments to determine the effects of nitrogen and phosphorus concentrations typical in ATWs on biomass, species dominance, and the N:P ratio. The first experiment, in 2002, explored the effects of NH_4^+ alone of the two species planted in polyculture. Five ammonia levels were selected using observed ammonia tolerances from Clarke (1999) and Humenik *et al.* (1999) for monoculture ammonia-N tolerances for the two species: 0, 75, 150, 225, and 300 mgL^{-1}. Fertilizer solution was replaced weekly and monitored to assure appropriate treatment concentrations, and above- and below-ground biomass was harvested at the end of eleven weeks. Ammonia effects on total biomass, above- and below-ground biomass, and stress tolerance were evaluated using analysis of variance (ANOVA) tools in SAS packages (SAS Institute, Cary, North Carolina, USA).

The second experiment, in 2003, explored the effects of NH_4^+ and PO_4^{-3} on polycultures of *Juncus* and *Typha*. Four ammonia levels were selected from the previous year's data: 75, 150, 225, and 300 mgL^{-1}, and phosphate was added such that solution N:P ratios of 5:1, 15:1, and 25:1 resulted. Fertilizer solutions were replaced weekly and monitored to assure appropriate treatment concentrations, and above- and below-ground biomass was harvested at the end of ten weeks. Effects of varying N:P ratios and ammonia on total biomass, above- and below-ground biomass, and stress tolerance were evaluated using analysis of variance (ANOVA) tools in SAS packages (SAS Institute, Cary, North Carolina, USA).

In attempting to answer the objectives listed above, the following assessments were made after biomass harvesting during the two experiments described above. To test whether tissue N:P ratios reflect environmental N:P ratios under high [NH_3] (objective 1), total tissue nitrogen and phosphorus concentrations for all tissues at each treatment, and the mean tissue nitrogen and phosphorus concentrations of *Juncus* and *Typha* were compared to the environmental N:P ratio as determined by solute addition. Comparisons were made using blocked ANOVA and data was adjusted for normality and homogeneity of variance. To test whether high productivity is maintained in phytotoxic [NH_3] given phosphate additions (objective 2), mean biomass of *Typha* and *Juncus* was compared between N treatment levels at equal N:P ratios with data adjusted as required to assure normality and homogeneity of residuals. Phytotoxic concentrations were assumed given the work of previous researchers (Clarke 1999, Humenik *et al.* 1999). Comparisons between N:P ratios within N treatments were also made for mean total, above-ground, and below-ground biomass for *Juncus* and *Typha*. To test differences in phytotoxicity in poly- and mono-culture (objective 3), mean total biomass for *Juncus* and *Typha* planted in polyculture (2002 data) was compared within species between treatments using blocked ANOVA with data adjusted as necessary.

CHAPTER TWO:

EFFECT OF AMMONIA ON BIOMASS IN MESOCOSMS OF *TYPHA LATIFOLIA* AND *JUNCUS EFFUSUS*

OBJECTIVE

The objective of this study was to investigate differences in productivity responses of mesocosms of mixtures of *Juncus effusus* L. and *Typha latifolia* L. to ammonia under controlled conditions. This was accomplished in a one greenhouse experiment, during which plants were exposed to levels of 0, 75, 150, 225, and 300 mg NH_4^+ L^{-1} over an 11 week period. In order to supply micronutrients without compromising experimental control, plants were cultured in sterilized soil-less media (Metromix, Scotts, Marysville, Ohio, USA), and treatments were applied by batch-loading with appropriate concentrations (Wang 1991). *Typha latifolia* (hereafter *Typha*) and *Juncus effusus* (hereafter *Juncus*) were selected for this study based on the work of Clarke (1999), Clarke and Baldwin (2002), Humenik *et al.* (1999), and Surrency (1993), which indicated high ammonia-N tolerances for these species. Using *Juncus* and *Typha* allowed us to investigate changes in the proportion of biomass allocated to each species over the given range of treatments, as well as to investigate changes in the mesocosm biomass on the whole.

METHODS

Preparation of Mesocosms

Plant material was purchased from Environmental Concern, Inc. (St. Michaels, Maryland) in May of 2002 as 125 quart pots of each species, grown from seed under greenhouse conditions. Plants were transported from St. Michaels to the Harrison Laboratory Greenhouses at the University of Maryland, College Park (UMCP) in a covered vehicle, where they were immediately transplanted with their original soil and accompanying microorganisms into 50 mesocosms of approximately 6 L in volume and watered thoroughly to prevent potential transplant shock. Each "mesocosm" was planted with two quart pots of each species, in a design minimizing North-South and East-West effects (Figure 2.1). Following 2 weeks of acclimatization to UMCP conditions, water, temperature, and transplant, the 6 L pots were then placed within 10 L pots lined with 4 mil. polypropylene and filled with 6 L of greenhouse tap water, creating a pot-within-pot design (Figure 2.1.) such that the water flooded each mesocosm (experimental unit) to the soil surface. These mesocosms were randomly placed in five columns of 10 rows, with columns aligned North-South (labeled A through E) and rows aligned East-West (labeled 1 through 10). Treatments of ammonia concentrations were assigned such that each treatment occurred once in each row and twice in each column, a block design to account for potential effects of shading due to rapid and dense growth of either species (Figure 2.1.). Treatments were applied such that ammonia concentrations of 0, 75, 150, 225, and 300 mgL^{-1} of NH_4^+ were established (Table 2.1.).

Ammonia treatments for the 2002 study were prepared using reagent-grade ammonium chloride (Fisher Scientific, Fair Lawn, New Jersey, USA) and greenhouse tap water. Tap water was used due to the large volumes of water changes necessary as a batch-loaded system. Batch loading was chosen over continuous flow based on Wang (1991) and Clarke and Baldwin (2002). Treatment solutions were prepared by the addition of appropriate mass of NH_4Cl-N and greenhouse tap water to 6 L, and were

A. Column

Row	A	B	C	D	E
1	300	150	75	225	0
2	150	75	300	0	225
3	225	0	150	75	300
4	0	300	225	150	75
5	75	225	0	300	150
6	225	300	75	150	0
7	75	0	150	300	225
8	300	150	225	0	75
9	0	75	300	225	150
10	150	225	0	75	300

B. Column

Row	A	B	C	D
1	25:1	25:1	25:1	25:1
2	15:1	15:1	15:1	15:1
3	5:1	5:1	5:1	5:1
4	25:1	25:1	25:1	25:1
5	15:1	15:1	15:1	15:1
6	5:1	5:1	5:1	5:1
7	25:1	25:1	25:1	25:1
8	15:1	15:1	15:1	15:1
9	5:1	5:1	5:1	5:1
Treatment (mgL^{-1})	300	225	150	75

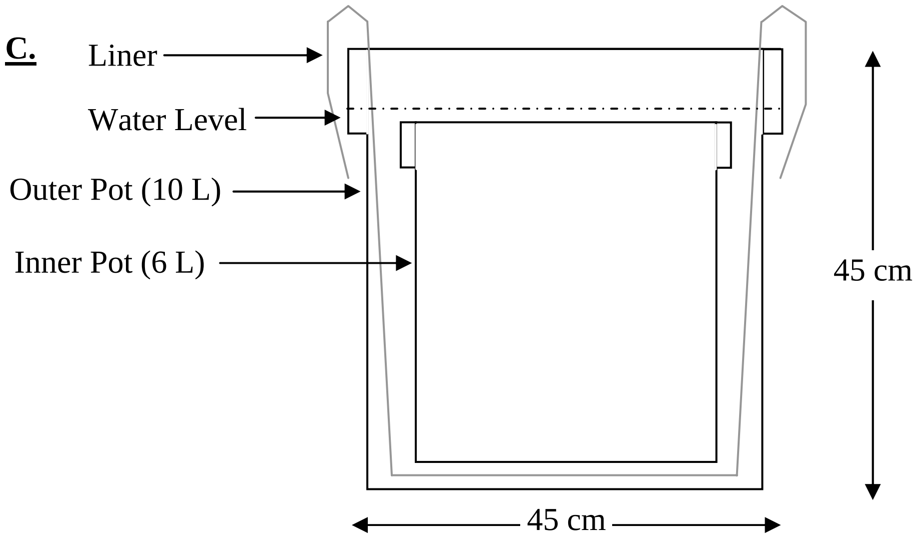

Figure 2.1. Aspects pertaining to greenhouse treatment design (North is towards the top of the page): (**A.**) indicates ammonia concentration (mgL^{-1}) treatment design with row and column squares used in 2002; (**B.**) indicates ammonia concentration (mgL^{-1}) treatment design with N:P ratio inclusion used in 2003; (**C.**) indicates pot-within pot design used for mesocosm setup in both years.

Table 2.1. Summary of nutrient additions to prepare treatment solutions in 6 L increments in 2002, and the associated total mass of ammonium chloride and nitrogen for each treatment.

[Ammonia] Treatment	NH_4Cl (g)	N (g)
0	0	0
75	1.41	0.37
150	2.83	0.74
225	4.24	1.11
300	5.65	1.48

replaced once per week. Solution replacements consisted of draining previously existing solution and replenishment with freshly mixed solution of appropriate concentration.

Treatment Solution, Plant Growth, and Statistical Analyses

Interstitial water was sampled weekly in each experimental unit during solution replacement to insure appropriate ammonium concentration. Due to the number and frequency of measurements and the corresponding frequency of recalibration necessary for such, it was deemed impractical to attempt the use of [NH_3] electronic meters. The Orion Ammonia Electrode (Model 95-12) warrants a suggested recalibration after each measurement, requires five to ten minutes per measurement, and suggests measurements not be taken *in situ* (Thermo Corp., Waltham, Massachusetts, USA). Conductivity meters are robust against need for recalibration, and serve as a useful surrogate for measurements of cation concentration. As ammonium was the only cation added to solution, changes in conductivities were affected primarily by this cation (Clarke 1999). Weekly variations in the initial greenhouse tap water cation-derived conductivity were noted. Conductivities were expected to deviate from desired treatment levels due to evapotranspiration, uptake, nitrification, volatilization, and accumulation on cation-exchange sites in the soil solution. Conductivities for each treatment level in 2002 clearly increased with increasing ammonium concentration, may be found in Figure 2.2; overall greenhouse tap water conductivity averaged 0.7±0.3 microsiemens.

Average above-and below-ground biomass of each species was determined for non-experimental plants prior to application of treatments. In 2002, mean above- and

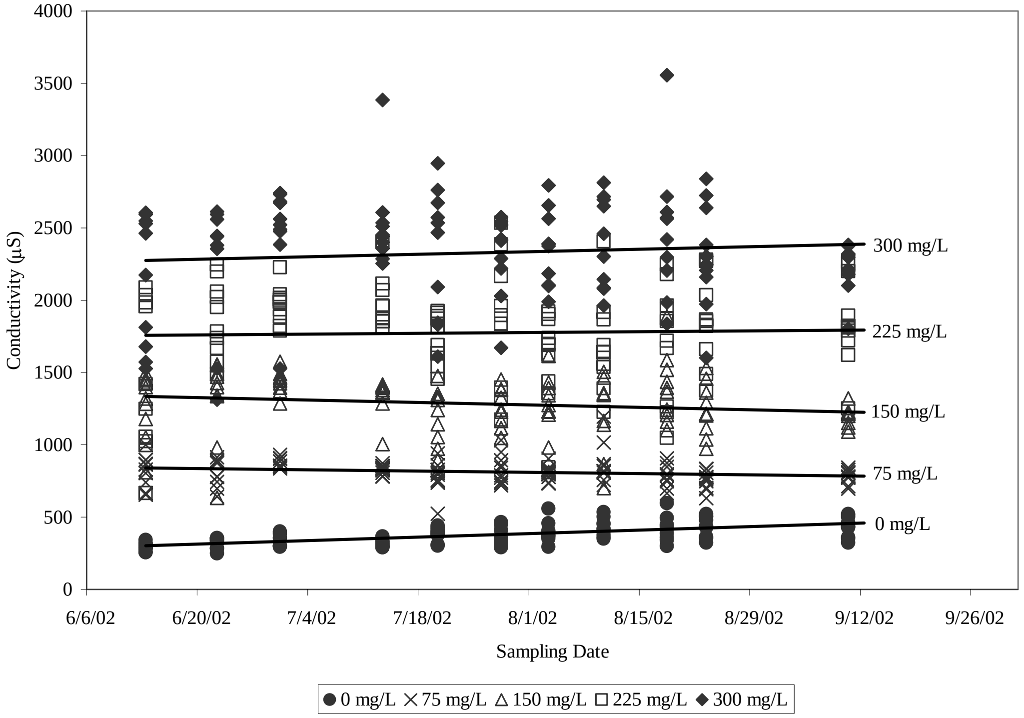

Figure 2.2. Interstitial water conductivities (µS) for each mesocosm at sampling dates in 2002 at experimental ammonia concentrations.

below-ground biomass per *Typha* plant (n=25) were 3.2 ±0.3g and 5.3± 0.2g, respectively (reported as mean ± SE). Mean above- and below-ground biomass per *Juncus* plant (n=25) were 4.3±0.9g and 6.4±0.2g, respectively. Mesocosms were destructively harvested in mid-September after eleven weeks of treatment application. Above-ground material for each species was removed to soil surface; below-ground material was retrieved by removing the root ball from each mesocosm, removing particles of the planting medium, and carefully separating *Juncus* and *Typha* roots, which were clearly discernible based on size and color. Thus, each mesocosm (experimental unit) was separated into four distinct sampling units. Biomass was dried at 27 degrees Centigrade and weighed until stabilization of mass (approximately three days for above-ground and five days for below-ground samples).

Analyses of variance (ANOVAs) were conducted for above- and below-ground biomass and total biomass for each mesocosm and within and between each species. ANOVAs were conducted using SAS Version 8.2 (SAS Institute, Cary, North Carolina, USA). In each case, biomass was the dependant variable; effects of species, column placement (blocking effect), and effects of ammonia treatments were assessed as independent variables. Blocking effect interactions were not analyzed due to significance of first-order blocking effects; significance of first order terms tends to reduce resolution of second order terms. Analyses of variance were likewise conducted for root-to-shoot ratios for *Typha* and *Juncus*, both between the species, and within the species at each ammonia concentration. Normality and homogeneity of variances were examined in each case, and log transformations of dependant data were made when necessary. Significant differences were determined at $\alpha=0.05$.

Because experiment-wise error rates would be elevated by using paired t-test to investigate treatment means and mean root-to-shoot ratios, pair-wise comparisons using ANOVA were selected to allow for a more robust analysis of the interactive effects of the independent variables. Additionally, multiple sampling units were assigned to each experimental unit, and ANOVA minimizes the effect of any potential oversampling complications.

RESULTS AND DISCUSSION

Ammonia concentration significantly affected total, above- and below-ground biomass for each mesocosm at the 5% level in 2002 (Table 2.2). In the case of total biomass for each mesocosm, a definite fertilization effect occurred between the 0 mgL^{-1} and the 75 mgL^{-1} ammonia treatments. The average mesocosm biomass for these treatments was 229.4±25.8 g and 508.2±30.1 g, respectively. A statistically apparent toxicity effect occurred in the 225 mgL^{-1} and the 300mgL^{-1} ammonia treatment levels (Figure 2.3). Toxicity effects may be seen as an overall decrease in the amount of biomass found at these higher treatment levels. This overall trend may be found in mesocosm above-ground biomass (Figure 2.4), although statistically significant toxicity effect begins at the 225 mgL^{-1} treatment level. This trend may be found as well in mesocosm below-ground biomass (Figure 2.5).

Ammonia concentration likewise significantly affected total, above- and below-ground biomass production of *Typha* at the 5% level in all years (Table 2.3). Total *Typha* biomass exhibited a statistically evident fertilization trend in the 75 mgL^{-1} and 150 mgL^{-1} ammonia treatments (Figure 2.6). Mean biomass production at these levels was

Table 2.2. Tests of fixed effects on total, above-ground, and below-ground biomass of mesocosms of *Typha latifolia* and *Juncus effusus* in 2002. Please refer to appendix I for SAS code.

Measured Biomass	**Effect**	**d.f.**	**F Value**	**P Level**
Total Mesocosm	[Ammonia]	4	16.2	p<0.0001
Total Mesocosm	Species	1	98.9	p<0.0001
Total Mesocosm	[Ammonia] x Species	4	6.9	p=0.0002
Total Mesocosm	Column	4	5.1	p=0.0023
Above-ground Mesocosm	[Ammonia]	4	23.2	p<0.0001
Above-ground Mesocosm	Species	1	145.7	p<0.0001
Above-ground Mesocosm	[Ammonia] x Species	4	7.59	p<0.0001
Above-ground Mesocosm	Column	4	2.1	p=0.0775
Below-ground Mesocosm	[Ammonia]	4	5.4	p=0.0006
Below-ground Mesocosm	Species	1	5.5	p=0.0001
Below-ground Mesocosm	[Ammonia] x Species	4	16.3	p=0.0066
Below-ground Mesocosm	Column	4	3.82	p=0.0005

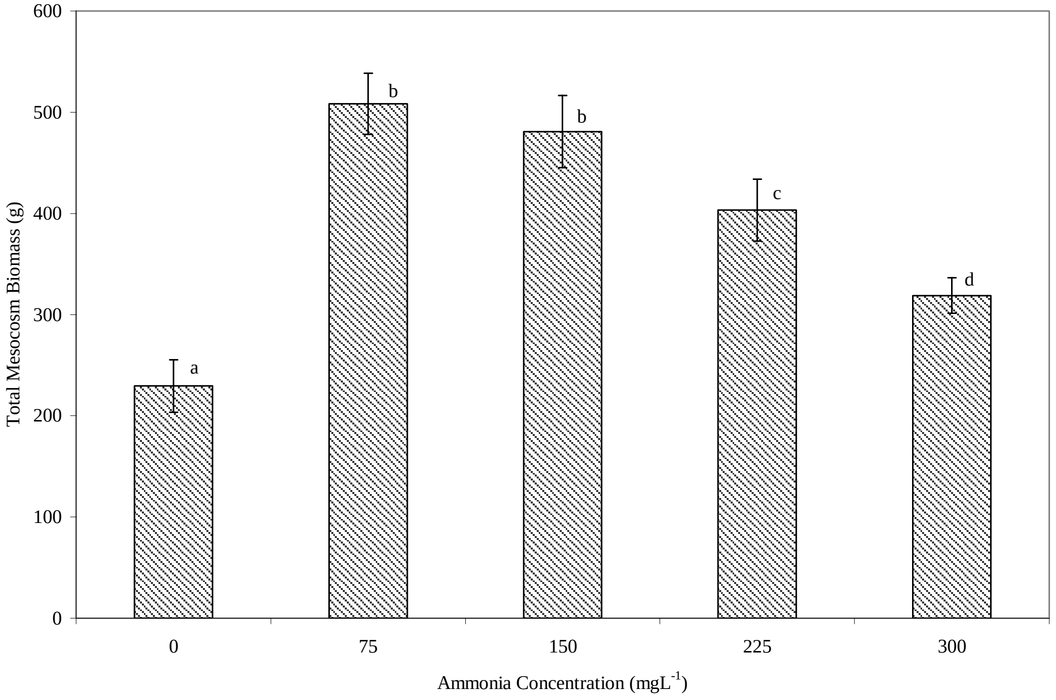

Figure 2.3. Total mesocosm biomass along a gradient of ammonia concentration treatments in 2002. Error bars indicate means ± S.E. Means with different letters indicate significant differences at the 5% level. Due to normality of data and homogeneity of residuals from the ANOVA, transformation was not necessary (see appendix I for SAS code).

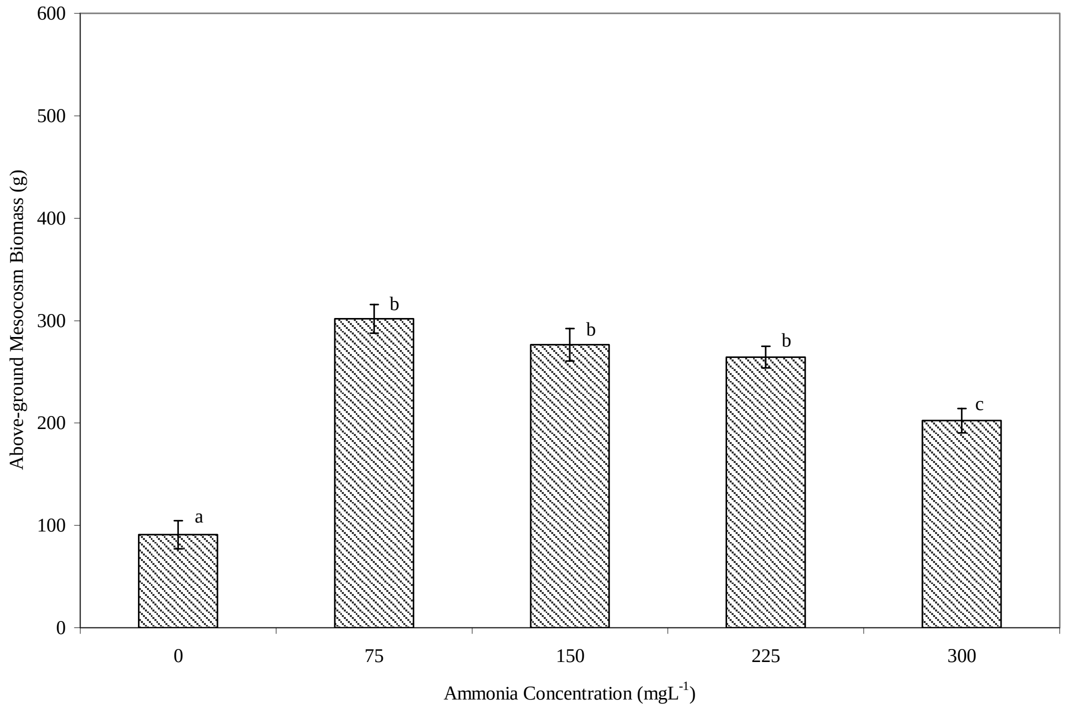

Figure 2.4. Total above-ground mesocosm biomass along a gradient of ammonia concentration treatments in 2002. Error bars indicate means ± S.E. Means with different letters indicate significant differences at the 5% level. Due to normality of data and homogeneity of residuals from the ANOVA, transformation was not necessary (see appendix I for SAS code).

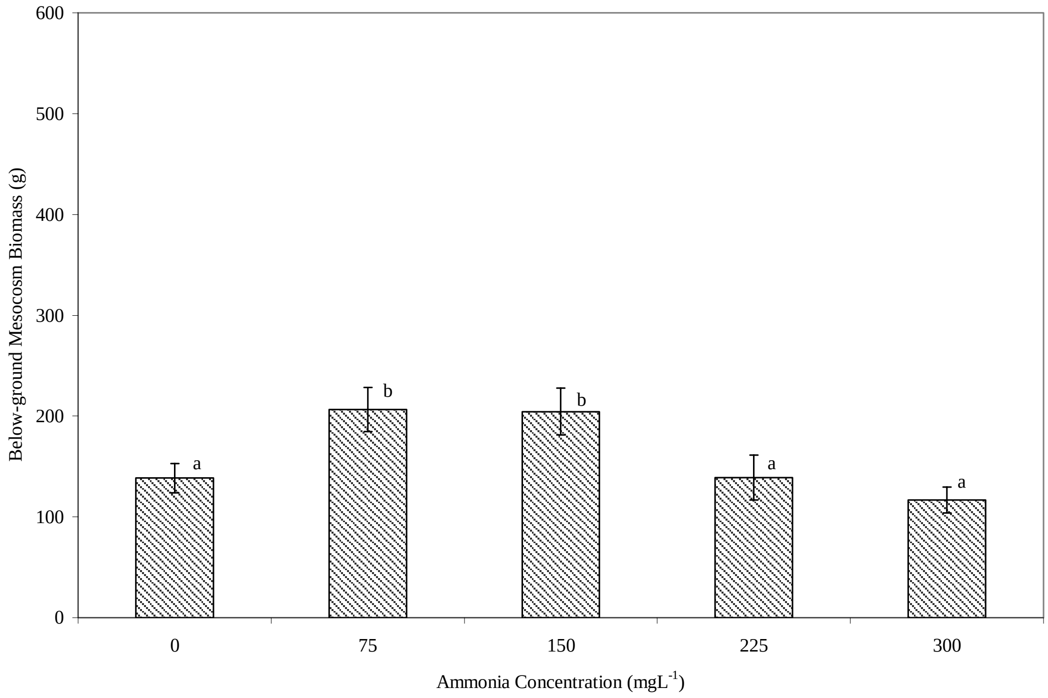

Figure 2.5. Total below-ground mesocosm biomass along a gradient of ammonia concentration treatments in 2002. Error bars indicate means ± S.E. Means with different letters indicate significant differences at the 5% level. Due to normality of data and homogeneity of residuals from the ANOVA, transformation was not necessary (see appendix I for SAS code).

Table 2.3. Tests of fixed effects on total, above-ground, and below-ground biomass of *Typha latifolia* in 2002. Please refer to appendix I for SAS code.

Measured Biomass	**Effect**	**d.f.**	**F Value**	**P Level**
Total *Typha latifolia*	[Ammonia]	4	19.2	p<0.0001
Total *Typha latifolia*	Column	4	2.2	p=0.0859
Above-ground *Typha latifolia*	[Ammonia]	4	14.8	p<0.0001
Above-ground *Typha latifolia*	Column	4	2.3	p=0.0726
Below-ground *Typha latifolia*	[Ammonia]	4	10.71	p<0.0001
Below-ground *Typha latifolia*	Column	4	1.8	p=0.1420

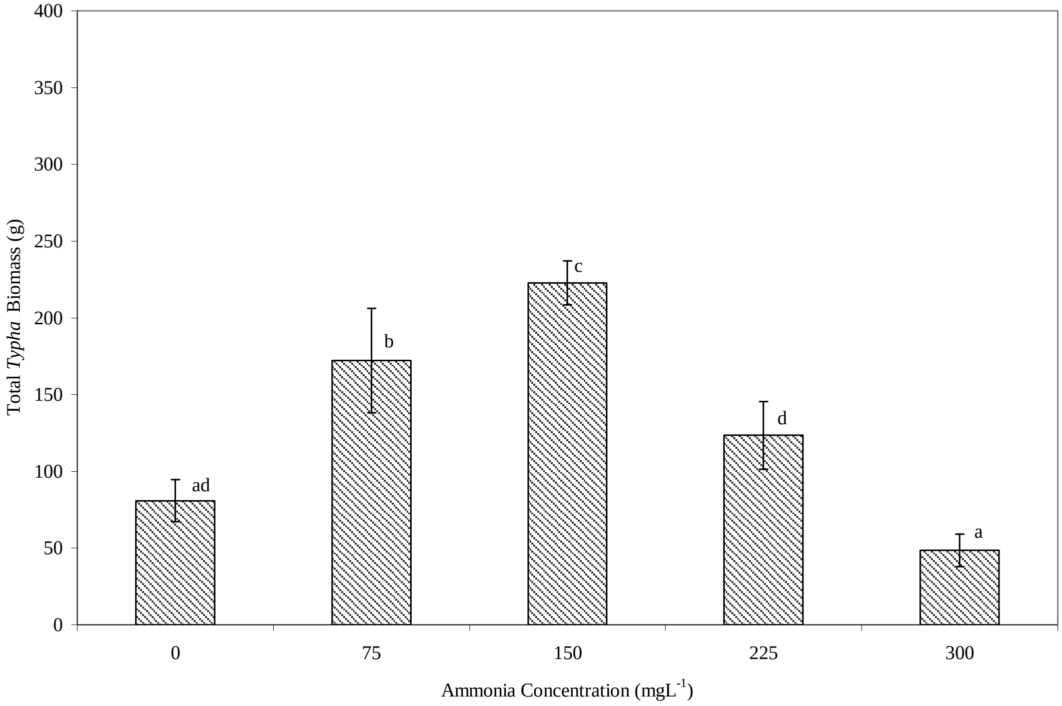

Figure 2.6. Total *Typha latifolia* biomass along a gradient of ammonia concentration treatments in 2002. Error bars indicate means ± S.E. Means with different letters indicate significant differences at the 5% level. Due to normality of data and homogeneity of residuals from the ANOVA, transformation was not necessary (see appendix I for SAS code).

172.2±33.9 g and 222.9±13.3 g respectively, which is elevated from the average 80.9±13.7 g at 0 mgL^{-1} and the toxicity effect displayed at 300mgL^{-1} ammonia, with an average of 48.6±10.7 g. Fertilization effects at between 75 mgL^{-1} and 150 mgL^{-1} may be seen in above-ground biomass production (Figure 2.7) and below-ground biomass production (Figure 2.8) in *Typha* as well. The observable trend in total *Typha* biomass production is similar to the effect produced in overall mesocosm biomass.

Juncus effusus total biomass production and above-ground biomass production were significantly affected by ammonia treatment at the 5% level (Table 2.4). Fertilization of *Juncus* occurred at the 75 mgL^{-1} ammonia treatment, where an average of 336.1±33.8 g of dry tissue was produced over the course of the experiment; at 0 mgL^{-1} ammonia, insufficient nitrogen was available, demonstrated by significant chlorosis at this treatment level, and by the low average biomass of 148.6±14.5 g. Above 75 mgL^{-1}, biomass production was reduced potentially due to ammonia toxicity (Figure 2.9), although differences between biomass production between 75 mgL^{-1} and 225 mgL^{-1} and 300 mgL^{-1} ammonia are not statistically significant; this indicates that, over an ammonia gradient of 150-300 mgL^{-1}, differential effects on growth are non-significant. The trend in above-ground biomass production is similar, with an observable biomass peak existing at the 75 mgL^{-1} treatment level (Figure 2.10). However, below-ground biomass production in *Juncus* was apparently not affected by ammonia concentration (Figure 2.11). This statistically non-significant effect could demonstrate a potential immunity to ammonia toxicity in the roots of *Juncus effusus*. *Juncus* root tissue tends to be denser than *Typha* root tissue, which could allow it to be more tolerant of variable ammonia concentrations. The large ammonia molecule may have difficulty diffusing through this

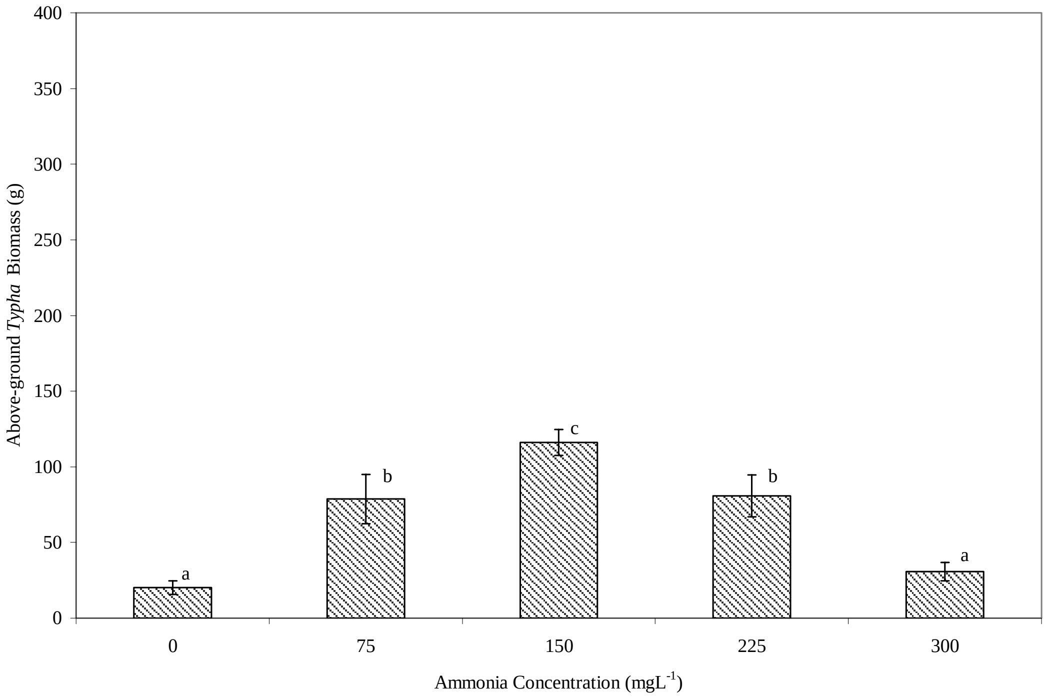

Figure 2.7. Above-ground *Typha latifolia* biomass along a gradient of ammonia concentration treatments in 2002. Error bars indicate means ± S.E. Means with different letters indicate significant differences at the 5% level. Due to normality of data and homogeneity of residuals from the ANOVA, transformation was not necessary (see appendix I for SAS code).

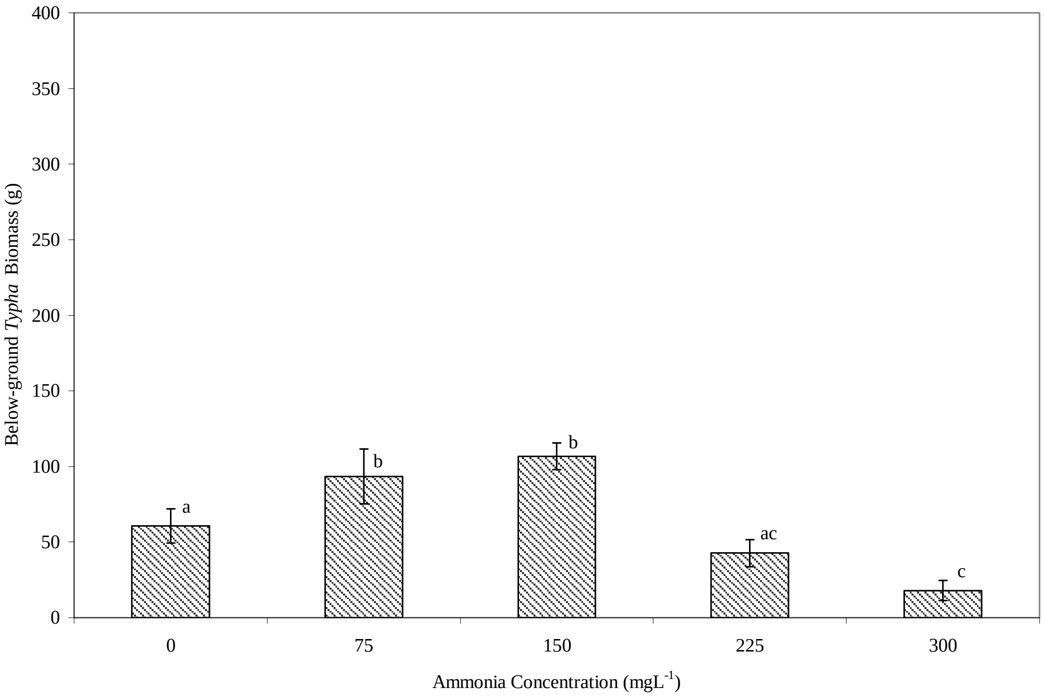

Figure 2.8. Below-ground *Typha latifolia* biomass along a gradient of ammonia concentration treatments in 2002. . Error bars indicate means ± S.E. Means with different letters indicate significant differences at the 5% level. Due to normality of data and homogeneity of residuals from the ANOVA, transformation was not necessary (see appendix I for SAS code).

Table 2.4. Tests of fixed effects on total, above-ground, and below-ground biomass of *Juncus effusus* in 2002. Please refer to appendix I for SAS code.

Measured Biomass	Effect	d.f.	F Value	P Level
Total *Juncus effusus*	[Ammonia]	4	10.08	p<0.0001
Total *Juncus effusus*	Column	4	4.16	p=0.0146
Above-ground *Juncus effusus*	[Ammonia]	4	14.6	p<0.0001
Above-ground *Juncus effusus*	Column	4	0.8	p=0.5651
Below-ground *Juncus effusus*	[Ammonia]	4	0.93	p=0.4578
Below-ground *Juncus effusus*	Column	4	6.5	p=0.0004

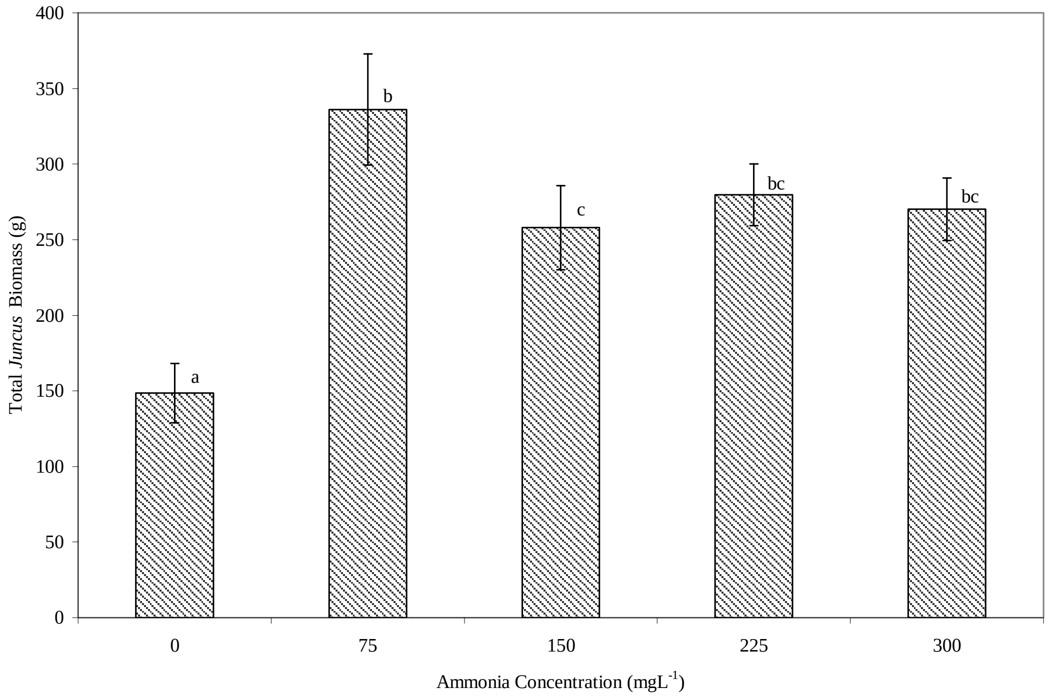

Figure 2.9. Total *Juncus effusus* biomass along a gradient of ammonia concentration treatments in 2002. Error bars indicate means ± S.E. Means with different letters indicate significant differences at the 5% level. Due to normality of data and homogeneity of residuals from the ANOVA, transformation was not necessary (see appendix I for SAS code).

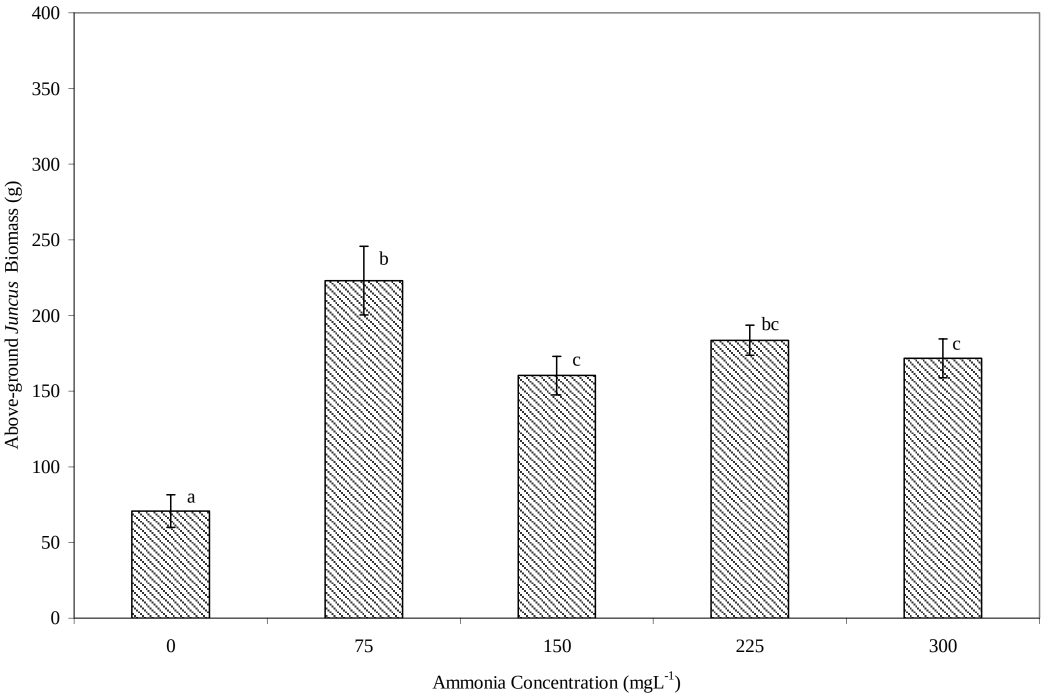

Figure 2.10. Above-ground *Juncus effusus* biomass along a gradient of ammonia concentration treatments in 2002. Error bars indicate means ± S.E. Means with different letters indicate significant differences at the 5% level. Due to normality of data and homogeneity of residuals from the ANOVA, transformation was not necessary (see appendix I for SAS code).

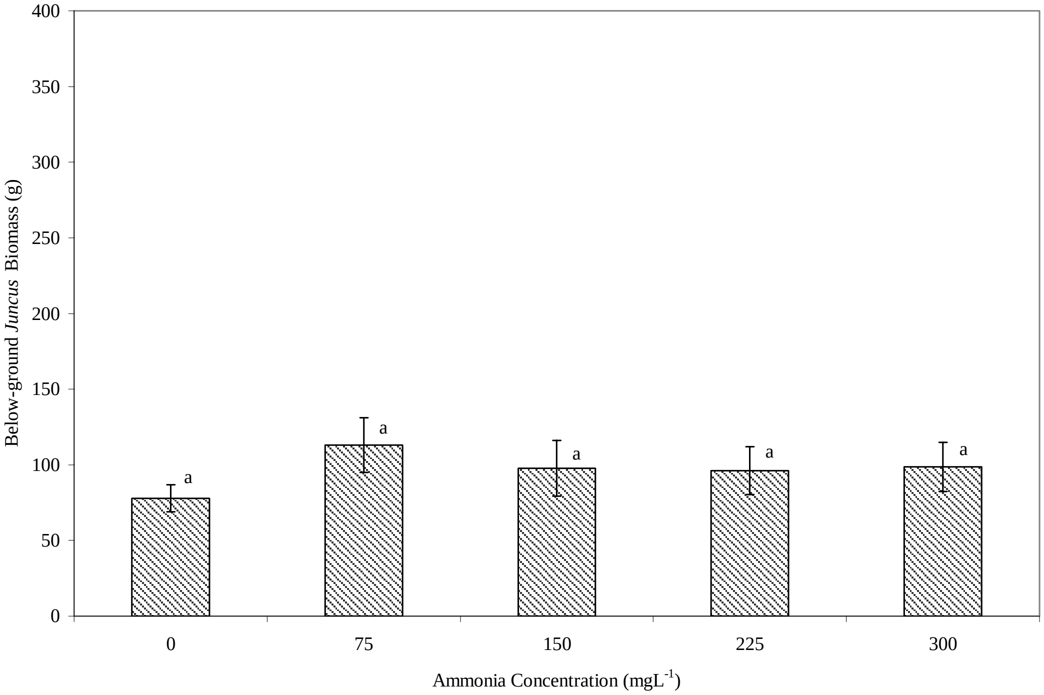

Figure 2.11. Below-ground *Juncus effusus* biomass along a gradient of ammonia concentration treatments in 2002. Error bars indicate means ± S.E. Means with different letters indicate significant differences at the 5% level. Due to normality of data and homogeneity of residuals from the ANOVA, transformation was not necessary (see appendix I for SAS code).

dense root tissue. Also of note is a consistently higher observable overall biomass production and especially overall below-ground biomass, when compared to *Typha* over all treatment conditions. These results indicate that *Juncus* is relatively robust under high ammonia concentrations, and optimal growth is maintained even at high ammonia concentrations, eclipsing previous growth inhibitory effects noted at 110 mgL^{-1} (Clarke 1999, Clarke and Baldwin 2002), and agreeing with Humenik *et al.* (1999) .

Between species total biomass differed significantly at all ammonia concentrations except in the below-ground biomass at low concentrations of ammonia (Table 2.5). Figure 2.12 illustrates significant differences between species within treatments for total biomass. *Typha latifolia* exhibited impaired growth at higher treatment levels, to a low of 48.6±10.5 g at 300 mgL^{-1}, with a peak of 222.8±14.3 g at 150 mgL^{-1}. At this peak alone is there no statistically significant difference between *Typha* biomass and *Juncus* biomass. *Juncus* exhibits peak growth at 75 mgL^{-1}, and diminishes somewhat at higher concentrations. Compared with *Typha, Juncus* appears to have higher ammonia tolerance. Figure 2.13 illustrates significant differences in *Juncus* and *Typha* in above-ground biomass, with a peak of *Typha* production at 150mgL^{-1}, and minimums at 0 and 300 mgL^{-1}, and a peak of *Juncus* production at 75 mgL^{-1}, but without excessive decline thereafter. Below-ground biomass for the species follows the same general observable trend, with significant differences occurring in the 225 mgL^{-1} to 300 mgL^{-1} ammonia concentration levels (Figure 2.14).

Negative response of biomass production to ammonia levels of 300 mgL^{-1} was statistically evident for all measured biomass, with decreasing biomass trends of varying statistical magnitude for all ammonia concentrations above 75 mgL^{-1}. These trends, with

Table 2.5. Summary of statistical analyses of differences in biomass production between *Typha latifolia* and *Juncus effusus* over an ammonia gradient for 2002. At each ammonia treatment, the results of pairwise comparisons of treatment means of *Juncus* and *Typha* are presented. Please refer to Table 2.2 for analysis of the effect of species on biomass production on the whole. Please refer to appendix I for SAS code.

Measured Biomass	**[Ammonia] Treatment**	**d.f.**	**T Value**	**P Level**
Total Mesocosm	0	185	2.2	p=0.0307
Total Mesocosm	75	185	5.7	p<0.0001
Total Mesocosm	150	185	1.1	p=0.2575
Total Mesocosm	225	185	5.3	p<0.0001
Total Mesocosm	300	185	7.7	p<0.0001
Above-ground Mesocosm	0	86	2.9	p=0.0053
Above-ground Mesocosm	75	86	8.2	p<0.0001
Above-ground Mesocosm	150	86	2.5	p=0.0141
Above-ground Mesocosm	225	86	5.8	p<0.0001
Above-ground Mesocosm	300	86	8.0	p<0.0001
Below-ground Mesocosm	0	86	1.0	p=0.3401
Below-ground Mesocosm	75	86	1.1	p=0.2763
Below-ground Mesocosm	150	86	0.5	p=0.6107
Below-ground Mesocosm	225	86	3.0	p=0.0036
Below-ground Mesocosm	300	86	4.5	p<0.0001

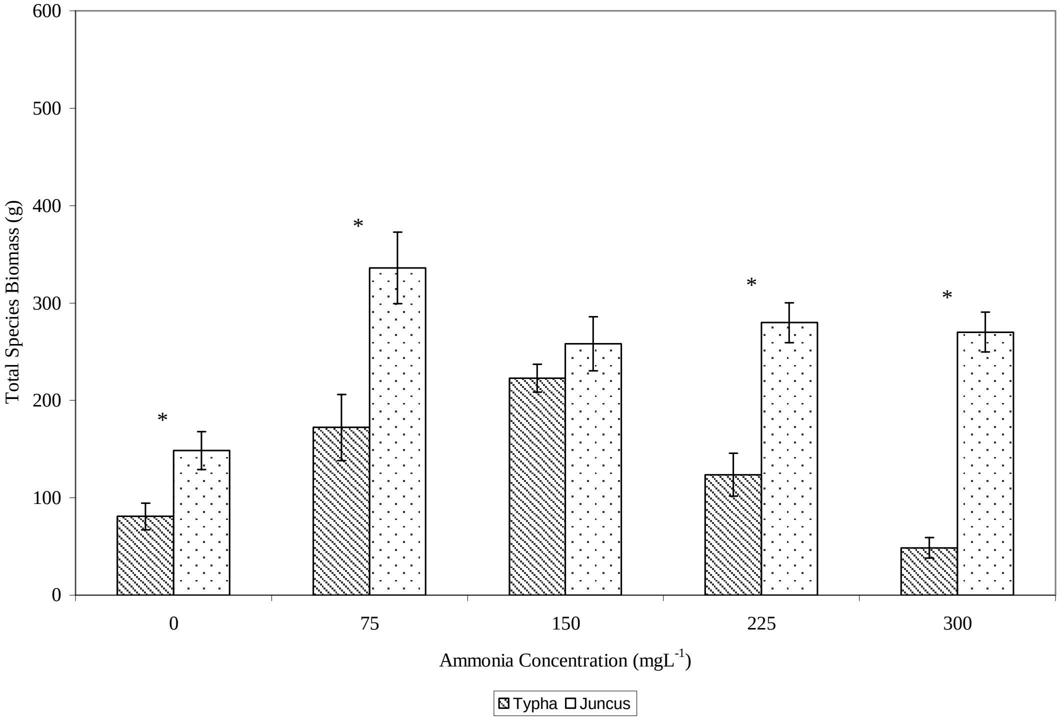

Figure 2.12. Total species biomass along a gradient of ammonia concentration treatments in 2002. Error bars indicate means ± S.E. Paired means with an asterisk indicate significant differences at the 5% level. Due to normality of data and homogeneity of residuals from the ANOVA, transformation was not necessary (see appendix I for SAS code).

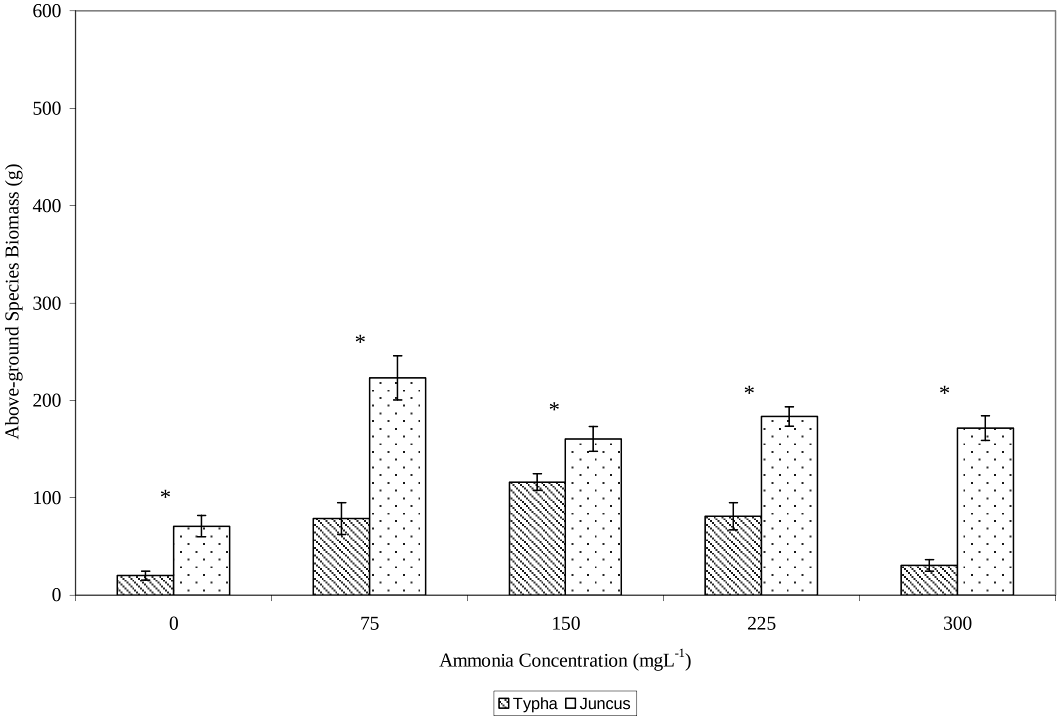

Figure 2.13. Above-ground species biomass along a gradient of ammonia concentration treatments in 2002. Error bars indicate means ± S.E. Paired means with an asterisk indicate significant differences at the 5% level. Due to normality of data and homogeneity of residuals from the ANOVA, transformation was not necessary (see appendix I for SAS code).

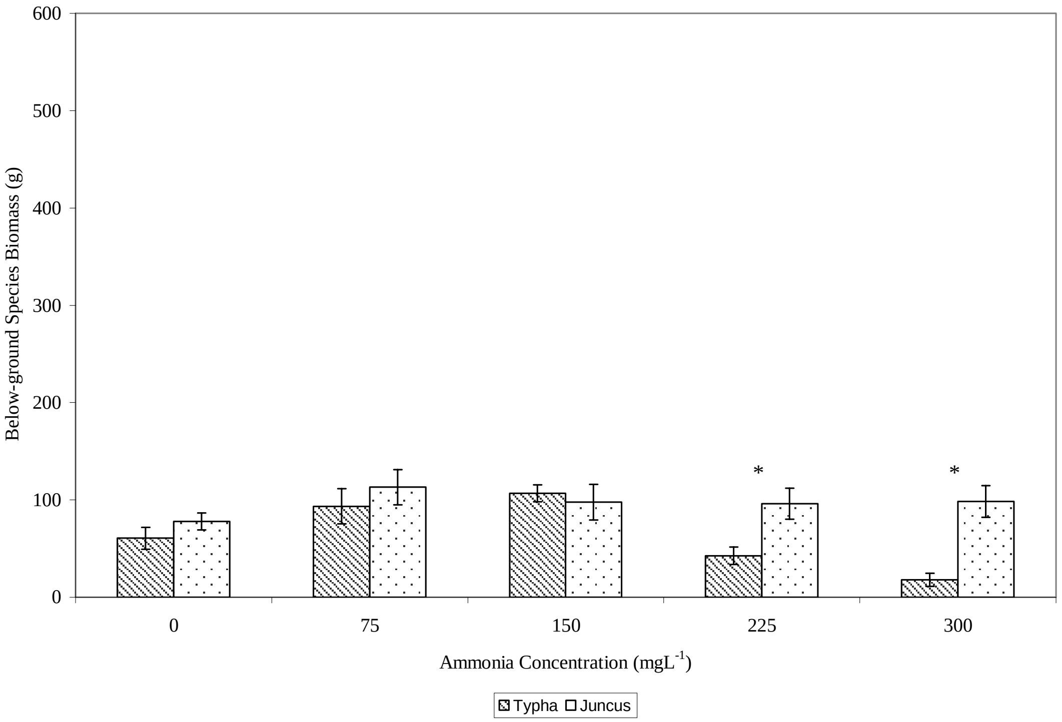

Figure 2.14. Below-ground species biomass along a gradient of ammonia concentration treatments in 2002. Error bars indicate means ± S.E. Paired means with an asterisk indicate significant differences at the 5% level. Due to normality of data and homogeneity of residuals from the ANOVA, transformation was not necessary (see appendix I for SAS code).

some fertilization effect occurring at moderate ammonia-N levels followed by toxic response at higher levels, are in agreement with studies investigating responses of species to varying ammonia levels with similar species (Surrency 1997, Hill *et al.* 1997, Humenik *et al.* 1999, Clarke and Baldwin 2002). Overall, *Typha* appears to grow optimally in the range of 75-100 mgL^{-1}, which corresponds well with the work of previous researchers (Hill *et al.* 1997, Clarke and Baldwin 2002). Other authors have noted stresses for *Typha* at levels approaching 170 mgL^{-1} (Surrency 1993), or a fertilization effect up to 83 mgL^{-1} (Hill *et al.* 1997) and 100 mgL^{-1} (Clarke and Baldwin 2002). Authors have noted tolerances of up to 110 mgL^{-1} for *Juncus* (Clarke 1999), but others note inhibition at relatively low ammonia levels (20.8 mgL^{-1}; Hill *et al.* 1997). The tolerance of *Juncus* tissue, especially of below-ground tissue, may confirm that *Juncus* is a "stress tolerator" moreso than *Typha*, and could therefore be considered dominant under toxic regimes (Emery *et al.* 2001).

Analysis of the root-to-shoot (R:S) ratio is important in considering the role of cytokinins in plant growth. Cytokinin depletion in the rhizosphere corresponds to decreased cytokinin transport to the leaves; low leaf cytokinin levels inhibit cell division (Walch-Liu *et al.* 2000, Taiz and Zeiger 2002). The role of increased levels of ammonia in decreasing shoot growth may be due to disruption of cytokinin transport and cytokinin synthesis in root apical meristem (Taiz and Zeiger 2002, Vojtiskova *et al.* 2004); this results in a reduction of cell division and of leaf elongation (Walch-Liu *et al.* 2000). Root-to-shoot ratio was significantly affected by ammonia, species, and the ammonia by species interaction (Table 2.6). Overall decreases in R:S ratio in the mesocosm are evident in Figure 2.15, but may be found between the 0 mgL^{-1} and the 75 mgL^{-1} ammonia

Table 2.6. Test of fixed effects on the root-to-shoot ratio in mesocosms and in *Typha latifolia* and *Juncus effusus* in 2002. Please refer to appendix I for SAS code.

Measured Root-to-Shoot Ratio	**Effect**	**d.f.**	**F Value**	**P Level**
Total Mesocosm	[Ammonia]	4	11.3	p<0.0001
Total Mesocosm	Species	1	17.8	p<0.0001
Total Mesocosm	[Ammonia] x Species	4	5.3	p=0.0008
Total Mesocosm	Column	4	1.8	p=0.1268
Total *Typha latifolia*	[Ammonia]	4	8.7	p<0.0001
Total *Typha latifolia*	Column	4	2.1	p=0.0978
Total *Juncus effusus*	[Ammonia]	4	12.1	p<0.0001
Total *Juncus effusus*	Column	4	3.1	p=0.0271

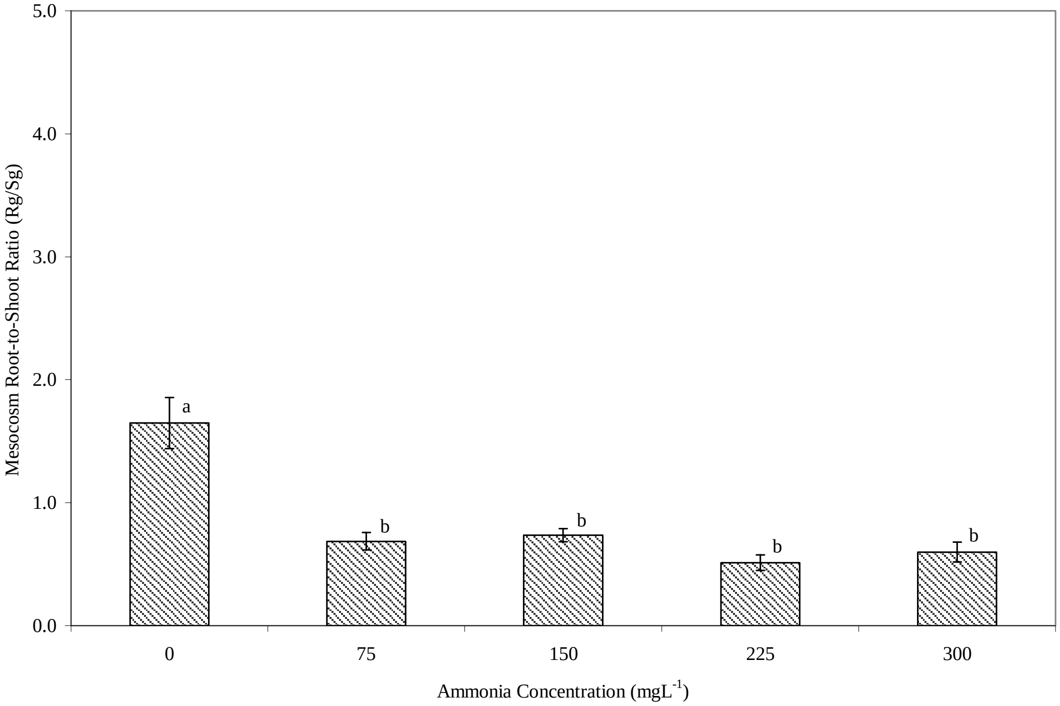

Figure 2.15. Root-to-shoot ratios for mesocosms along a gradient of ammonia concentration treatments in 2002. Error bars indicate means ± S.E. Means with different letters indicate significant differences at the 5% level. Due to normality of data and homogeneity of residuals from the ANOVA, transformation was not necessary (see appendix I for SAS code).

treatments, where overall biomass fertilization effect is occurring. Both species show the same general effect, with differences in the R:S ratio evident only in the 0 mgL^{-1} treatment (Figure 2.18). This demonstrates similarity in the effects of ammonium toxicity on the two species. Decreases in the R:S ratio in *Juncus* and in *Typha* individually are statistically evident as well (Table 2.6, Figures 2.16 and 2.17), and indicate suppression of root formation, increased shoot production, or a combination of the two. Since *Juncus* below-ground biomass is not significantly affected by ammonia concentration, increased shoot production must have occurred to generate this statistically significant decrease in R:S ratio. Variation in the R:S ratio is due primarily to ammonia treatment and the species considered, but may also be due to the interaction of the terms, indicating some level of interaction between the species. Increases in ammonia concentration and subsequent reduction of the R:S ratio corresponds well with similar responses in other species (Walch-Liu *et al.* 2000, Vojtiskova *et al.* 2004), although direct measurement of R:S ratio inhibition by ammonia has not been noted in *Juncus* and *Typha* prior to this study.

The significance of the blocking effect in this analysis was a demonstration of the effects of North-South positioning in the greenhouse (the reader is directed to Tables 2.2 through 2.6 for those instances where the blocking effect is statistically significant). Some variation in biomass production was caused as a result of the orientation of the mesocosms along the axis of the greenhouse, and it was therefore advisable to utilize this fixed effect in experimental design. It is of interest to note that the column effect, that is, the effect of the rising and setting of the sun on providing enough light for the plants, is more evident in *Juncus,* and especially in below-ground *Juncus* biomass, where it

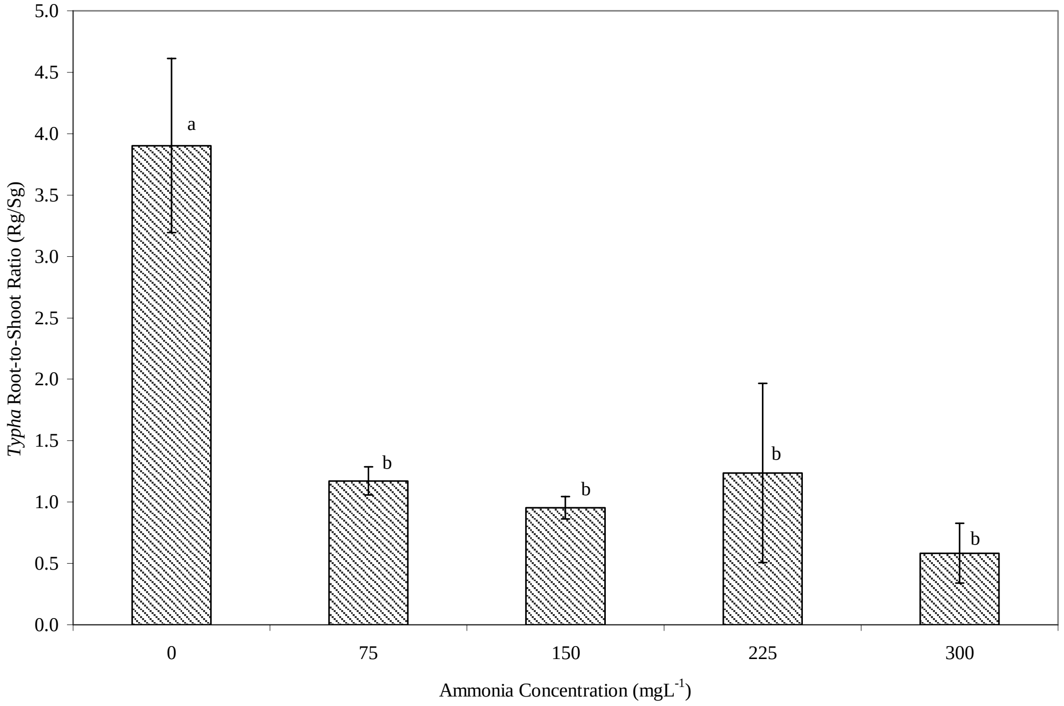

Figure 2.16. Root-to-shoot ratio for *Typha latifolia* along a gradient of ammonia concentration treatments in 2002. Error bars indicate means ± S.E. Means with different letters indicate significant differences at the 5% level. Due to normality of data and homogeneity of residuals from the ANOVA, transformation was not necessary (see appendix I for SAS code).

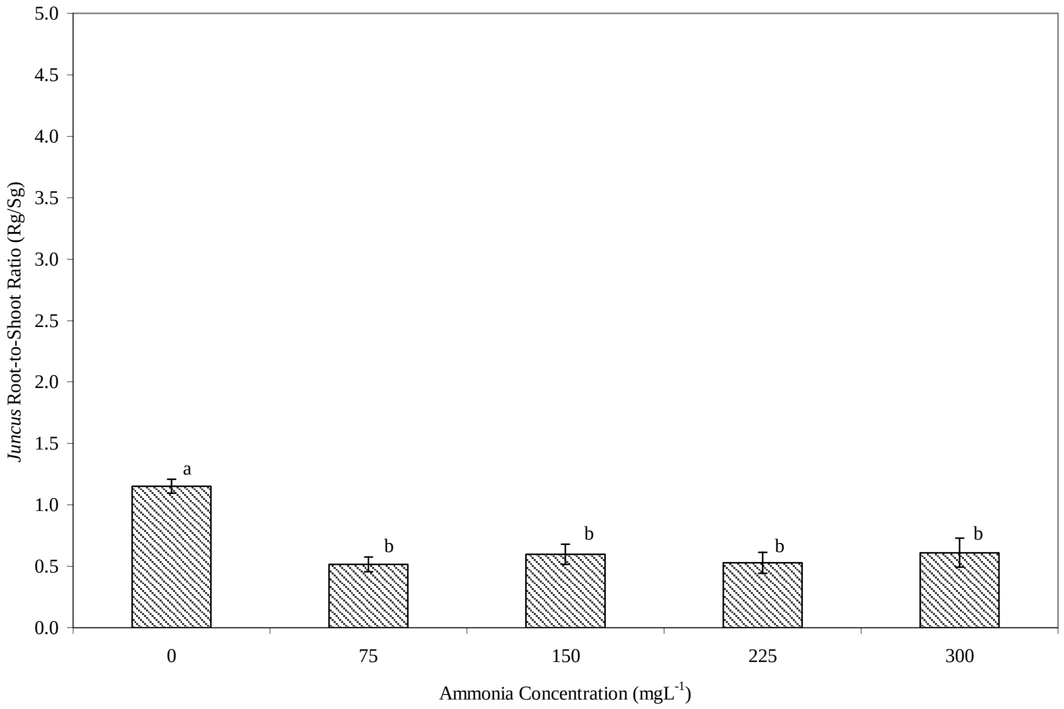

Figure 2.17. Root-to-shoot ratio for *Juncus effusus* along a gradient of ammonia concentration treatments in 2002. Error bars indicate means ± S.E. Means with different letters indicate significant differences at the 5% level. Due to normality of data and homogeneity of residuals from the ANOVA, transformation was not necessary (see appendix I for SAS code).

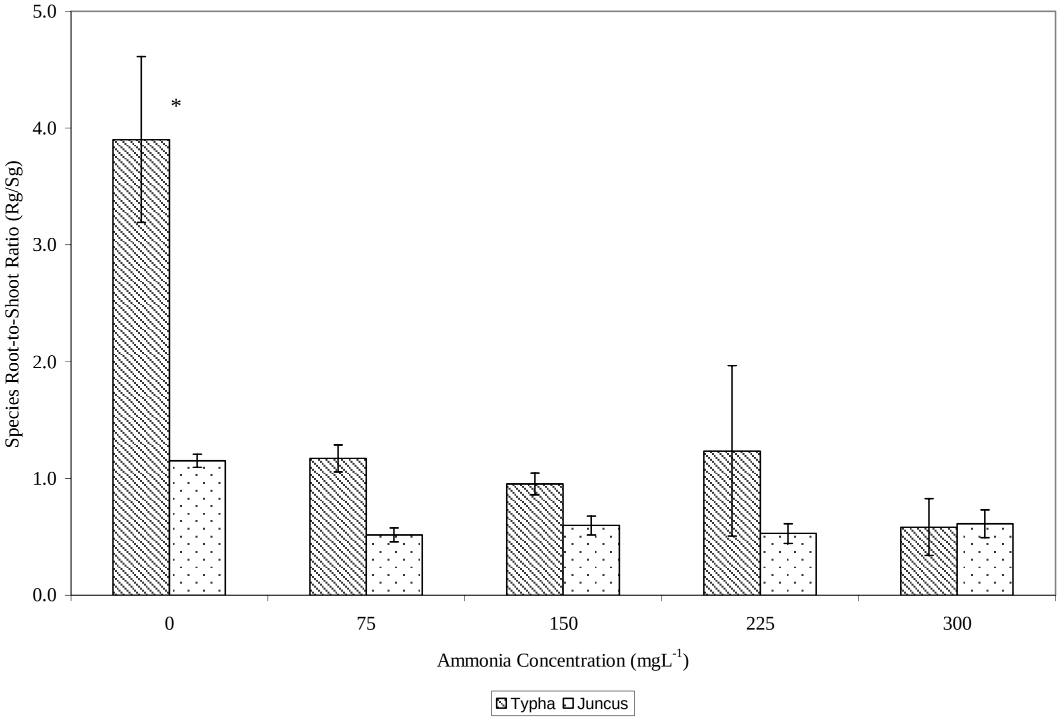

Figure 2.18. Species root-to-shoot ratios along a gradient of ammonia concentration treatments in 2002. Error bars indicate means ± S.E. Paired means with an asterisk indicate significant differences at the 5% level. Due to normality of data and homogeneity of residuals from the ANOVA, transformation was not necessary (see appendix I for SAS code).

accounted for more variation than did the ammonia treatment itself (Table 2.4). *Juncus* is a plant of shorter stature, and was shaded by the taller *Typha* to produce this effect.

The effects of *Juncus* and *Typha* upon biomass production or either species are important to note as a result of this study. Although Tilman (1982) found *Typha* to be a better competitor under N-fertilization regimes, intrageneric competition along a phytotoxic nutrient gradient is a little known trend. The interactive effects of toxicity and competition are poorly documented. Most work to date has focused on field-scale studies along much lower nutrient gradients for fertilization effects typical of natural ecosystems (e.g. Tilman 1982, Tilman *et al.* 1999, Wilson and Tilman 1995, Mamolos and Veresoglou 2000). In this study, *Juncus* produced more total biomass than *Typha* for all ammonia concentrations in 2002 (Figure 2.12) In no case did *Juncus* produce significantly less biomass than *Typha* in any biomass measurement and at any ammonia treatment, despite similar initial biomass at the inception of this study.

The ability of *Juncus* to tolerate stress better than *Typha* may occur as a result of plant morphology in a confined system. Pot size could lead to an inability to establish new culms, and although other studies have indicated that *Typha* can establish quickly in confined situations (Clarke 1999, Svensouk and Mitch 2000), none of these were conducted in a 10 L pot size. *Typha latifolia* tends to occur in relatively deep water conditions (Grace and Wetzel 1981); the presence of high amounts of aerenchymous tissue in the stems and the presence of numerous fine root hairs are likely to facilitate high rates of gas exchange through anoxic sediments (Brix *et al.* 1992). Additionally, the presence of a large rhizome in *Typha* indicates a high storage capacity for nutrients. *Juncus*, on the other hand, has aerenchymous tissue with significantly smaller gas volume, and also fewer root hairs; it is typically found in clumps in oxygen-poor but not necessarily anoxic sediments. *Juncus* has a significantly smaller base culm, certainly without the starchy tuber of *Typha latifolia*, and therefore uses nutrients as they become available in lieu of storing them. *Typha* stores nutrients; this capacity may allow it to outcompete other species in times of nutrient stress, but will also force accumulation of excess nutrients in times of overabundance of nutrients. In this study, rhizomes of *Typha* in high ammonia concentrations tended to be smaller than those of lower concentrations, where they existed at all. Often, stunted, smaller rhizomes were found in higher nutrient situations, exhibiting much necrotic tissue and decay. This finding appears to be unique in the literature; for the most part, managers have focused on eliminating *Typha* in order to increase diversity in treatment systems (Kadlec and Knight 1996, Mitsch and Gosselink 2000) or developing planting schemes to enhance the competitive abilities of other species (Svengsouk and Mitsch 2000). Extremely elevated ammonia concentrations here helped to limit the dominance of *Typha*.

CONCLUSIONS

Above-ground, below-ground, and total biomass for the mesocosms were significantly affected by ammonia concentrations at the 5% level. Biomass measurements were maximized in the 75 to 150 mgL^{-1} ammonia concentration levels, and declined for higher concentrations of ammonia. Above-, below-, and total biomass of *Typha latifolia* were significantly affected by ammonia concentrations over a one-year period. Above-ground and total biomass of *Juncus effusus* were affected by ammonia concentrations at the as well, although in many cases *Juncus* grew at productive levels even at higher ammonia concentrations. Decreases in cytokinin production due to elevated ammonia levels may have limited cell division, leaf elongation, and overall biomass production in these species are trends shown by a decrease in the root-to-shoot ratio. Evident trends in biomass production demonstrate a high stress tolerance ability of *Juncus effusus* in high ammonia concentrations typical of constructed wetlands for wastewater treatment; the species demonstrated significantly higher ammonia tolerance than previously reported. *Juncus* has often been overlooked by managers hoping to create a diverse wetland community (Kadlec and Knight 1996, Mitsch and Gosselink 2000); its performance here may call for a re-evaluation of its competitive dynamic.

Observation of the effects of additional limiting or phytotoxic nutrient concentrations may further elaborate competitive performance differences between these two species and others, as differences in tissue ratios often indicate differences in uptake mechanisms and potential effects of limitation.

Potential increased genetic knowledge of enzyme control of ammonia toxicity in, for example, *Arabidopsis*, may lead to better control of toxic effects (Kronzucker *et al.* 2001, Glass *et al.* 2002), but application to wetland species known to have specific enzyme adaptations to anoxia and its affect on cation uptake pathways (Mitsch and Gosselink 1993) may not occur for some time. Until the problem of ammonia toxicity can be better understood, pre-treatment of wastes prior to introduction into ATWs probably remains the best policy in maximizing biomass production and subsequent nitrogen removal.

CHAPTER THREE:

THE VEGETATION N:P RATIO IN MESOCOSMS SUBJECTED TO HIGH AMMONIA CONCENTRATIONS

OBJECTIVE

The objective of this study was to investigate the role of the vegetation N:P ratio in illustrating relationships in *Juncus effusus* L. and *Typha latifolia* L. to mixtures of ammonia and phosphorus under controlled conditions. The specific relationships of interest were the overall biomass production by each species, the effect of the N:P ratio on the nitrogen uptake of each species, and the effect of the experimental N:P ratio on actual vegetation N:P ratio. These objectives were accomplished in a one-year greenhouse study during which plants were exposed to levels of 75, 150, 225 and 300 mgL^{-1} of ammonia amended with phosphate-P concentrations at N:P ratios of 5:1, 15:1 and 25:1 over a ten week period. Plants were cultured as in Chapter Two. Using *Juncus* and *Typha* following the rationale explained above allowed investigation of changes in N:P ratios, such that the N:P ratio may be used to determine species fitness over a range of treatment levels. Growth in these species was tested for limitation by N, P or co-limitation, and the utility of indications of growth limitation by the N:P ratio was examined.

METHODS

Preparation of Mesocosms

Plant material was purchased, transported, and prepared as described in Chapter Two in May of 2003. After an initial acclimation period, each planted mesocosm was placed in a lined 10 L pot (Figure 2.1). These mesocosms (experimental units) were placed in four columns aligned North-South (labeled A through D), and nine rows aligned East-West (labeled 1 through 9). Treatments of ammonia concentrations were applied such that each column received a single ammonia concentration for ease of treatment application (the same concentrations as used in the 2002 experiment, except the 0 mgL^{-1} treatment, Figure 2.1). Treatments of phosphorus were applied such that, in each row, each N:P concentration was applied three times (Figure 2.1). For this experimental design, each N:P ratio occurred in each ammonia concentration treatment three times. Nitrogen to phosphorus ratios were chosen based on Koerselman and Meuleman (1996) with categories of N-limited, co-limited, and P-limited at ratios of 5:1, 15:1 and 25:1, respectively. The ammonia concentration of 0 mgL^{-1} was removed as appropriate N:P ratios to a 0 mgL^{-1} ammonia treatment do not exist. Mass values of total nitrogen, ammonia chloride, sodium phosphate, total phosphate, and total phosphorus, and accompanying N:P ratios are found in Table 3.1.

Ammonia treatments were prepared using the 2002 protocol. Treatments of N:P ratio were prepared by adding appropriate amounts of reagent grade $Na_3(PO_4)_2$ (Fisher Scientific, Fair Lawn, New Jersey, USA) to ammonia treatments and greenhouse tap water to 6 L (see Table 3.1). Solutions were replaced once weekly following the above protocol for 2002 with the addition of the N:P ratio treatment described above.

Treatment Solution, Vegetation N:P Ratio, and Statistical Analyses

Interstitial water sampling was carried out using the methodology described above for experiments occurring in 2002. As in 2002, conductivities clearly increased with

Table 3.1. Summary of nutrient additions to prepare treatment solutions in 6 L increments in 2003, and the associated total mass of ammonium chloride, nitrogen, phosphate, and total phosphorus, and accompanying N:P ratios for each treatment.

[Ammonia] Treatment	NH_4Cl (g)	N (g)	N:P Ratio Treatment	P (g) Required	NaH_2PO_4 (g)	[Phosphorus]
75	1.41	0.37	5	0.07	0.29	12.34
75	1.41	0.37	15	0.02	0.10	4.11
75	1.41	0.37	25	0.01	0.06	2.47
150	2.83	0.74	5	0.15	0.57	24.67
150	2.83	0.74	15	0.05	0.19	8.22
150	2.83	0.74	25	0.03	0.11	4.93
225	4.24	1.11	5	0.22	0.86	37.01
225	4.24	1.11	15	0.07	0.29	12.34
225	4.24	1.11	25	0.04	0.17	7.40
300	5.65	1.48	5	0.30	1.15	49.35
300	5.65	1.48	15	0.10	0.38	16.45
300	5.65	1.48	25	0.06	0.23	9.87

increasing ammonium addition (Figure 3.1A); additions of phosphorus did not significantly affect conductivity; slopes of the lines for conductivity of the three N:P ratio treatments are not significantly different (Figure 3.1B).

Hydrogen ion concentration (pH) was measured twice monthly in the 2003 experiment to ensure that the addition of a weak diprotic acid ($Na_3(PO_4)_2$) did not result in significant pH differences between treatments; phosphate concentration was measured twice monthly to identify and correct any deviations from the proposed ratios that may have occurred due to evaporation of solvent, adsorption of phosphate ions, or other forms of accumulation using the appropriate colorimetry protocol (Spectrophotometer Model DR/2500, Hach Co., Loveland, Colorado, USA).

Average above- and below-ground biomass of each species was determined for non-experimental plants prior to application of treatments. In 2003, mean above- and below-ground biomass per *Typha* plant (n=28) were 2.3± 0.4g and 3.3± 0.3g, respectively (reported as mean ± SE). Mean above- and below-ground biomass per *Juncus* plant (n=28) were 3.9±0.2g and 6.4±0.6g, respectively. Mesocosms were destructively harvested in mid-September after ten weeks of treatment application. Above-ground material for each species was removed to soil surface; below-ground material was retrieved by removing the root ball from each mesocosm, removing particles of planting medium, and carefully separating *Juncus* and *Typha* roots, which were clearly discernible based on size and color. Thus, each mesocosm was separated into four distinct sampling units. Biomass was dried at 80 degrees Centigrade and weighed until stabilization of mass (approximately 3 days for above-ground and five days for below-ground samples).

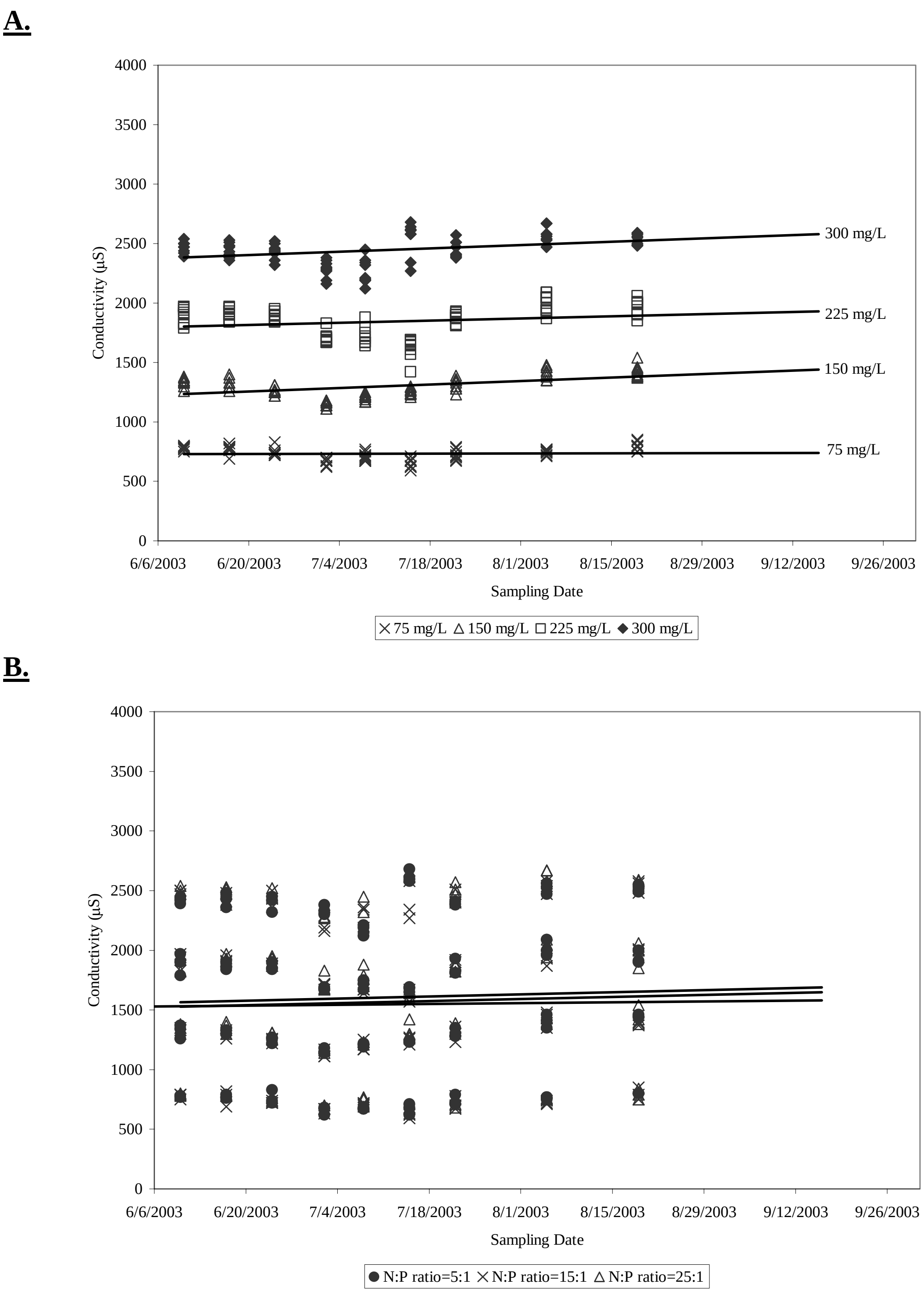

Figure 3.1. Interstitial water conductivities (µS) for each mesocosm at sampling dates in 2003: (**A.**) indicates conductivities based on experimental ammonia concentrations; (**B.**) indicates conductivities based on experimental N:P ratios.

Tissue nitrogen and phosphorus concentrations for each species were measured by dry matter combustion and color spectroscopy. Tissue samples from above- and below-ground biomass of each species were removed from the biomass following measurement for the above studies. For nitrogen and phosphorus analysis, 0.05 grams were removed from the initial 5 g samples following grinding for homogenization. The use of only 0.05 grams in lieu of the typical 0.1 grams was necessary due to problems with samples sticking in the tube prior to the spectroscopy runs. Samples were purged of atmospheric gases entering during sample loading, combusted in series at 950 and 850 degrees Centigrade, and measured following written protocols for LECO TruSpec CN Elemental Determiner (LECO Inc., St. Joseph, Missouri, USA). For phosphorus samples CT Kjeldahl Digestion Mixture was used following like protocols.

Analyses of variance (ANOVA) were conducted for several effects using SAS Version 8.2 (SAS Institute, Cary, North Carolina, USA). Above- and below-ground biomass ANOVAs were conducted using biomass as the dependant variable, and as independent variables, effects of experimental N:P ratio treatment, ammonia concentration treatment, species, and the interactions of N:P ratio treatment, ammonia treatment, and species were tested. Tissue nitrogen concentration ANOVAs were conducted using above- and below-ground tissue nitrogen concentration as the dependant variable, and as independent variables, effects of experimental N:P ratio treatment, ammonia concentration treatment, species, and the interactions of N:P ratio treatment, ammonia treatment, and species were tested. Interaction terms are of importance due to the fact that a significant N:P ratio by ammonia interaction could potentially infer that biomass production is maintained at high ammonia concentrations given phosphorus fertilization. Total tissue mass nitrogen ANOVAs were conducted using above- and below-ground tissue total mass nitrogen as the dependant variable, and as independent variables, effects of experimental N:P ratio treatment, ammonia concentration treatment, species, and the interactions of N:P ratio treatment, ammonia treatment, and species were tested. Tissue phosphorus concentration ANOVAs were conducted using above- and below-ground tissue phosphorus concentration as the dependant variable, and as independent variables, effects of experimental N:P ratio treatment, ammonia concentration treatment, species, and the interactions of N:P ratio treatment, ammonia treatment, and species were tested. Interaction terms were tested in these cases as well to test if phosphorus additions at different ammonia concentrations significantly affected uptake or total mass of nitrogen and uptake of phosphorus. In each ANOVA, normality and homogeneity of residuals were examined for each variable, and data were log-transformed when necessary. Significant differences were determined at $\alpha=0.05$.

RESULTS AND DISCUSSION

Mesocosm Biomass Production

Total biomass was significantly affected by ammonia concentration treatment and species tested (Table 3.2). Total mesocosm biomass declined from an average of 373.0±14.0 g at the 75 mgL^{-1} ammonia treatment level to an average of 277.5±12.4 g at the 300 mgL^{-1} ammonia treatment (Figure 3.2). This concurs with the 2002 results, in which biomass declined from the 75 mgL^{-1} ammonia treatment level to the 300 mgL^{-1} treatment level. *Juncus* again exhibited significantly higher overall average biomass than *Typha*, with an average of 223.5±14.0 g for *Juncus* versus 83.1±11.1 g for *Typha*.

Table 3.2. Tests of fixed effects on total, above-ground, and below-ground biomass of mesocosms of *Typha latifolia* and *Juncus effusus* in 2003. Please refer to appendix II for SAS code.

Measured Biomass	**Effect**	**d.f.**	**F Value**	**P Level**
Total Mesocosm	Species	1	3.96	0.0538
Total Mesocosm	[Ammonia]	3	7.58	0.0004
Total Mesocosm	Species x [Ammonia]	3	0.69	0.5622
Total Mesocosm	N:P Ratio	2	0.57	0.5691
Total Mesocosm	N:P Ratio x Species	2	0.25	0.7824
Total Mesocosm	N:P Ratio x [Ammonia]	6	0.42	0.8608
Total Mesocosm	Species x N:P Ratio x [Ammonia]	6	1.1	0.3807
Above-ground Mesocosm	Species	1	6.22	0.0171
Above-ground Mesocosm	[Ammonia]	3	11.22	<0.0001
Above-ground Mesocosm	Species x [Ammonia]	3	0.74	0.5328
Above-ground Mesocosm	N:P Ratio	2	0.16	0.8557
Above-ground Mesocosm	N:P Ratio x Species	2	1.07	0.3527
Above-ground Mesocosm	N:P Ratio x [Ammonia]	6	0.5	0.8033
Above-ground Mesocosm	Species x N:P Ratio x [Ammonia]	6	1.14	0.3564
Below-ground Mesocosm	Species	1	6.84	0.0128
Below-ground Mesocosm	[Ammonia]	3	8.53	0.0002
Below-ground Mesocosm	Species x [Ammonia]	3	0.42	0.7364
Below-ground Mesocosm	N:P Ratio	2	0.32	0.7268
Below-ground Mesocosm	N:P Ratio x Species	2	0.85	0.4351
Below-ground Mesocosm	N:P Ratio x [Ammonia]	6	0.41	0.8683
Below-ground Mesocosm	Species x N:P Ratio x [Ammonia]	6	0.66	0.6815

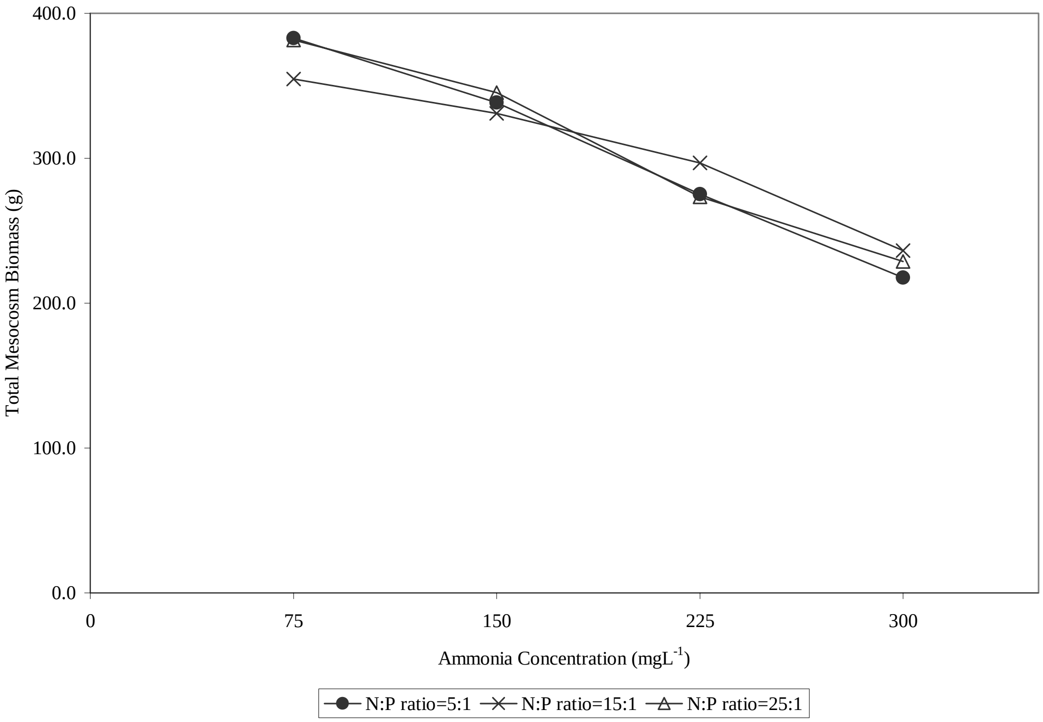

Figure 3.2. Total mesocosm biomass production along a gradient of ammonia concentration treatments and at three N:P ratios in 2003. Standard errors are not presented due to graphic difficulty in their interpretation. Due to normality of data and homogeneity of residuals from the ANOVA, log transformation was not necessary (see appendix II for SAS code).

Observable, although not statistically significant differences between the species at each ammonia concentration level corresponds well to the 2002 data (Table 2.5).

Total above-ground mesocosm biomass was significantly affected by species and ammonia concentration treatment level (Table 3.2). A similar trend as for overall mesocosm biomass is evident, with steadily declining biomass production for higher levels of ammonia concentration (Figure 3.3); likewise overall *Juncus* above-ground biomass, 161.7±11.0 g, is significantly higher than for *Typha* above-ground biomass, 53.7±7.2 g. Of interest, however, is the slight increase in biomass production between the 75 mg/L^{-1} ammonia concentration and the 150 mg/L^{-1} ammonia concentration treatments for the 15:1 N:P ratio. It appears that at co-limited N:P levels, biomass production has a peak at higher nitrogen concentrations, although the peak production itself is not statistically different than the biomass production for other N:P ratios.

Total below-ground biomass was significantly affected by ammonia concentration treatment and species tested (Table 3.2). Like above-ground biomass, a similar trend to that of total mesocosm biomass is evident, with toxicity effects at ammonia concentrations of above 75 mgL^{-1} treatment levels. *Juncus effusus* biomass production was 60.3±4.9 g, while *Typha* biomass was just 29.4±4.7 g. At all levels of ammonia treatment, *Juncus* produced significantly more biomass. Production levels of below-ground biomass were observably (but not statistically) greater at N:P levels of 15:1 for ammonia treatment concentrations of 225 mgL^{-1} and 300 mgL^{-1} (Figure 3.4).

Of particular interest in this study is the effect of additions of phosphorus in varying ratios to ammonia-N in alleviating potentially toxic ammonia concentrations. Although in overall mesocosm biomass analysis, N:P ratio treatments are not a

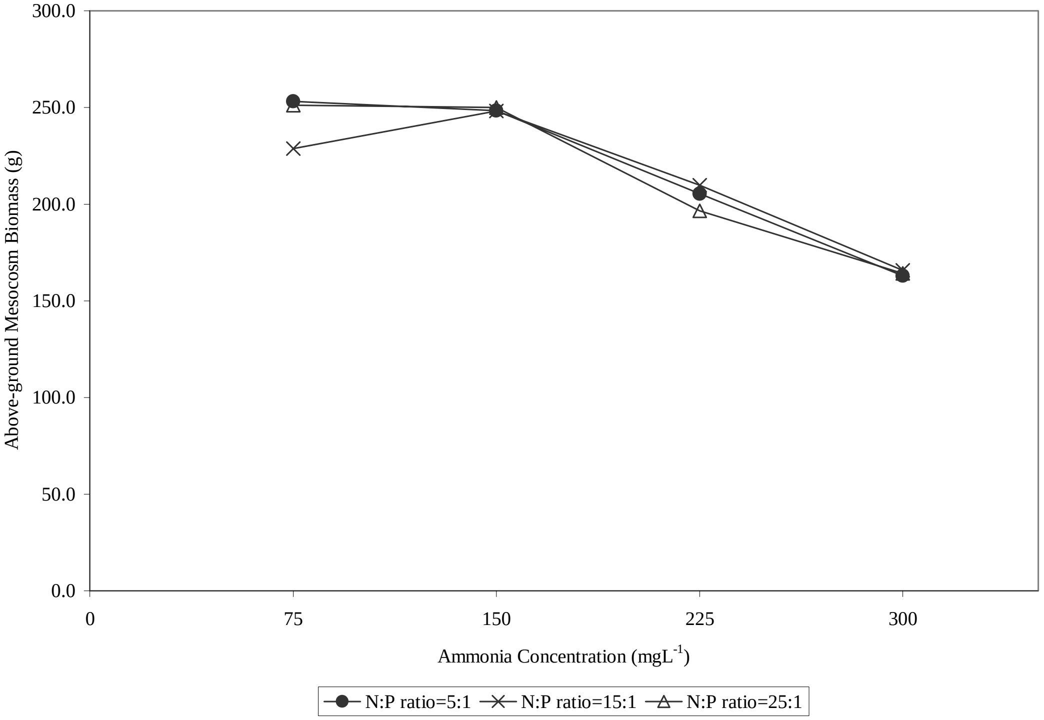

Figure 3.3. Total above-ground mesocosm biomass production along a gradient of ammonia concentration treatments and at three N:P ratios in 2003. Standard errors are not presented due to graphic difficulty in their interpretation. Due to normality of data and homogeneity of residuals from the ANOVA, log transformation was not necessary (see appendix II for SAS code).

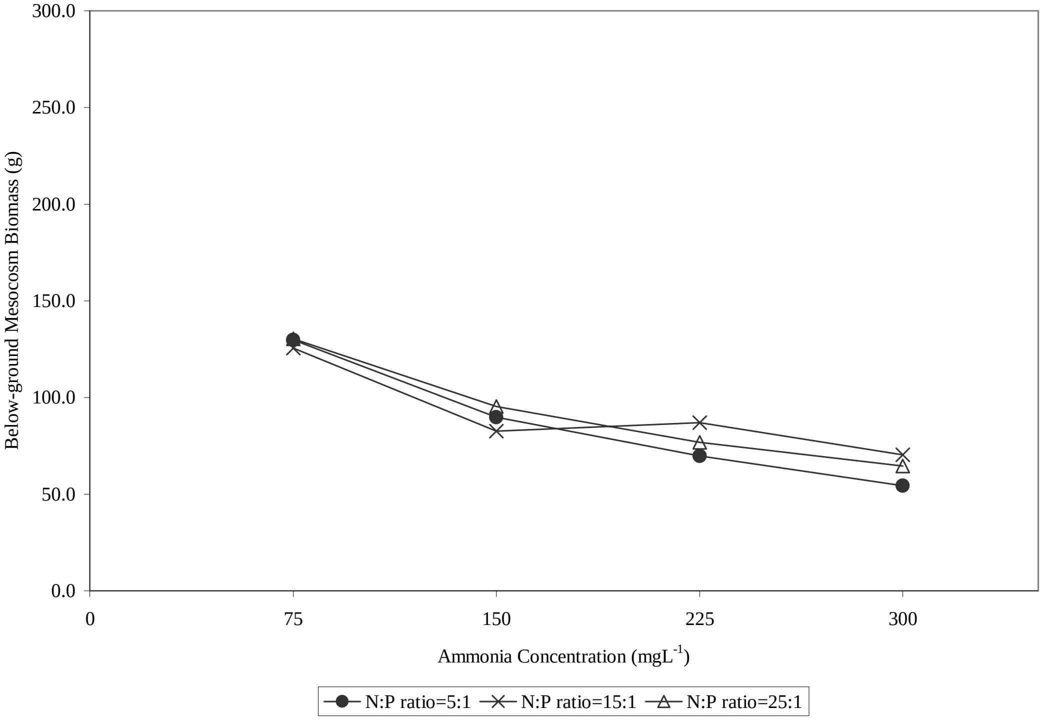

Figure 3.4. Total below-ground mesocosm biomass production along a gradient of ammonia concentration treatments and at three N:P ratios in 2003. Standard errors are not presented due to graphic difficulty in their interpretation. Due to normality of data and homogeneity of residuals from the ANOVA, log transformation was not necessary (see appendix II for SAS code).

significant source of variation, some interesting trends are evident. Trends in N:P ratios within ammonia treatments indicate highest biomass production at levels other than 15:1 at lower treatments, and highest biomass production at levels of 15:1 at higher, toxic treatments. Although variability induced by N:P ratio is not statistically significant, the indication of higher biomass production when nutrients are co-limited is in agreement with other authors (Tilman 1982, Mamolos *et al.* 1995), although it has never been tested in phyto-inhibitory ammonia concentration levels. This unclear result may be elucidated by further testing; the initial indication that N:P ratio may be involved with reduction of ammonia toxicity is encouraging, although acceptance of non-significant data is problematic.

Total *Typha latifolia* biomass production was significantly affected by ammonia concentration treatment alone (Table 3.3). Differences in biomass between ammonia treatments is likewise consistent with previous findings, and illustrate a clear toxic effect of ammonia concentrations higher than 75 mgL^{-1} (Figure 3.5); although 150 mgL^{-1} ammonia demonstrated a fertilization effect in the 2002 data. It is possible that year-to-year differences in such variables as temperature or photon flux density could affect nitrogen utilization in *Typha*. The N:P ratio of 5:1 produced observably higher biomass than the 15:1 or 25:1 N:P ratio, with an overall average of 96.2±11.4 g versus 76.2±14.1 g and 77.0±7.9 g, respectively. This may indicate that at N limitation, higher biomass is produced at all ammonia treatment levels, on average. Observably, the N:P ratio of 15:1 appears have less negative slope than the other lines at higher levels of ammonia. This could indicate that at even higher levels of ammonia, an N:P level of 15:1 may result in lower toxicity and more biomass production (Figure 3.5). Coupled with the statistical

Table 3.3. Tests of fixed effects on total, above-ground, and below-ground biomass of mesocosms of *Typha latifolia* in 2003. Please refer to appendix II for SAS code.

Measured Biomass	**Effect**	**d.f.**	**F Value**	**P Level**
Total *Typha latifolia*	[Ammonia]	3	3.24	0.0452
Total *Typha latifolia*	N:P Ratio	2	0.11	0.8961
Total *Typha latifolia*	N:P Ratio x [Ammonia]	6	0.71	0.6430
Above-ground *Typha latifolia*	[Ammonia]	3	5.4	0.0074
Above-ground *Typha latifolia*	N:P Ratio	2	0.34	0.7191
Above-ground *Typha latifolia*	N:P Ratio x [Ammonia]	6	0.75	0.6190
Below-ground *Typha latifolia*	[Ammonia]	3	3.47	0.0378
Below-ground *Typha latifolia*	N:P Ratio	2	0.28	0.7604
Below-ground *Typha latifolia*	N:P Ratio x [Ammonia]	6	0.46	0.8263

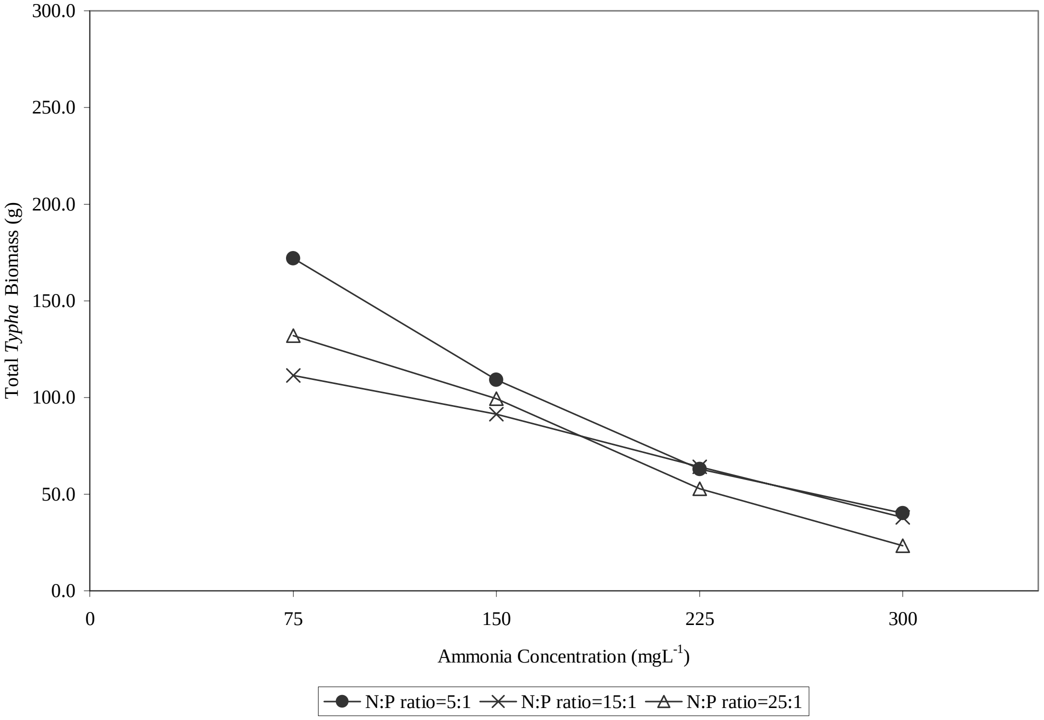

Figure 3.5. Total *Typha latifolia* biomass production along a gradient of ammonia concentration treatments and at three N:P ratios in 2003. Standard errors are not presented due to graphic difficulty in their interpretation. Due to normality of data and homogeneity of residuals from the ANOVA, log transformation was not necessary (see appendix II for SAS code).

non-significance of differences in biomass production at N:P ratios of 15:1 and 25:1, this finding may indicate that at N:P levels of 15:1, there is less effect of ammonia toxicity.

Above-ground *Typha* biomass production is significantly affected by ammonia concentration treatment (Table 3.3). Difference in biomass production between ammonia treatments is consistent with the overall effect in *Typha* in 2003 and 2002 and with the above-ground effect in 2002 as well, although the 2002 data indicate toxic effects begin at more than 150 mgL^{-1}, while the 2003 data indicate toxicity at a lower ammonia concentration (Figure 3.6). The N:P ratio of 5:1 produced observably higher above-ground biomass, similar to the total *Typha* biomass results as well. The slope of the line of the 15:1 N:P ratio appears to be less negative at higher ammonia concentrations, although the interactive effect of N:P and ammonia concentration is not significant.

Below-ground *Typha* biomass is significantly affected by ammonia treatment alone (Table 3.3), with the overall trend in biomass production observably similar to the total and above-ground *Typha* biomass curves (Figure 3.7). Highest production occurs at the 75 mgL^{-1} ammonia concentration, with toxic effects apparent thereafter. Although the N:P ratio is not significant in determining biomass production in *Typha*, the observable trend of the 15:1 ratio having the lowest negative slope is again potentially meaningful, especially at higher ammonia concentrations.

Findings for *Typha* biomass production concur with the results of other researchers, who indicate a fertilization effect up to around 75 mgL^{-1} ammonia concentration (Hill *et al.* 1997). The effects of the N:P ratio in *Typha* are not particularly statistically apparent; higher biomass production occurs overall at an N:P ratio of 5:1, but the interactive effects of ammonia and the N:P ratio are unclear. The observable trend in

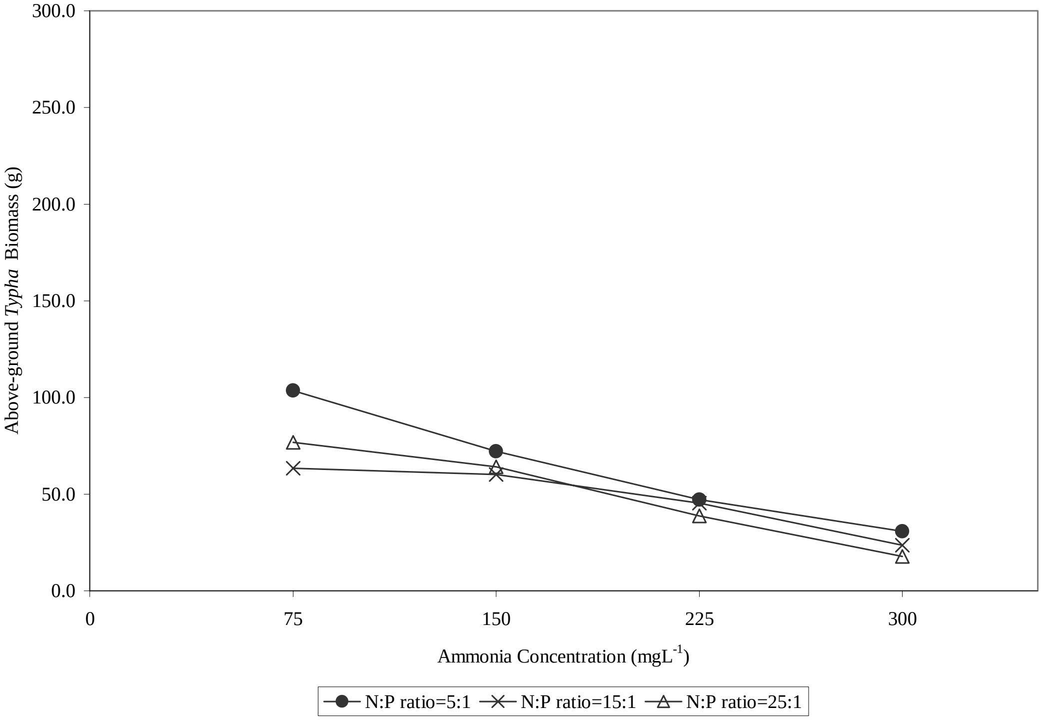

Figure 3.6. Above-ground *Typha latifolia* biomass production along a gradient of ammonia concentration treatments and at three N:P ratios in 2003. Standard errors are not presented due to graphic difficulty in their interpretation. Due to normality of data and homogeneity of residuals from the ANOVA, log transformation was not necessary (see appendix II for SAS code).

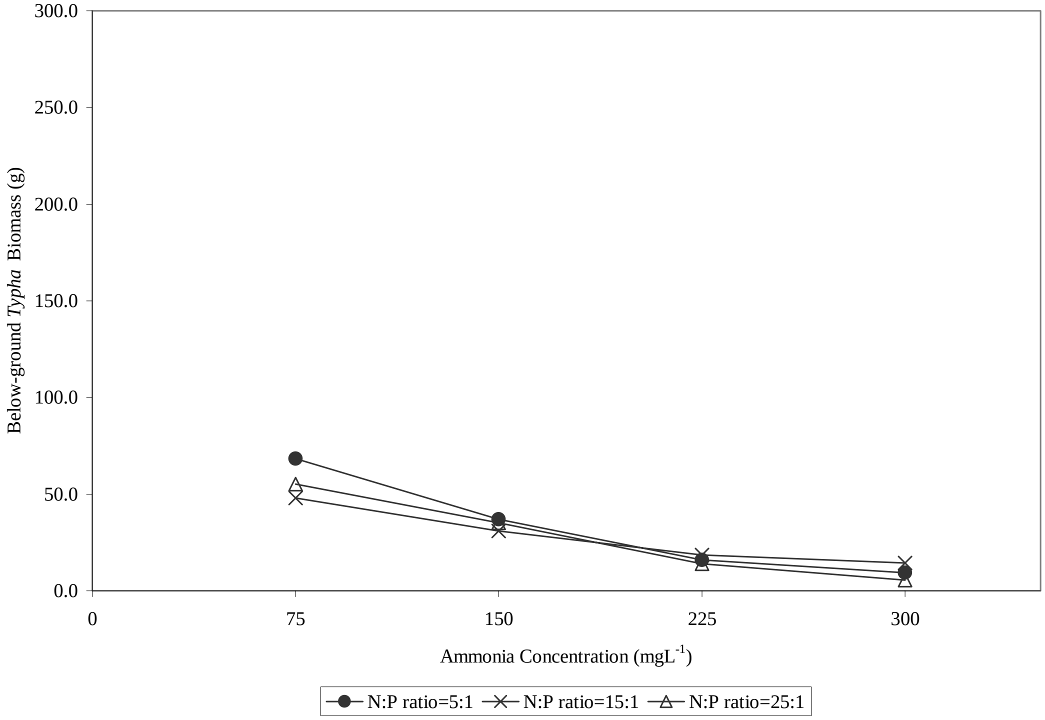

Figure 3.7. Below-ground *Typha latifolia* biomass production along a gradient of ammonia concentration treatments and at three N:P ratios in 2003. Standard errors are not presented due to graphic difficulty in their interpretation. Due to normality of data and homogeneity of residuals from the ANOVA, log transformation was not necessary (see appendix II for SAS code).

the effect of the N:P ratio is similar to that of the entire mesocosm, with higher biomass production at co-limited N:P ratios at high ammonia concentrations (Tilman 1982, Mamolos *et al.* 1995). Again, the lack of statistical significance of the N:P ratio is problematic in making determinations such as these.

Total *Juncus effusus* biomass was significantly affected by ammonia concentration treatments (Table 3.4). Differences in biomass based on ammonia treatments are likewise consistent with results from other portions of this study, and illustrate toxicity in *Juncus* at approximately the 150 mgL^{-1} ammonia concentration. The peak biomass production of 238.2±8.9 g occurs at 150 mgL^{-1} ammonia treatment level, and is followed by a decline to a low of 193.5±9.9 g at 300 mgL^{-1} ammonia (Figure 3.8). Similar to the 2002 data, little statistically significant differences exist at higher ammonia treatment levels. The N:P ratio of 25:1 produced 230.3±10.0 g biomass, which is higher (although not statistically) than that produced by the 15:1 N:P ratio treatment level, 228.4±19.0 g, and significantly higher than that produced at an N:P ratio of 5:1, 207.3±9.9 g. Observably, the N:P ratio of 15:1 appears once again to have the least negative slope of the three, which could potentially indicate that at higher levels of ammonia, a co-limited N:P ratio would produce higher biomass, which is consistent with findings from *Typha* and for the mesocosms on the whole.

Above-ground *Juncus* biomass production is significantly affected by ammonia concentration treatment alone, although N:P ratio treatment almost produces an observable effect as well (Table 3.4). Differences in biomass production across ammonia concentrations is consistent with the overall effect in *Juncus* in 2002 and 2003, although peak biomass production in 2002 occurs at the 75 mgL^{-1} ammonia concentration and

Table 3.4. Tests of fixed effects on total, above-ground, and below-ground biomass of mesocosms of *Juncus effusus* in 2003. Please refer to appendix II for SAS code.

Measured Biomass	**Effect**	**d.f.**	**F Value**	**P Level**
Total *Juncus effusus*	[Ammonia]	3	9.46	0.0005
Total *Juncus effusus*	N:P Ratio	2	2.03	0.1595
Total *Juncus effusus*	N:P Ratio x [Ammonia]	6	1.11	0.3949
Above-ground *Juncus effusus*	[Ammonia]	3	9.46	0.0005
Above-ground *Juncus effusus*	N:P Ratio	2	2.03	0.1595
Above-ground *Juncus effusus*	N:P Ratio x [Ammonia]	6	1.11	0.3949
Below-ground *Juncus effusus*	[Ammonia]	3	10.35	0.0003
Below-ground *Juncus effusus*	N:P Ratio	2	2.44	0.1143
Below-ground *Juncus effusus*	N:P Ratio x [Ammonia]	6	1.01	0.4483

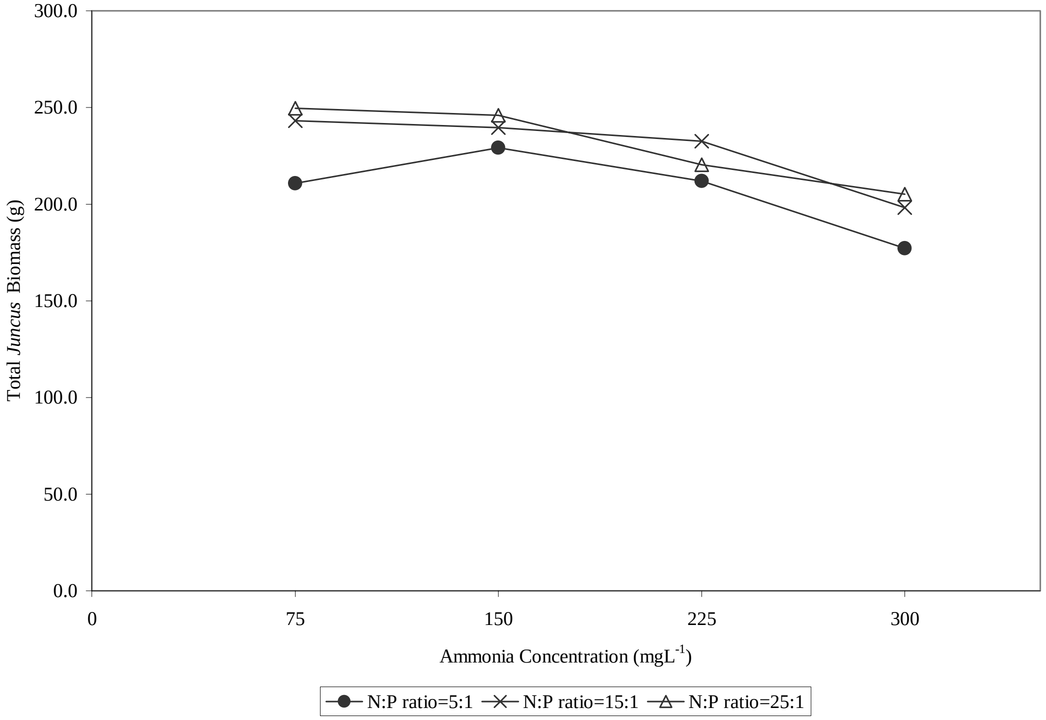

Figure 3.8. Total *Juncus effusus* biomass production along a gradient of ammonia concentration treatments and at three N:P ratios in 2003. Standard errors are not presented due to graphic difficulty in their interpretation. Due to normality of data and homogeneity of residuals from the ANOVA, log transformation was not necessary (see appendix II for SAS code).

occurs in 2003 at the 150 mgL^{-1} ammonia concentration (Figure 3.9). Although the N:P ratio is not a statistically significant effect in determining biomass production, there is an observable trend of higher biomass production at a level of 15:1 as predicted by Tilman (1982), Mamolos *et al.* (1995) and by the observable trends in this study.

Below-ground *Juncus* biomass production is significantly affected by the ammonia treatment alone (Table 3.4). The highest production occurs at the 75 mgL^{-1} ammonia concentration, with lowest production at 300 mgL^{-1} although observable toxic effects do not seem overly evident (Figure 3.10). An observable trend of high biomass production along the 15:1 N:P ratio may be found in Figure 3.10, although N:P ratio is not a statistically significant source of variation.

Findings for *Juncus* biomass production do concur with the results of Clarke (1999), but yield overall higher tolerances for ammonia than some (Hill *et al.* 1997); results from the 2003 study corroborate well with the 2002 study in indicating relatively high ammonia tolerance in *Juncus*. Overall, *Juncus* again produced more biomass than *Typha* at all ammonia treatment concentrations, and appeared to be less affected by increased ammonia concentrations, based on observable patterns in the slopes of the lines in Figures 3.8 to 3.10. The trend in the effect of the N:P ratio is much more difficult to observe in *Juncus*, but appears to agree with that of the entire mesocosm and with that of *Typha* biomass production, with more biomass produced at high ammonia concentrations if the experimental N:P ratio is co-limited.

If the application of phosphorus in varying N:P ratios could alleviate potentially toxic ammonia concentrations, interactions between the N:P ratio treatments and the ammonia concentration treatments should be statistically significant sources of variation.

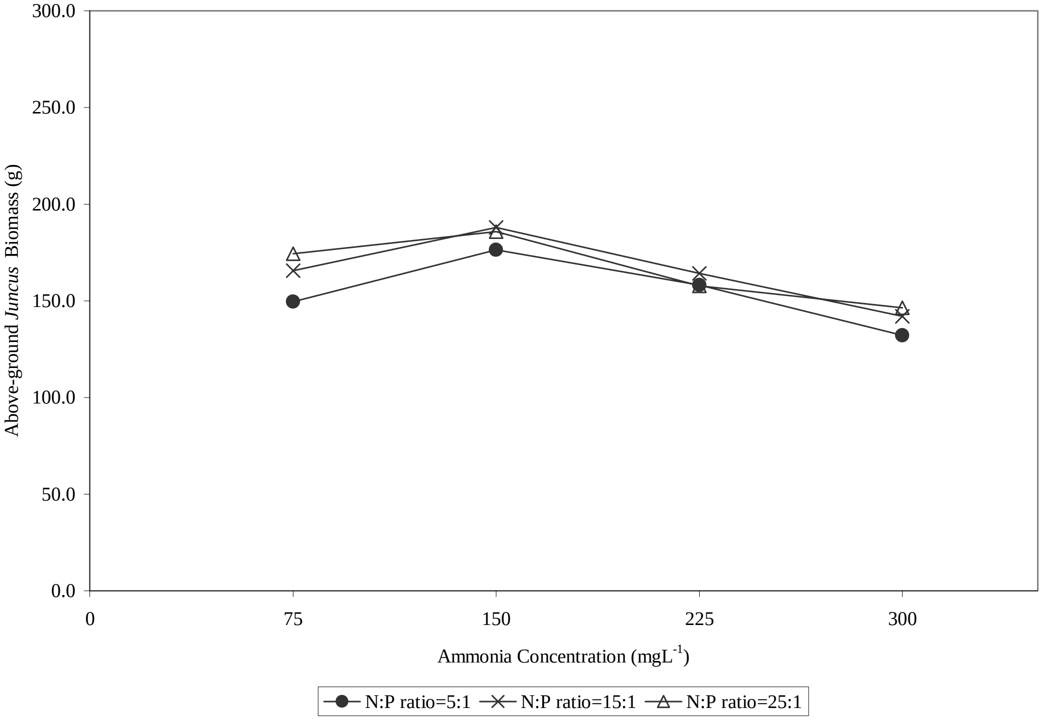

Figure 3.9. Above-ground *Juncus effusus* biomass production along a gradient of ammonia concentration treatments and at three N:P ratios in 2003. Standard errors are not presented due to graphic difficulty in their interpretation. Due to normality of data and homogeneity of residuals from the ANOVA, log transformation was not necessary (see appendix II for SAS code).

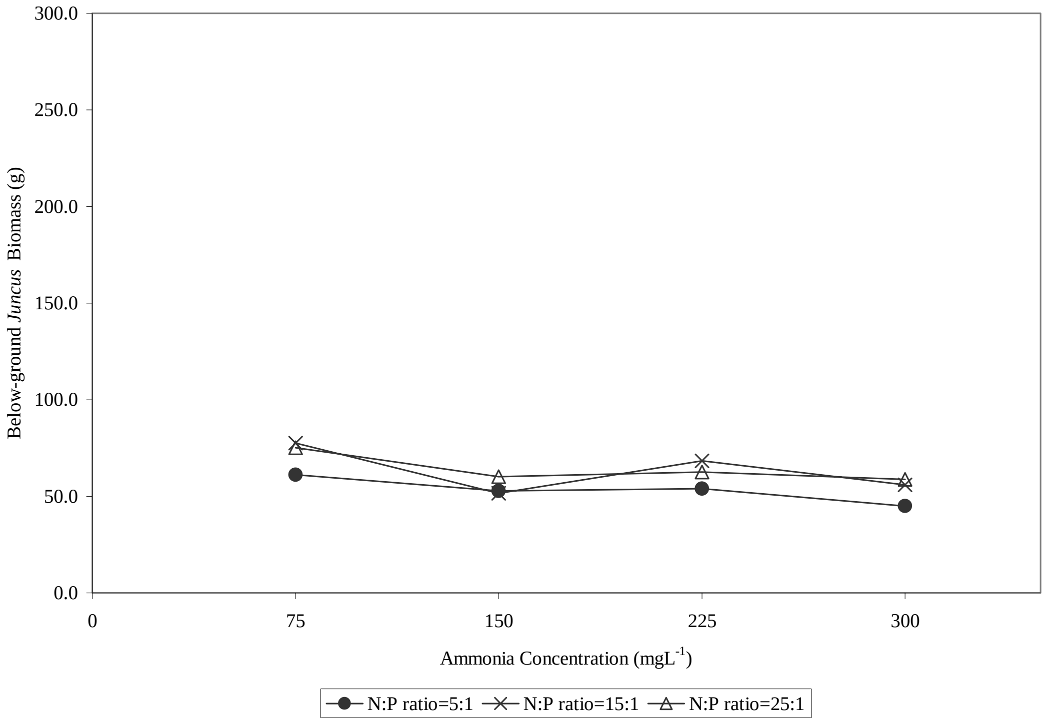

Figure 3.10. Below-ground *Juncus effusus* biomass production along a gradient of ammonia concentration treatments and at three N:P ratios in 2003. Standard errors are not presented due to graphic difficulty in their interpretation. Due to normality of data and homogeneity of residuals from the ANOVA, log transformation was not necessary (see appendix II for SAS code).

This is not the case, but some interesting trends are evident that could be further investigated. It appears that for *Juncus*, as well as for *Typha*, that under low ammonia concentrations, best growth is achieved when the N:P ratio treatment is 5:1 or 25:1. Under high ammonia concentrations, best growth is achieved when N and P are co-limiting. A potential competitive effect could account for this trend in biomass production; Tilman *et al.* (1999) found in field plot studies (using significantly lower concentrations of nutrients than used in this study) that the competitor with the best mechanism for acquiring a limiting nutrient will be favored in the absence of that nutrient. It is possible (although Tilman 1982 states otherwise, again in field plot studies) that *Typha* is a more efficient competitor for both N and P at lower nutrient concentrations, and is therefore inhibited in growth when N and P are co-limited. On the other hand, *Juncus* produces biomass relatively well at high ammonia concentrations regardless of P fertilization, which may indicate that *Juncus* is the better competitor for phosphorus in P-limiting situations.

Mesocosm Root-to-Shoot Ratios

Mesocosm root-to-shoot ratios were significantly affected by ammonia concentration treatment, N:P ratio treatment, species tested, and the interaction of species and ammonia concentration treatment (Table 3.5). Decreases were evident in the R:S ratio in the mesocosm from 0.53±0.1 at the 75 mgL^{-1} ammonia treatment to 0.4±0.0 at the 300 mgL^{-1} ammonia treatment; R:S ratio was maximized at 0.4±0.0 at the N:P ratio treatment of 15:1 (Figure 3.11). *Typha* exhibited a higher mean R:S ratio at 0.5±0.1 than *Juncus*, at 0.4±0.0. Decreases following the same trend as for the mesocosm are evident

Table 3.5. Test of fixed effects on the root-to-shoot ratio in mesocosms and in *Typha latifolia* and *Juncus effusus* in 2003. Please refer to appendix II for SAS code.

Measured Root-to-Shoot Ratio	Effect	d.f.	F Value	P Level
Total Mesocosm	Species	1	26.31	<0.0001
Total Mesocosm	[Ammonia]	3	13.1	<0.0001
Total Mesocosm	Species x [Ammonia]	3	7.16	0.0005
Total Mesocosm	N:P Ratio	2	5.62	0.0064
Total Mesocosm	N:P Ratio x Species	2	0.95	0.3946
Total Mesocosm	N:P Ratio x [Ammonia]	6	0.9	0.5030
Total Mesocosm	Species x N:P Ratio x [Ammonia]	6	1.82	0.1157
Total *Typha latifolia*	[Ammonia]	3	10.07	0.0002
Total *Typha latifolia*	N:P Ratio	2	2.26	0.1258
Total *Typha latifolia*	N:P Ratio x [Ammonia]	6	1.13	0.3749
Total *Juncus effusus*	[Ammonia]	3	10.5	0.0001
Total *Juncus effusus*	N:P Ratio	2	9.91	0.0007
Total *Juncus effusus*	N:P Ratio x [Ammonia]	6	2.83	0.0314

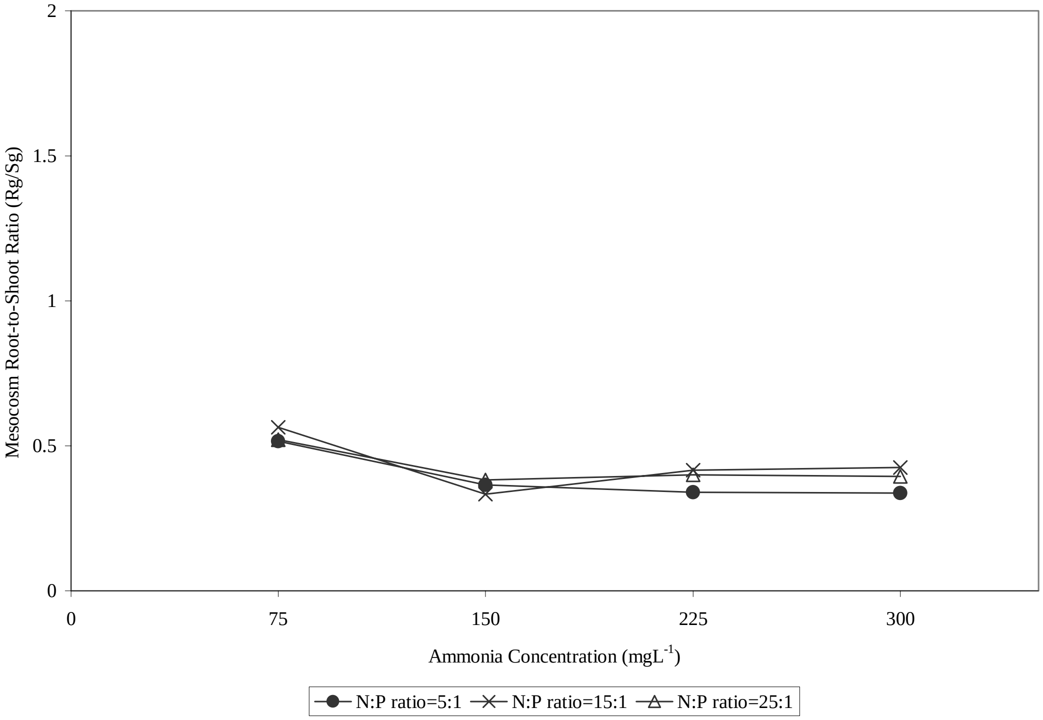

Figure 3.11. Root-to-shoot ratios for mesocosms along a gradient of ammonia concentration treatments and at three N:P ratios in 2003. Standard errors are not presented due to graphic difficulty in their interpretation. Due to normality of data and homogeneity of residuals from the ANOVA, log transformation was not necessary (see appendix II for SAS code).

for *Typha* (Figure 3.12) and *Juncus* (Figure 3.13), although N:P ratio is not significant in determining the R:S ratio in *Typha* (Table 3.5). These data concur with the 2002 data and indicate suppression of root formation, increased shoot production, or a combination of the two with increasing ammonia concentration. The fact that the interaction term of species and ammonia concentration is present indicates differences in the level of stress tolerance between the species. Overall decreases in R:S ratio with increasing ammonia concentration concur with similar responses in other species (Walch-Liu *et al.* 2000, Vojtiskova *et al.* 2004). It is not to be expected that biomass allocations to roots and shoots will be the same, based on a number of factors which include fertilization (Vojtiskova *et al.* 2004, Tilman 1997). The fact that the 15:1 N:P ratio alleviated some of the reduction in cell division and leaf elongation typical of phytotoxic concentrations of ammonia is consistent with the observable trend in increased overall biomass production at the 15:1 N:P level in mesocosms, *Typha*, and *Juncus*.

Mesocosm Tissue Nitrogen Concentration

Total mesocosm nitrogen tissue concentration was significantly affected by species tested, ammonia concentration, N:P ratio treatment, and the interaction of species and ammonia concentration. (Table 3.6). Total tissue nitrogen concentration increased from an average of 19.3±2.1 mg g^{-1} at the 75 mgL^{-1} ammonia treatment level to an average of 26.7±0.5 mg g^{-1} at the 300 mgL^{-1} ammonia treatment level (Figure 3.14). This trend is to be expected and has been referred to as “luxury consumption”; as the available nitrogen increases, nitrogen concentration in the biomass should increase as well (Tripler *et al.* 2002). This finding is one of the main reasons why plants are used in treatment

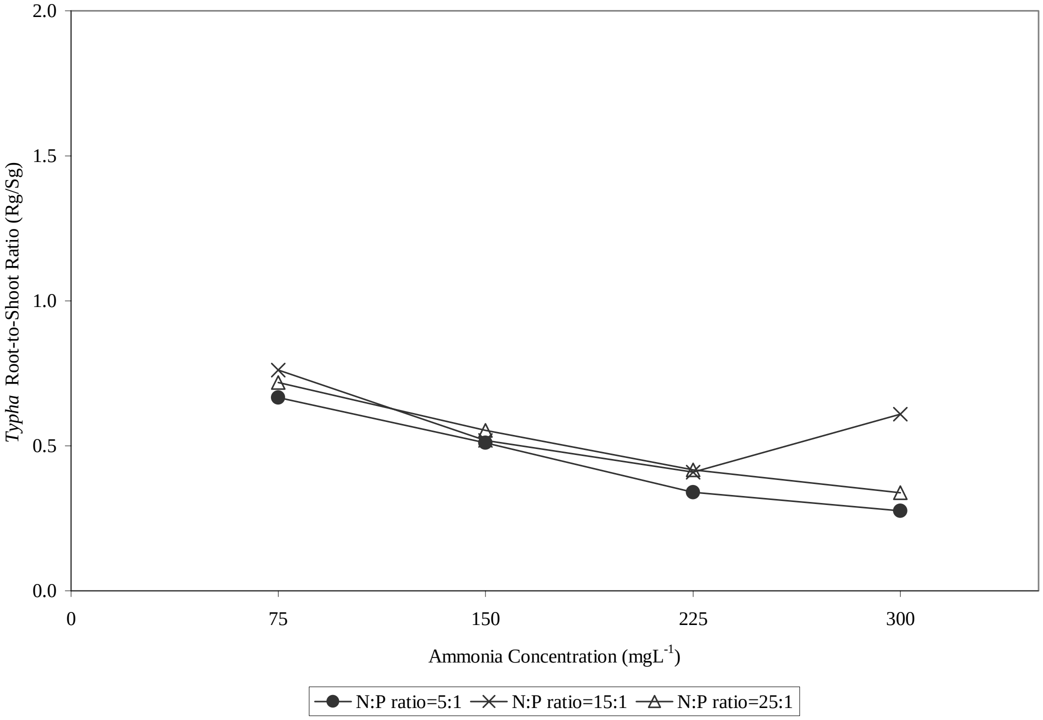

Figure 3.12. Root-to-shoot ratios for *Typha latifolia* along a gradient of ammonia concentration treatments and at three N:P ratios in 2003. Standard errors are not presented due to graphic difficulty in their interpretation. Due to normality of data and homogeneity of residuals from the ANOVA, log transformation was not necessary (see appendix II for SAS code).

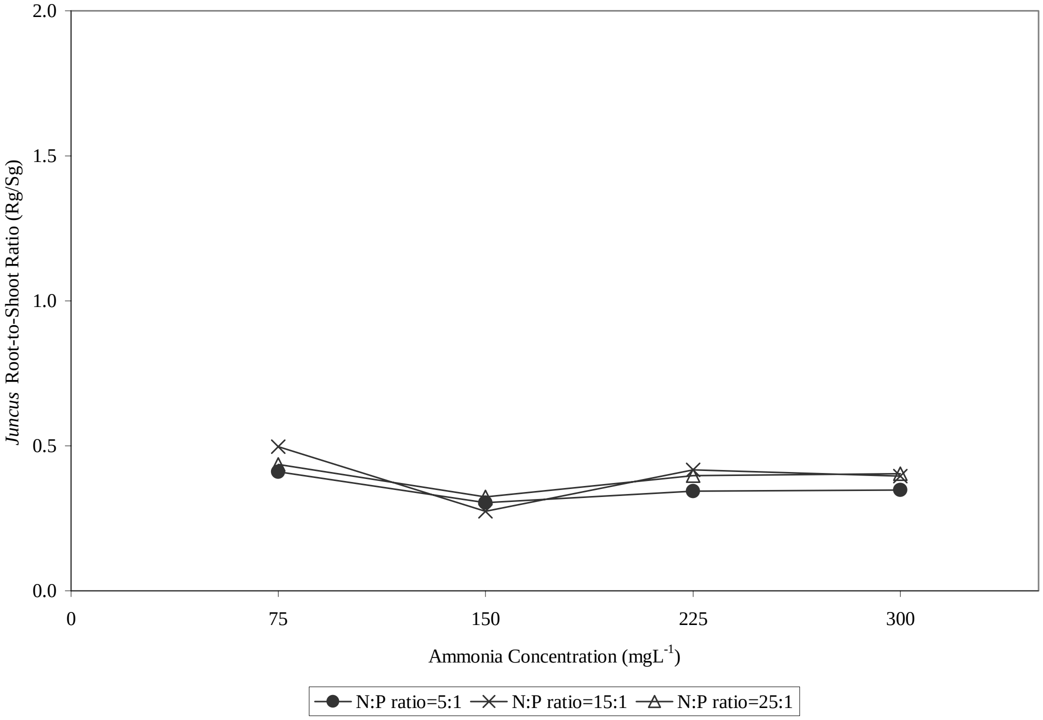

Figure 3.13. Root-to-shoot ratios for *Juncus effusus* along a gradient of ammonia concentration treatments and at three N:P ratios in 2003. Standard errors are not presented due to graphic difficulty in their interpretation. Due to normality of data and homogeneity of residuals from the ANOVA, log transformation was not necessary (see appendix II for SAS code).

Table 3.6. Tests of fixed effects on total, above-ground, and below-ground tissue nitrogen concentration of mesocosms of *Juncus effusus* and *Typha latifolia* in 2003. Please refer to appendix II for SAS code.

Measured Biomass	Effect	d.f.	F Value	P Level
Total Mesocosm	Species	1	7.9	0.0078
Total Mesocosm	[Ammonia]	3	10.32	<0.0001
Total Mesocosm	Species x [Ammonia]	3	3.69	0.02
Total Mesocosm	N:P Ratio	2	11.37	0.0001
Total Mesocosm	N:P Ratio x Species	2	1.89	0.1644
Total Mesocosm	N:P Ratio x [Ammonia]	6	1.39	0.2426
Total Mesocosm	Species x N:P Ratio x [Ammonia]	6	0.71	0.6426
Above-ground Mesocosm	Species	1	10.42	0.0026
Above-ground Mesocosm	[Ammonia]	3	12.7	<0.0001
Above-ground Mesocosm	Species x [Ammonia]	3	4.79	0.0063
Above-ground Mesocosm	N:P Ratio	2	12.94	<0.0001
Above-ground Mesocosm	N:P Ratio x Species	2	2.09	0.1374
Above-ground Mesocosm	N:P Ratio x [Ammonia]	6	1.51	0.2
Above-ground Mesocosm	Species x N:P Ratio x [Ammonia]	6	0.6	0.7309
Below-ground Mesocosm	Species	1	16.42	0.0003
Below-ground Mesocosm	[Ammonia]	3	17.2	<0.0001
Below-ground Mesocosm	Species x [Ammonia]	3	7.38	0.0005
Below-ground Mesocosm	N:P Ratio	2	15.35	<0.0001
Below-ground Mesocosm	N:P Ratio x Species	2	2.71	0.0794
Below-ground Mesocosm	N:P Ratio x [Ammonia]	6	1.99	0.0916
Below-ground Mesocosm	Species x N:P Ratio x [Ammonia]	6	0.53	0.7811

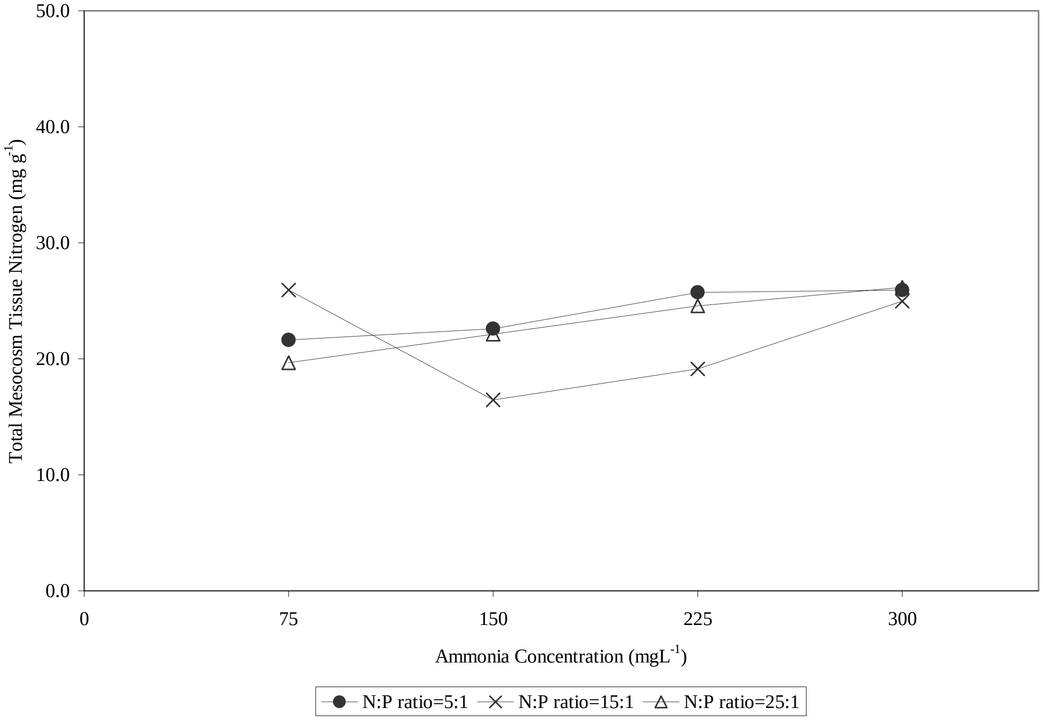

Figure 3.14. Total mesocosm tissue nitrogen concentration along a gradient of ammonia concentration treatments and at three N:P ratios in 2003. Standard errors are not presented due to graphic difficulty in their interpretation. Due to normality of data and homogeneity of residuals from the ANOVA, log transformation was not necessary (see appendix II for SAS code).

wetlands: their ability to remove designated elements from wastewater (e.g. Kadlec and Knight 1996). *Typha* averaged significantly higher tissue nitrogen content, 24.3±3.6 mg g^{-1}, than *Juncus*, which averaged 21.7±1.2 mg g^{-1} tissue nitrogen. Average tissue nitrogen content was highest at the 5:1 N:P ratio. The significant interactive effect of species and ammonia treatment reflects significant differences between tissue nitrogen concentrations in each species across ammonia concentrations. From this study, the hope was that a significant N:P ratio effect would develop; this would imply that as N:P ratios changed, more or less nitrogen could be sequestered in plant tissues. The line following the 15:1 N:P ratio in Figure 3.14 shows a trend towards less tissue nitrogen at low ammonia concentrations. This could be due to an inability of the mesocosms to uptake nitrogen, even under "ideal" N:P ratios, at low ammonia concentrations. Other authors (de Groot *et al.* 2003) found nitrogen uptake to be limited by phosphorus availability; at high N:P ratios where phosphorus is the limiting factor, N uptake was inhibited.

Total above-ground mesocosm tissue nitrogen concentration was significantly affected by the N:P ratio treatment, species, and ammonia concentration treatment level (Table 3.6). A similar trend as for overall mesocosm tissue nitrogen concentration is evident, with increasing tissue nitrogen with increasing ammonia treatment concentration (Figure 3.15); likewise, average *Typha* above-ground tissue nitrogen concentration, 24.5±2.9 mg g^{-1}, is higher than that for *Juncus*, 21.7±1.2 mg g^{-1}. Again the 5:1 N:P ratio results in highest tissue nitrogen concentration. The line representing the 15:1 N:P ratio demonstrates the same interesting trend as that for overall tissue nitrogen, an apparent inability of the plant tissues to sequester nitrogen at low ammonia concentrations. In fact, at this 15:1 N:P ratio, a significantly lower nitrogen tissue concentration occurs over the

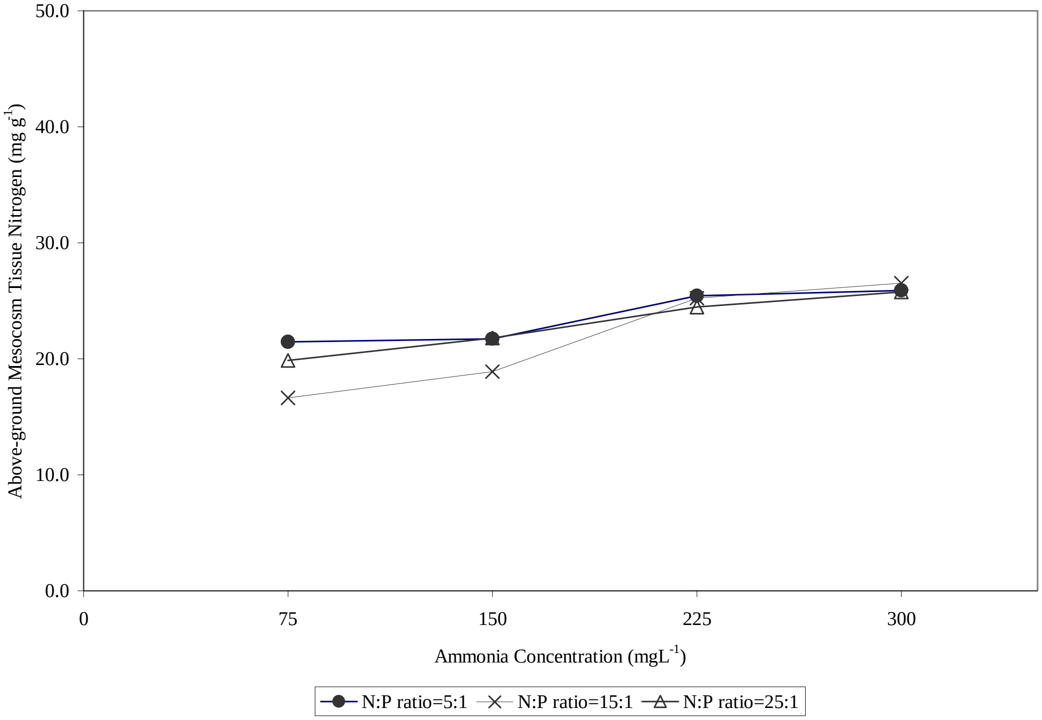

Figure 3.15. Total above-ground mesocosm tissue nitrogen concentration along a gradient of ammonia concentration treatments and at three N:P ratios in 2003. Standard errors are not presented due to graphic difficulty in their interpretation. Due to normality of data and homogeneity of residuals from the ANOVA, log transformation was not necessary (see appendix II for SAS code).

entire ammonia treatment regime,. At co-limited N:P levels, less tissue nitrogen uptake occurs.

Total below-ground tissue nitrogen concentration was significantly affected by N:P ratio treatment, species, and ammonia concentration treatment as well (Table 3.6). Like above-ground tissue nitrogen, a similar trend to that of overall mesocosm tissue nitrogen concentration is evident, with greater tissue nitrogen concentrations at higher ammonia concentrations and at the 5:1 N:P ratio (Figure 3.16), and a greater concentration of tissue nitrogen found in *Typha*. Of interest to note is the fact that average *Juncus* tissue nitrogen concentration is exactly the same in above- and below-ground tissues, 21.7±1.2 mg g^{-1}. This indicates that the plant sequesters nitrogen equally in above- and below-ground tissues, which may have implications as to how nitrogen is used in plant tissues and as to how ammonia toxicity occurs in wetland plants.

If the N:P ratio treatment was a useful means of alleviating ammonia toxicity, N:P ratio treatments should significantly affect the nitrogen stored and used in plant tissue. For ammonia toxicity to decrease, some of the nitrogen must be used by the biomass. In this experiment, N:P ratio did significantly affect tissue nitrogen concentration, with the N:P ratio of 5:1 producing significantly higher tissue N concentration. In fact, at the N:P ratio which is co-limited, 15:1, the highest stored tissue nitrogen was expected. At a co-limited N:P ratio, both nutrients should be used more quickly for the production of biomass (Tilman 1982), and, while the observable trend in biomass production did show this to an extent, the amount of nitrogen stored in the tissue is the lowest of all three N:P ratio treatments. At sub-optimal N:P ratios, plant tissues should not be able to use nutrients at an optimal rate. Perhaps the difference in this case is between “use” of

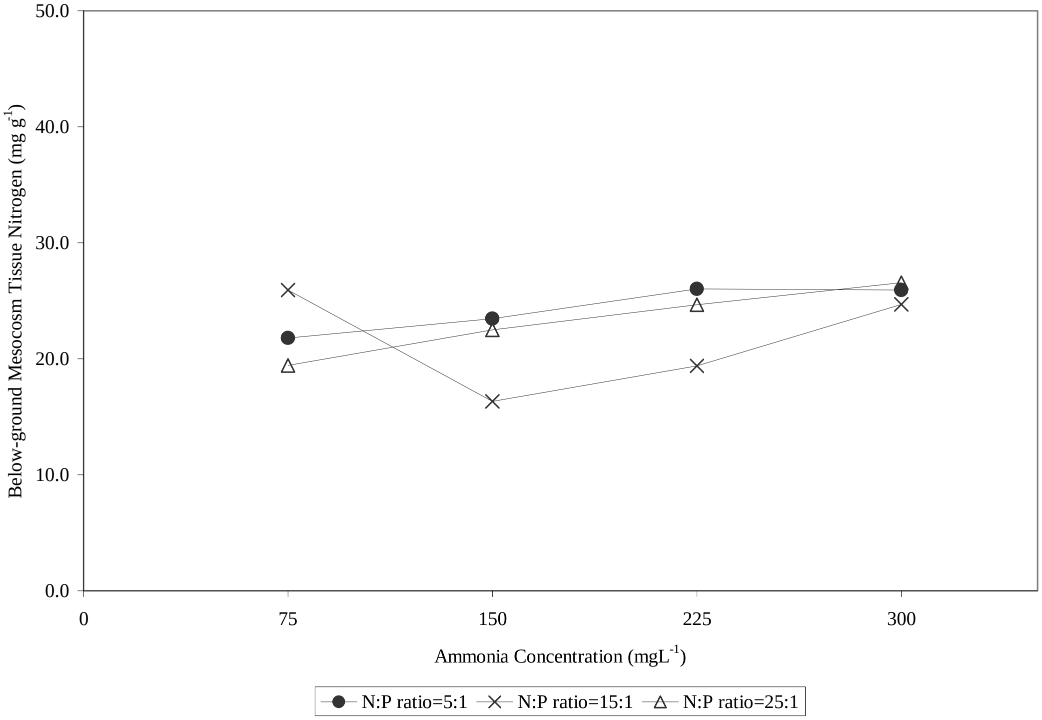

Figure 3.16. Total below-ground mesocosm tissue nitrogen concentration along a gradient of ammonia concentration treatments and at three N:P ratios in 2003. Standard errors are not presented due to graphic difficulty in their interpretation. Due to normality of data and homogeneity of residuals from the ANOVA, log transformation was not necessary (see appendix II for SAS code).

nutrients and “storage” of nutrients, which should be further explored. Aerts *et al.*(2001) indicate that N and P tissue concentration in *Sphagnum* is not controlled by the addition of nutrients to the site, although at significantly lower fertilization rates than in this study.

Total *Typha latifolia* tissue nitrogen concentration was significantly affected by ammonia concentration treatment and N:P ratio treatment (Table 3.7). Tissue nitrogen increased from an average of 19.3±3.6 mg g^{-1} at the 75 mgL^{-1} ammonia concentration treatment to 29.5±3.7 at the 225 mgL^{-1} ammonia concentration treatment, and then declined to the 300 mgL^{-1} ammonia concentration treatment (Figure 3.17). Tissue nitrogen was highest at the 15:1 N:P ratio. The fact that peak tissue nitrogen concentration appears at the 225 mgL^{-1} ammonia treatment could indicate the toxic effects of ammonia could potentially limit the overall uptake. Although 15:1 N:P ratio treatments were significant in determining high tissue nitrogen concentrations, this treatment again performed poorly at high ammonia concentrations. At the high phosphorus N:P ratio, the slope of the line indicates that tissue nitrogen concentration may continue to increase at even higher ammonia concentrations. The behavior of the above-ground *Typha* tissue nitrogen concentration (Figure 3.18) and below-ground tissue nitrogen concentration (Figure 3.19) demonstrate a similar trend; for each case, ammonia concentration and the N:P ratio account for differences in tissue nitrogen concentration. The overall N:P ratio trend is similar in each case as well, with the line representing the N:P ratio of 15:1 demonstrating a peak at 225 mgL^{-1} ammonia, and the 5:1 N:P ratio line increasing at 300 mgL^{-1} ammonia.

Total *Juncus effusus* tissue nitrogen concentration is affected by ammonia concentration treatment and the N:P ratio treatment (Table 3.8). Mean tissue nitrogen

Table 3.7. Tests of fixed effects on total, above-ground, and below-ground tissue nitrogen concentration of *Typha latifolia* in 2003. Please refer to appendix II for SAS code.

Measured Biomass	**Effect**	**d.f.**	**F Value**	**P Level**
Total *Typha latifolia*	[Ammonia]	3	7.74	0.0014
Total *Typha latifolia*	N:P Ratio	2	6.56	0.0068
Total *Typha latifolia*	N:P Ratio x [Ammonia]	6	0.86	0.5438
Above-ground *Typha latifolia*	[Ammonia]	3	9.92	0.0004
Above-ground *Typha latifolia*	N:P Ratio	2	7.59	0.0038
Above-ground *Typha latifolia*	N:P Ratio x [Ammonia]	6	0.84	0.5572
Below-ground *Typha latifolia*	[Ammonia]	3	13.52	<0.0001
Below-ground *Typha latifolia*	N:P Ratio	2	9.27	0.0017
Below-ground *Typha latifolia*	N:P Ratio x [Ammonia]	6	1.01	0.4517

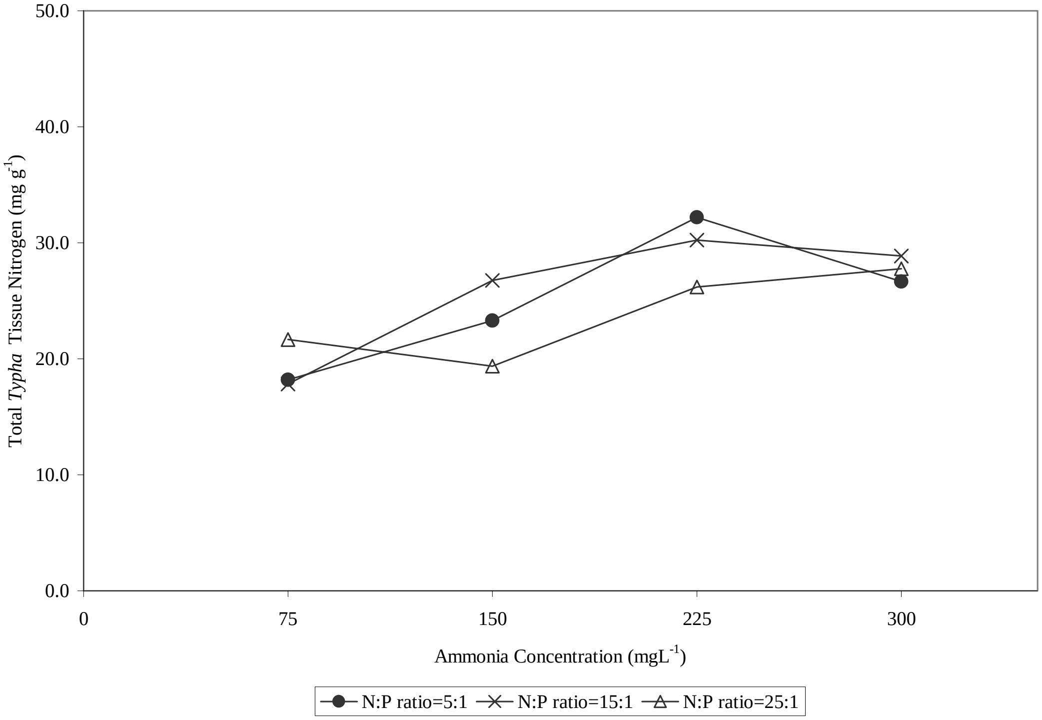

Figure 3.17. Total *Typha latifolia* tissue nitrogen concentration along a gradient of ammonia concentration treatments and at three N:P ratios in 2003. Standard errors are not presented due to graphic difficulty in their interpretation. Due to normality of data and homogeneity of residuals from the ANOVA, log transformation was not necessary (see appendix II for SAS code).

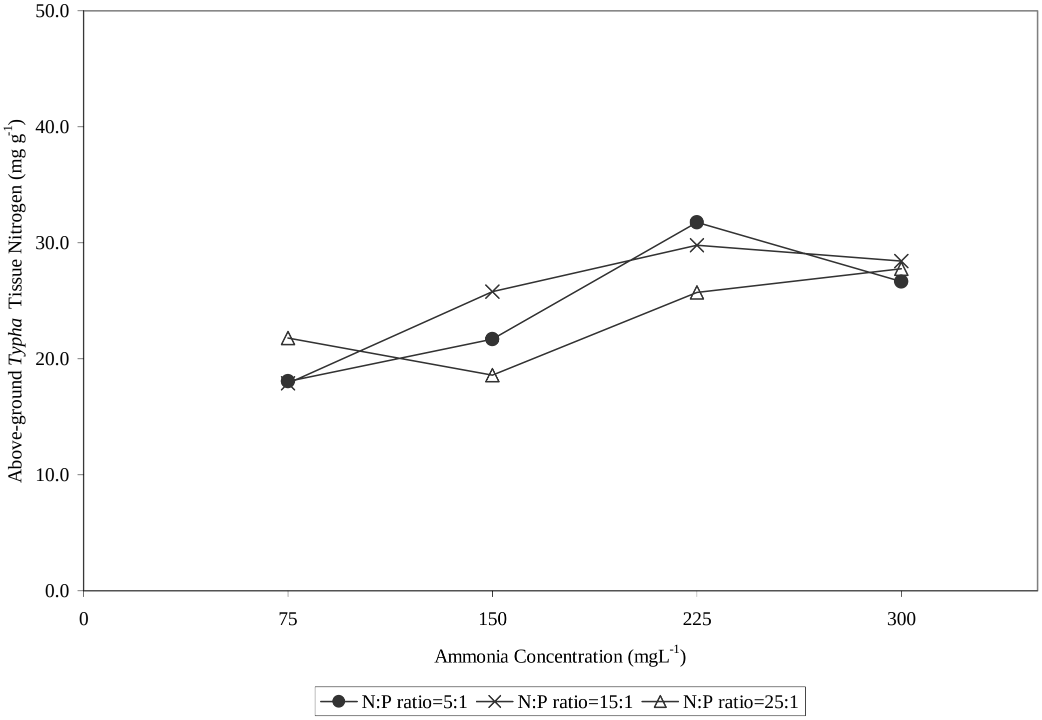

Figure 3.18. Above-ground *Typha latifolia* tissue nitrogen concentration along a gradient of ammonia concentration treatments and at three N:P ratios in 2003. Standard errors are not presented due to graphic difficulty in their interpretation. Due to normality of data and homogeneity of residuals from the ANOVA, log transformation was not necessary (see appendix II for SAS code).

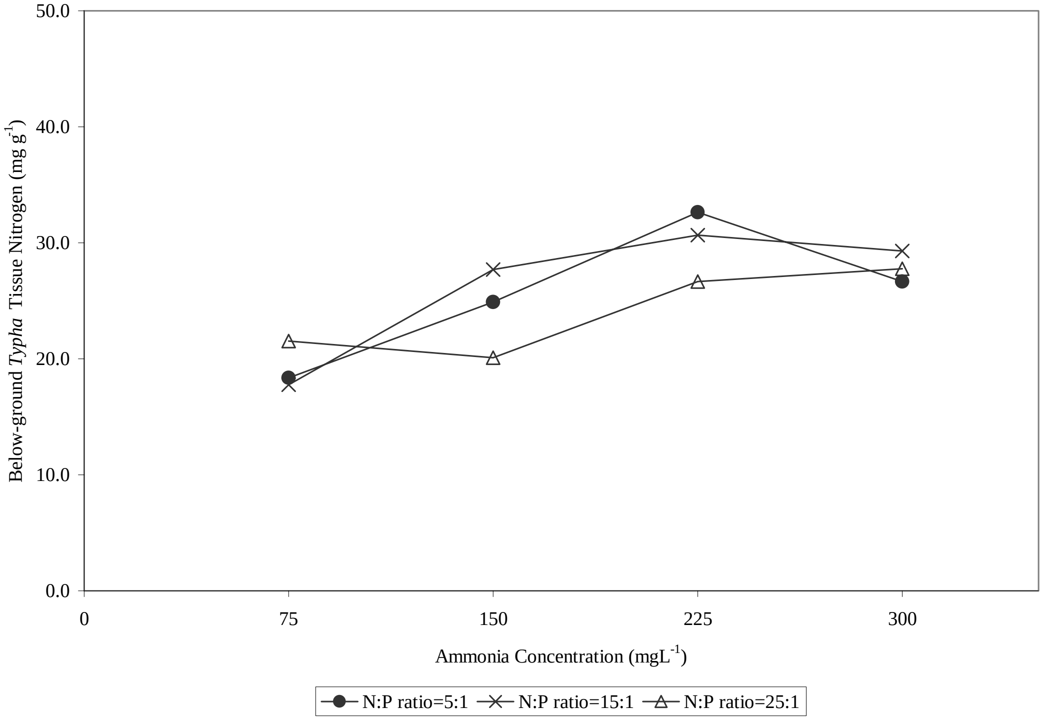

Figure 3.19. Below-ground *Typha latifolia* tissue nitrogen concentration along a gradient of ammonia concentration treatments and at three N:P ratios in 2003. Standard errors are not presented due to graphic difficulty in their interpretation. Due to normality of data and homogeneity of residuals from the ANOVA, log transformation was not necessary (see appendix II for SAS code).

Table 3.8. Tests of fixed effects on total, above-ground, and below-ground tissue nitrogen concentration of *Juncus effusus* in 2003. Please refer to appendix II for SAS code.

Measured Biomass	**Effect**	**d.f.**	**F Value**	**P Level**
Total *Juncus effusus*	[Ammonia]	3	3.03	0.0549
Total *Juncus effusus*	N:P Ratio	2	7.11	0.0049
Total *Juncus effusus*	N:P Ratio x [Ammonia]	6	2.17	0.0922
Above-ground *Juncus effusus*	[Ammonia]	3	3.03	0.0549
Above-ground *Juncus effusus*	N:P Ratio	2	7.11	0.0049
Above-ground *Juncus effusus*	N:P Ratio x [Ammonia]	6	2.17	0.0922
Below-ground *Juncus effusus*	[Ammonia]	3	3.03	0.0549
Below-ground *Juncus effusus*	N:P Ratio	2	7.11	0.0049
Below-ground *Juncus effusus*	N:P Ratio x [Ammonia]	6	2.17	0.0922

concentration increases from 17.5±1.0 mg g^{-1} at 75 mgL^{-1} ammonia to 24.5±0.6 mg g^{-1} at the 300 mgL^{-1} ammonia treatment. Mean tissue nitrogen concentration likewise increases from 20.2±1.7 mg g^{-1} at an N:P ratio of 15:1 to about 22 mg g^{-1} at N:P ratios of 5:1 and 25:1. Figure 3.20 demonstrates that, especially under low ammonia concentrations, tissue nitrogen is minimized by the 15:1 N:P ratio treatment. At N-limited and at P-limited N:P ratios, more nitrogen is stored in tissues in *Juncus*, especially in the lower ammonia concentrations, although this effect is significantly smaller than in *Typha*. In the related *Juncus roemerianus*, Brewer (2003) noted tissue nitrogen conservation and little increase in N uptake with fertilization. Emery *et al.* (2001) noted *Juncus effusus* to be a "stress tolerator" and could therefore potentially be dominant under unfavorable situations such as ammonia toxicity; they note that "stress tolerators" are not typically considered dominant in the presence of nutrients. This trend towards more consistent storage is not similar to the trend in *Typha*, where at higher ammonia concentrations, the N-limited and P-limited N:P ratios tend to cause less nitrogen storage in tissues. Curves are similar in above-ground *Juncus* tissue nitrogen concentration (Figure 3.21) and in below-ground nitrogen tissue concentration (Figure 3.22).

Mesocosm Total Tissue Mass Nitrogen

Total mesocosm tissue nitrogen uptake is affected by ammonia concentration, species, the N:P ratio treatment, and the interaction between species and ammonia concentration (Table 3.9). Total tissue nitrogen decreased with increasing ammonia concentration, from an average of 7301.2±834.3 mg at the 150 mgL^{-1} ammonia treatment level to 5981.1±854.3 mg at the 300 mgL^{-1} treatment (Figure 3.23). This indicates that

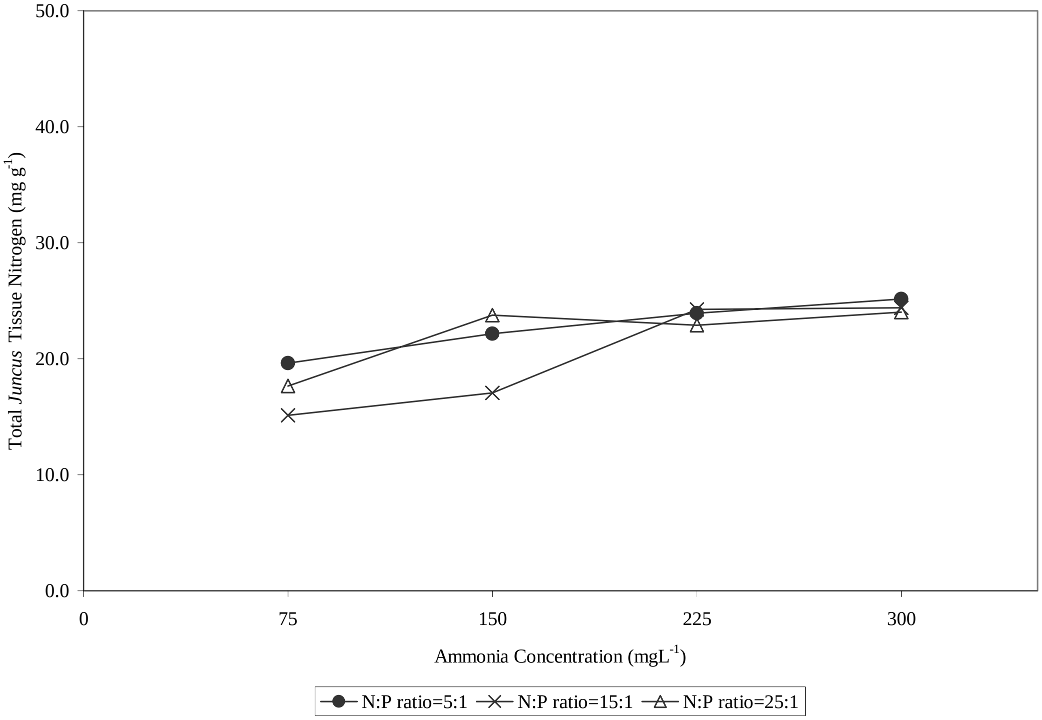

Figure 3.20. Total *Juncus effusus* tissue nitrogen concentration along a gradient of ammonia concentration treatments and at three N:P ratios in 2003. Standard errors are not presented due to graphic difficulty in their interpretation. Due to normality of data and homogeneity of residuals from the ANOVA, log transformation was not necessary (see appendix II for SAS code).

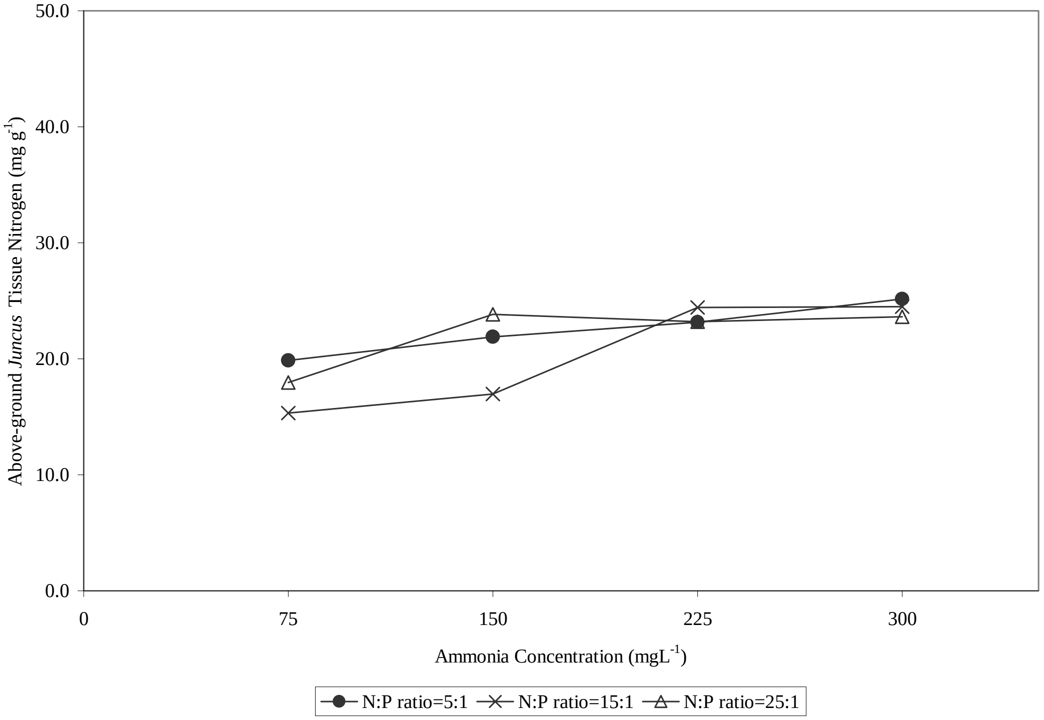

Figure 3.21. Above-ground *Juncus effusus* tissue nitrogen concentration along a gradient of ammonia concentration treatments and at three N:P ratios in 2003. Standard errors are not presented due to graphic difficulty in their interpretation. Due to normality of data and homogeneity of residuals from the ANOVA, log transformation was not necessary (see appendix II for SAS code).

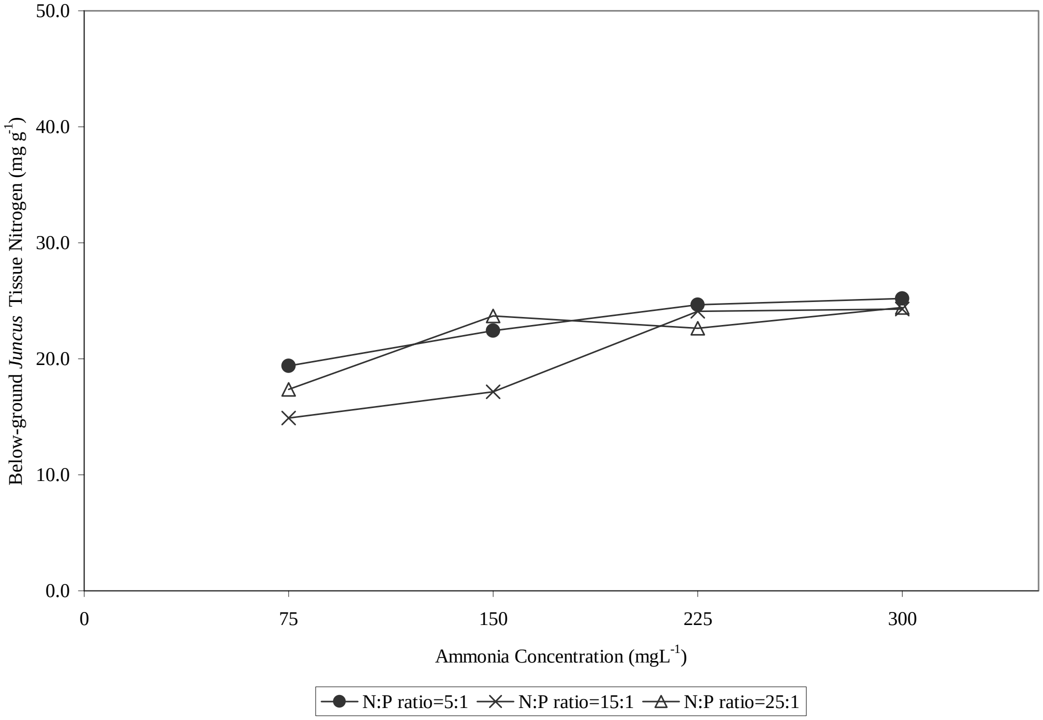

Figure 3.22. Below-ground *Juncus effusus* tissue nitrogen concentration along a gradient of ammonia concentration treatments and at three N:P ratios in 2003. Standard errors are not presented due to graphic difficulty in their interpretation. Due to normality of data and homogeneity of residuals from the ANOVA, log transformation was not necessary (see appendix II for SAS code).

Table 3.9. Tests of fixed effects on total, above-ground, and below-ground tissue total mass nitrogen in mesocosms of *Typha latifolia* and *Juncus effusus* in 2003. Please refer to appendix II for SAS code.

Measured Biomass	**Effect**	**d.f.**	**F Value**	**P Level**
Total Mesocosm	Species	1	10.09	0.003
Total Mesocosm	[Ammonia]	3	9.73	<0.0001
Total Mesocosm	Species x [Ammonia]	3	2.77	0.0548
Total Mesocosm	N:P Ratio	2	5.68	0.0069
Total Mesocosm	N:P Ratio x Species	2	1.3	0.2848
Total Mesocosm	N:P Ratio x [Ammonia]	6	1.17	0.3406
Total Mesocosm	Species x N:P Ratio x [Ammonia]	6	0.62	0.7147
Above-ground Mesocosm	Species	1	13.03	0.0009
Above-ground Mesocosm	[Ammonia]	3	12.62	<0.0001
Above-ground Mesocosm	Species x [Ammonia]	3	3.86	0.0167
Above-ground Mesocosm	N:P Ratio	2	6.4	0.004
Above-ground Mesocosm	N:P Ratio x Species	2	1.59	0.2179
Above-ground Mesocosm	N:P Ratio x [Ammonia]	6	1.31	0.2744
Above-ground Mesocosm	Species x N:P Ratio x [Ammonia]	6	0.61	0.721
Below-ground Mesocosm	Species	1	15.76	0.0003
Below-ground Mesocosm	[Ammonia]	3	12.33	<0.0001
Below-ground Mesocosm	Species x [Ammonia]	3	3.85	0.0171
Below-ground Mesocosm	N:P Ratio	2	6.45	0.004
Below-ground Mesocosm	N:P Ratio x Species	2	1.95	0.1561
Below-ground Mesocosm	N:P Ratio x [Ammonia]	6	1.43	0.23
Below-ground Mesocosm	Species x N:P Ratio x [Ammonia]	6	0.42	0.863

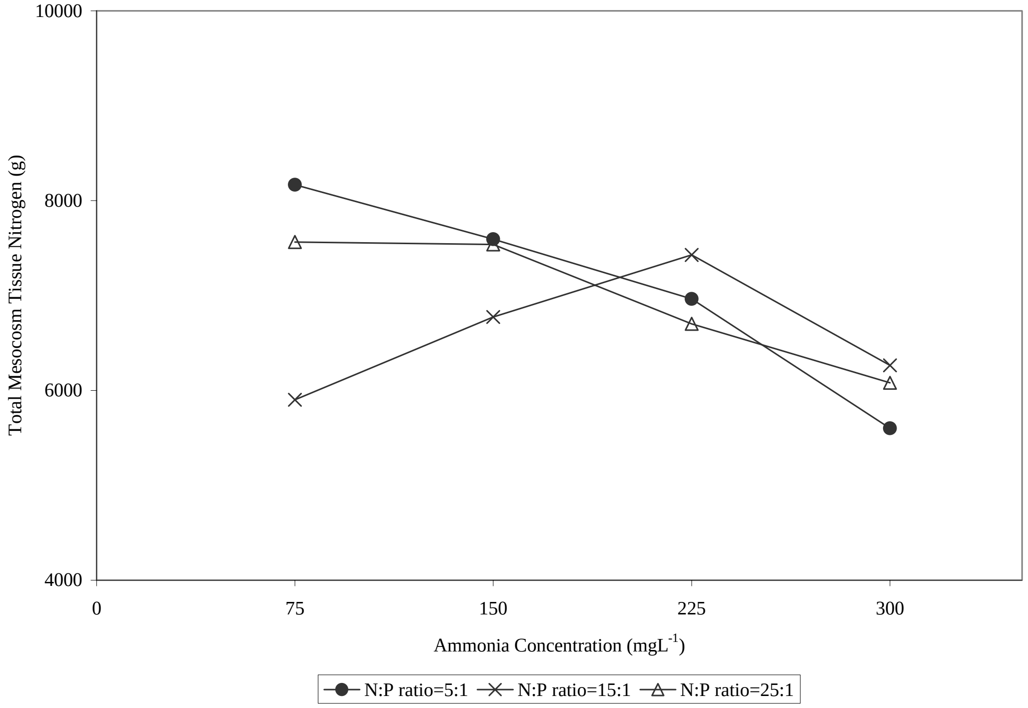

Figure 3.23. Total mesocosm tissue mass nitrogen along a gradient of ammonia concentration treatments and at three N:P ratios in 2003. Standard errors are not presented due to graphic difficulty in their interpretation. Due to normality of data and homogeneity of residuals from the ANOVA, log transformation was not necessary (see appendix II for SAS code).

total nitrogen uptake may be based primarily on total biomass rather than on tissue nitrogen concentration. Although more nitrogen is taken up at high ammonia treatment levels, more total nitrogen is removed from wastewater at peak biomass production. Total N uptake is greatest at the 5:1 N:P ratio treatment; this finding is similar to tissue N concentration results (Figures 3.23 and 3.14, respectively). Since *Juncus* produced more biomass than *Typha* at all treatment levels, it sequestered significantly higher mass of N in the tissue than *Typha*, although *Typha* has higher tissue N concentrations. Although this may seem to indicate that *Typha* and *Juncus* therefore operate in the same manner in terms of overall N removal, since *Juncus* produces more biomass and is more tolerant of high ammonia concentrations, it is able to remove more N from wastewater.

Total above-ground mesocosm tissue mass nitrogen was significantly affected by the same effects as total mesocosm tissue mass nitrogen, and in much the same way; ammonia concentration, species, the N:P ratio treatment, and the interaction between species and ammonia concentration affect total above-ground mesocosm tissue mass nitrogen (Table 3.9). Total above-ground tissue mass nitrogen decreased with increasing ammonia concentration, due in part to the fact that less biomass was produced at high ammonia concentrations, and despite higher tissue nitrogen concentrations at high ammonia concentrations (Figure 3.24). *Juncus* had higher above-ground tissue mass nitrogen at all treatment levels, and averaged 3526.4±409.6 mg over all treatments. *Typha* averaged 1242.3±286.7 mg over all treatments. Highest N uptake occurs at the 5:1 N:P ratio treatment; this is similar to overall mesocosm tissue N and to tissue N concentration results. This may indicate a result of a low N:P ratio, and demonstrates

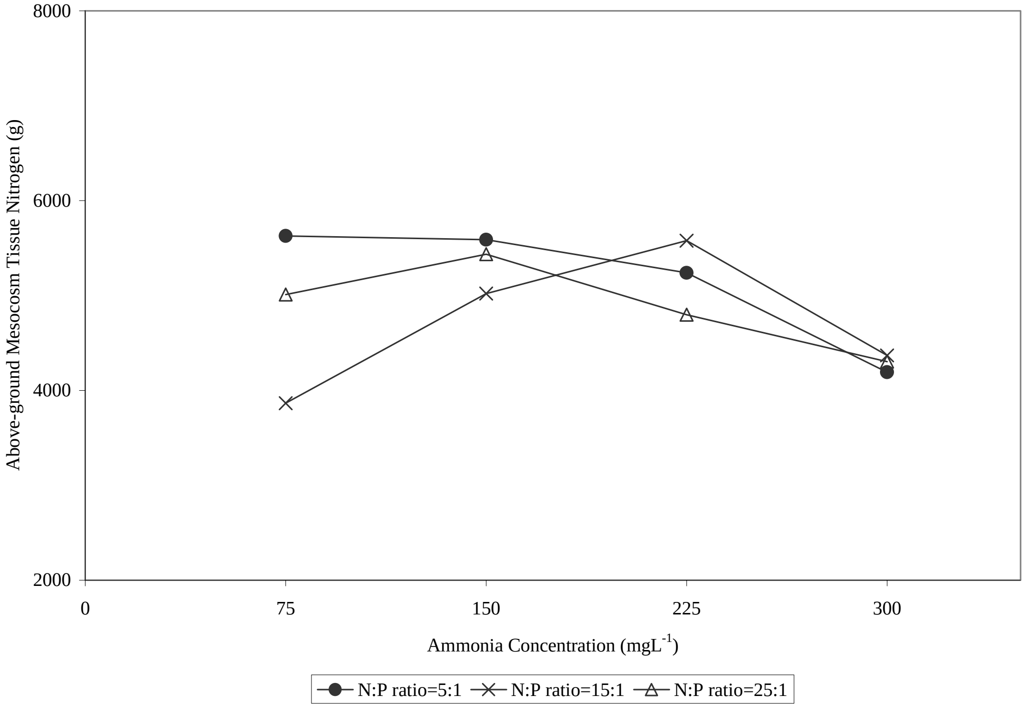

Figure 3.24. Total above-ground mesocosm tissue mass nitrogen along a gradient of ammonia concentration treatments and at three N:P ratios in 2003. Standard errors are not presented due to graphic difficulty in their interpretation. Due to normality of data and homogeneity of residuals from the ANOVA, log transformation was not necessary (see appendix II for SAS code).

that when less N is available, plant tissues sequester more overall N and sequester N in higher concentrations.

Total below-ground tissue mass nitrogen was significantly affected by N:P ratio treatment, species, and ammonia concentration treatment and by the interaction of species and ammonia concentration as well (Table 3.9). Like above-ground tissue mass nitrogen, a similar trend to overall mesocosm mass nitrogen is evident, with greater mass nitrogen at lower ammonia concentrations and at the 5:1 N:P ratio (Figure 3.25), and a greater mass of N found in *Juncus*. Again, this trend indicates a clear relationship between total biomass production and total N uptake; since *Juncus* produced more biomass, total N in *Juncus* tissue is higher than that of *Typha,* despite a higher N concentration in *Typha*.

If the amending the N:P ratio could improve ATW functioning, N:P ratio treatments should significantly affect the total mass N stored in plant tissues. In this experiment, the N:P ratio of 5:1 produced significantly higher N mass uptake in plant tissues, as well as producing higher overall tissue N concentration. At a co-limited N:P ratio, nutrients should be used for biomass production, and therefore should be evident in high tissue mass N (Tilman 1982). In fact, at the lower, N-limited N:P ratio (5:1), higher N mass uptake and higher N concentration occurred. If the 5:1 N:P ratio had likewise produced overall higher biomass, then a precedent could be set for managers to improve ATW function by setting an N:P ratio of 5:1. Despite the 5:1 N:P ratio not statistically influencing biomass, the fact that over-fertilization with phosphorus may serve to remove additional nitrogen from wastewater may be a practical means by which to control ammonia concentration in ATW effluent.

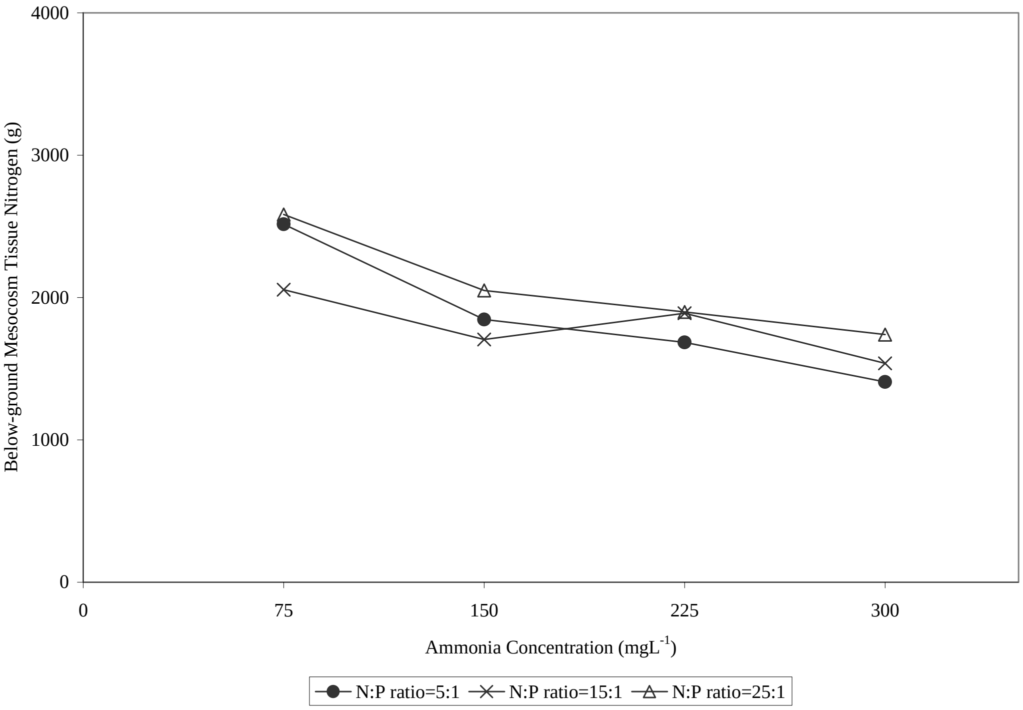

Figure 3.25. Total below-ground mesocosm tissue mass nitrogen along a gradient of ammonia concentration treatments and at three N:P ratios in 2003. Standard errors are not presented due to graphic difficulty in their interpretation. Due to normality of data and homogeneity of residuals from the ANOVA, log transformation was not necessary (see appendix II for SAS code).

Total *Typha latifolia* tissue mass nitrogen was significantly affected by ammonia concentration treatment alone (Table 3.10). Tissue mass N decreased with from an average of 2731.0±796.3 mg at the 75 mgL^{-1} ammonia concentration treatment to 787.5±89.3 mg at the 300 mgL^{-1} ammonia concentration treatment (Figure 3.26). This concurs well with the tissue N concentration results, and indicates N uptake problems at the higher ammonia concentrations, as well as suppressed biomass production at those levels. Although the N:P ratio treatment was not significant in determining *Typha* tissue N mass, the 5:1 N:P ratio produces observably higher N mass uptake, which is a similar overall trend to total mesocosm N uptake, and may as well indicate the management implications of this study. The behavior of above-ground *Typha* tissue mass N (Figure 3.27), and below-ground tissue mass N (Figure 3.28) demonstrates similar trends. In fact, the 5:1 N:P ratio produces statistically significant high N mass for these measurements (Table 3.10). Again, the decrease in biomass at high ammonia treatments appears to affect total N uptake in *Typha* above- and below-ground tissues; this corresponds well with total mesocosm effects.

Total *Juncus effusus* tissue mass nitrogen is affected by ammonia concentration treatment, the N:P ratio treatment, and their interaction (Table 3.11). Mean tissue mass N increases from 4068.9±588.2 mg at the 75 mgL^{-1} ammonia concentration to 5222.41±318.1 mg at the 225 mgL^{-1} ammonia concentration, then drops to 4798±333.8 mg at the 300 mgL^{-1} ammonia concentration. Highest tissue mass N is produced by the 25:1 N:P ratio treatment. Figure 3.29 illustrates the interactive effects of ammonia concentration and N:P ratio treatment in affecting tissue N mass, and shows the higher ammonia treatments have relatively similar overall N mass uptake. This corresponds

Table 3.10. Tests of fixed effects on total, above-ground, and below-ground tissue total mass nitrogen in *Typha latifolia* in 2003. Please refer to appendix II for SAS code.

Measured Biomass	**Effect**	**d.f.**	**F Value**	**P Level**
Total *Typha latifolia*	[Ammonia]	3	6.03	0.0046
Total *Typha latifolia*	N:P Ratio	2	3.19	0.0637
Total *Typha latifolia*	N:P Ratio x [Ammonia]	6	0.85	0.5493
Above-ground *Typha latifolia*	[Ammonia]	3	8.11	0.0011
Above-ground *Typha latifolia*	N:P Ratio	2	3.71	0.0435
Above-ground *Typha latifolia*	N:P Ratio x [Ammonia]	6	0.94	0.4915
Below-ground *Typha latifolia*	[Ammonia]	3	7.42	0.0019
Below-ground *Typha latifolia*	N:P Ratio	2	3.75	0.0437
Below-ground *Typha latifolia*	N:P Ratio x [Ammonia]	6	0.82	0.5655

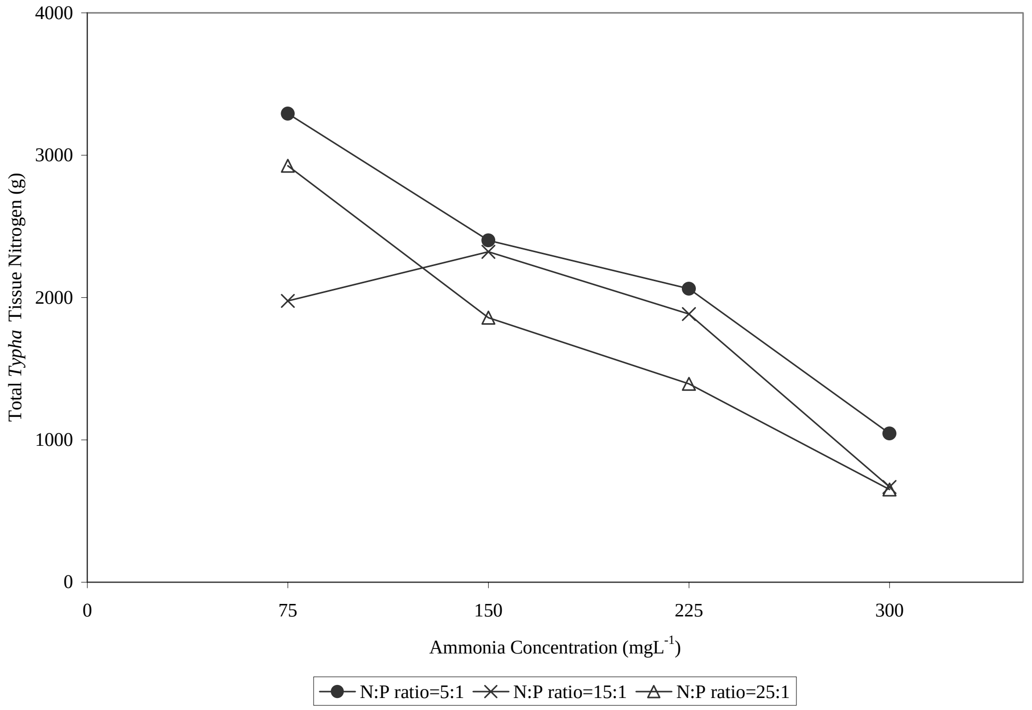

Figure 3.26. Total *Typha latifolia* tissue mass nitrogen along a gradient of ammonia concentration treatments and at three N:P ratios in 2003. Standard errors are not presented due to graphic difficulty in their interpretation. Due to normality of data and homogeneity of residuals from the ANOVA, log transformation was not necessary (see appendix II for SAS code).

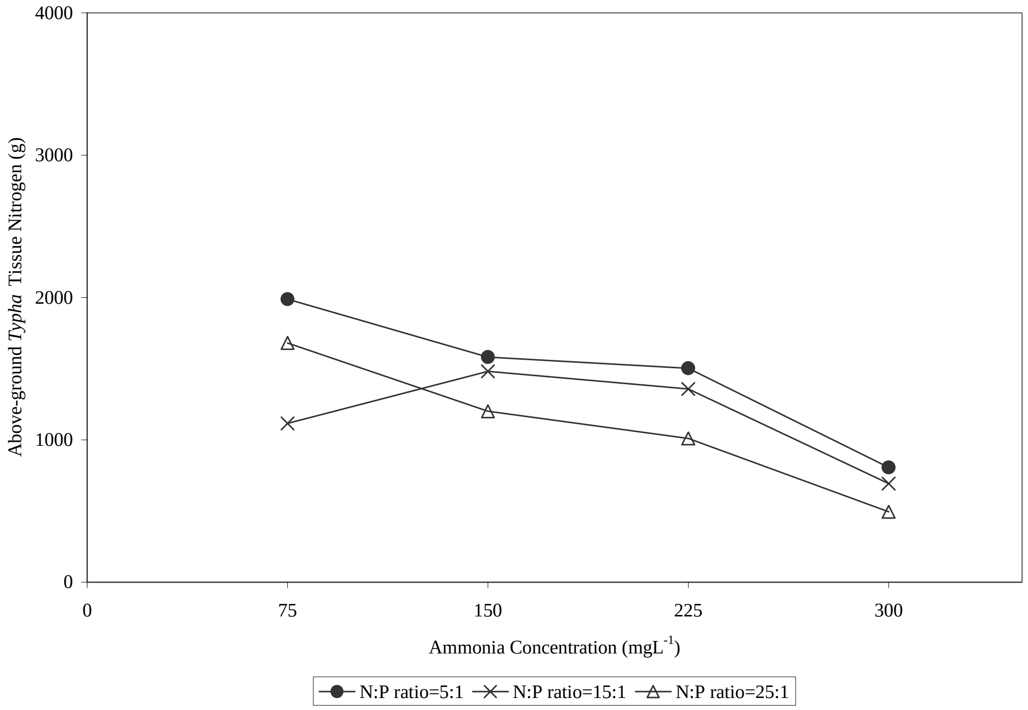

Figure 3.27. Above-ground *Typha latifolia* tissue mass nitrogen along a gradient of ammonia concentration treatments and at three N:P ratios in 2003. Standard errors are not presented due to graphic difficulty in their interpretation. Due to normality of data and homogeneity of residuals from the ANOVA, log transformation was not necessary (see appendix II for SAS code).

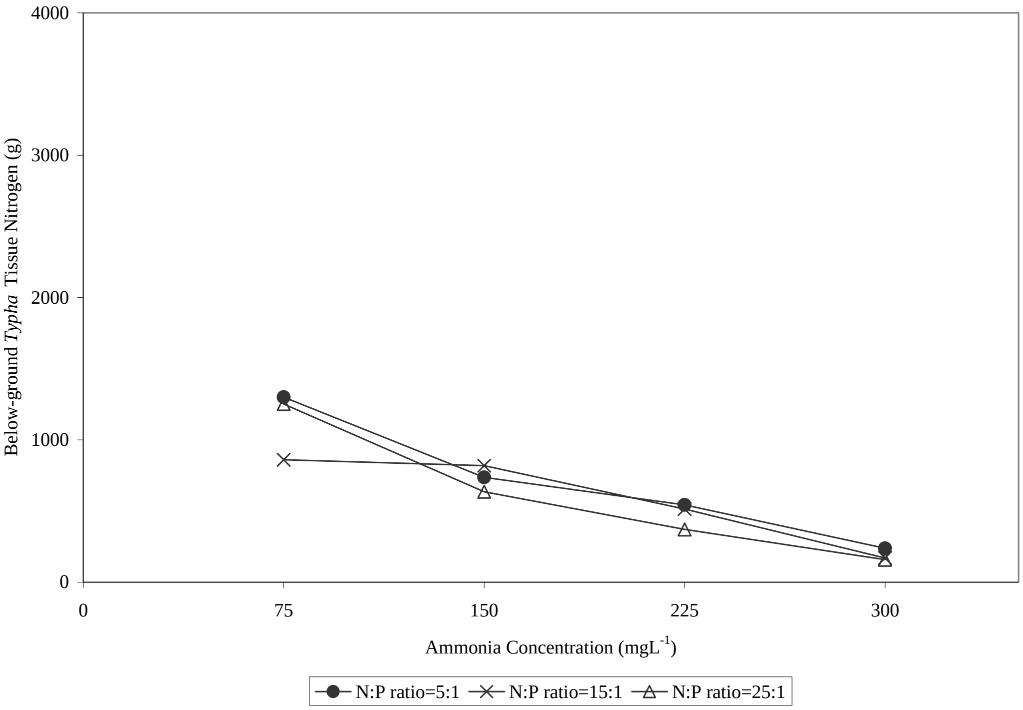

Figure 3.28. Below-ground *Typha latifolia* tissue mass nitrogen along a gradient of ammonia concentration treatments and at three N:P ratios in 2003. Standard errors are not presented due to graphic difficulty in their interpretation. Due to normality of data and homogeneity of residuals from the ANOVA, log transformation was not necessary (see appendix II for SAS code).

Table 3.11. Tests of fixed effects on total, above-ground, and below-ground tissue total mass nitrogen in *Juncus effusus* in 2003. Please refer to appendix II for SAS code.

Measured Biomass	**Effect**	**d.f.**	**F Value**	**P Level**
Total *Juncus effusus*	[Ammonia]	3	14.14	<0.0001
Total *Juncus effusus*	N:P Ratio	2	10.71	0.0008
Total *Juncus effusus*	N:P Ratio x [Ammonia]	6	2.83	0.0386
Above-ground *Juncus effusus*	[Ammonia]	3	12.31	0.0001
Above-ground *Juncus effusus*	N:P Ratio	2	8.98	0.0018
Above-ground *Juncus effusus*	N:P Ratio x [Ammonia]	6	2.27	0.0806
Below-ground *Juncus effusus*	[Ammonia]	3	16	<0.0001
Below-ground *Juncus effusus*	N:P Ratio	2	12.56	0.0003
Below-ground *Juncus effusus*	N:P Ratio x [Ammonia]	6	3.47	0.0173

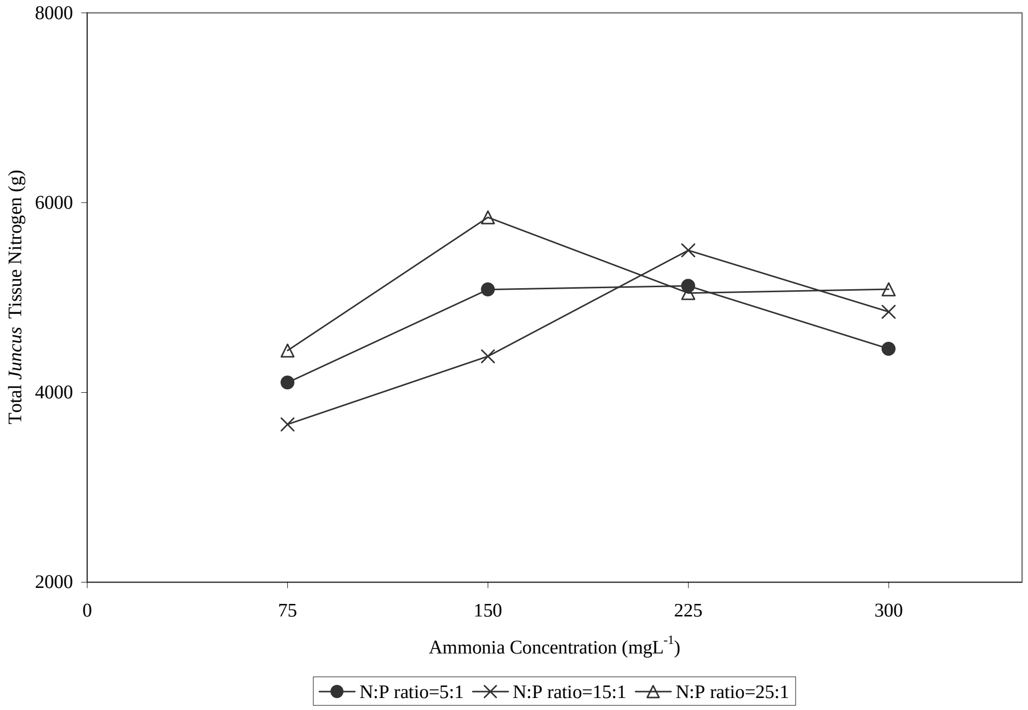

Figure 3.29. Total *Juncus effusus* tissue mass nitrogen along a gradient of ammonia concentration treatments and at three N:P ratios in 2003. Standard errors are not presented due to graphic difficulty in their interpretation. Due to normality of data and homogeneity of residuals from the ANOVA, log transformation was not necessary (see appendix II for SAS code).

well with overall biomass production results for *Juncus*, with little toxicity effect at higher ammonia concentrations. Of interest to note is that the P-limited 25:1 N:P ratio produced higher tissue N mass than co-limited or N-limited ratios, as in the case of overall mesocosm and *Typha* tissue mass N. This may indicate interactions with *Typha* growth at 5:1 and 15:1 N:P levels, and may also indicate the "stress tolerator" syndrome described above (Emery *et al*. 2001). Curves are similar for above-ground *Juncus* tissue mass N (Figure 3.30) and below-ground tissue mass N (Figure 3.31), although in below-ground tissue mass N, there is a less evident effect. This corresponds well with lower biomass and N concentration effects for *Juncus* previously noted.

Mesocosm Tissue Phosphorus Concentration

Total mesocosm tissue phosphorus concentration is affected by ammonia concentration, species, and the interactions between N:P ratio treatment and species and action between ammonia and species (Table 3.12). Since phosphorus administered by the N:P ratio is the only phosphorus available to the plant (other than phosphorus present in the tap water, which should be consistent between mesocosms), the non-significance of the N:P ratio is unclear and may represent additional phosphorus sources such as the greenhouse tap water or the medium in which the plants were grown. Mean tissue phosphorus concentration in *Juncus* is 2.1±0.2 mg g^{-1}, while that of *Typha* is 2.7±0.5 mg g-1, indicating that, like nitrogen, *Typha* typically sequesters more P in tissues. It is the significance of the ammonia concentration which is of particular interest here; tissue phosphorus concentrations increase from a low of 1.6±0.1 mg g^{-1} at the 75 mgL^{-1} ammonia treatment to 3.1±0.4 mg g^{-1} at the 300 mgL^{-1} ammonia treatment (Figure 3.32).

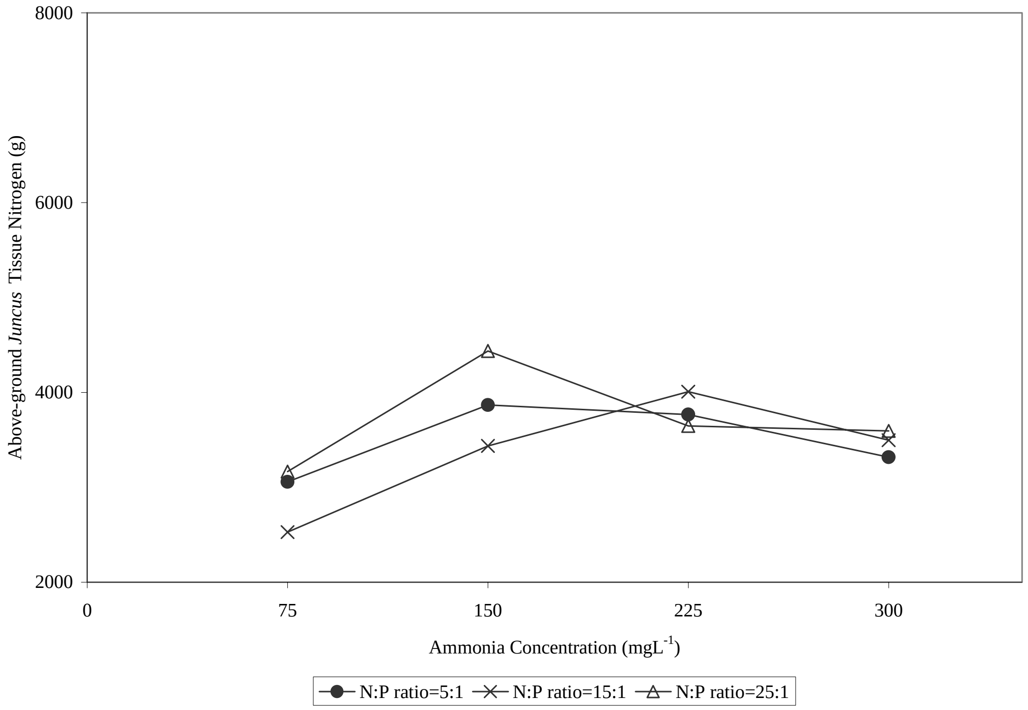

Figure 3.30. Above-ground *Juncus effusus* tissue mass nitrogen along a gradient of ammonia concentration treatments and at three N:P ratios in 2003. Standard errors are not presented due to graphic difficulty in their interpretation. Due to normality of data and homogeneity of residuals from the ANOVA, log transformation was not necessary (see appendix II for SAS code).

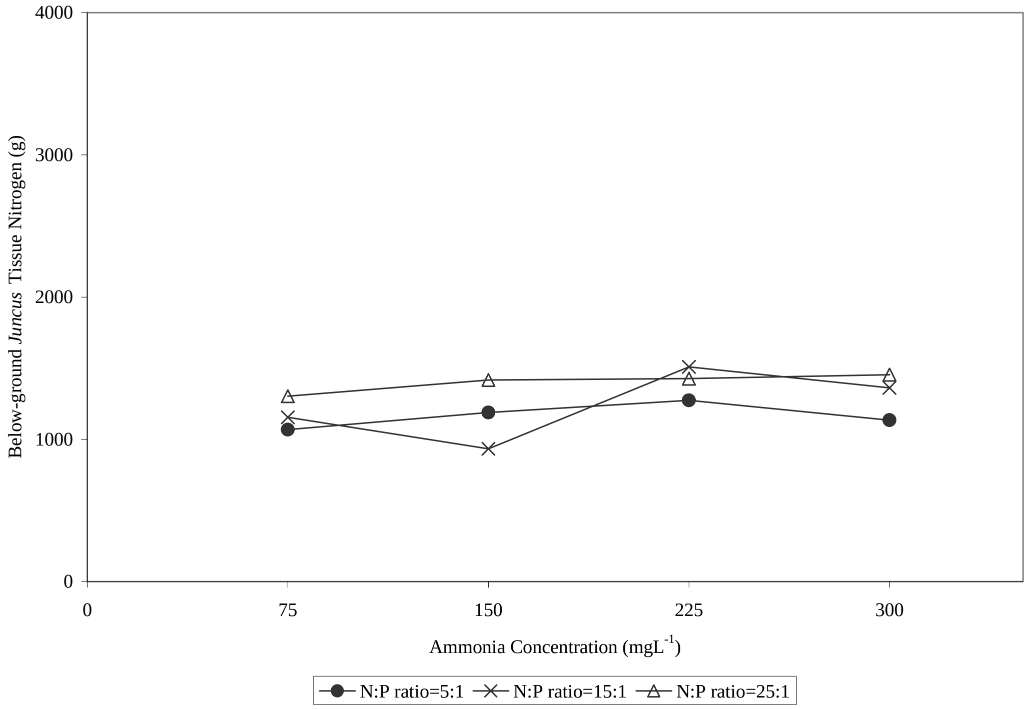

Figure 3.31. Below-ground *Juncus effusus* tissue mass nitrogen along a gradient of ammonia concentration treatments and at three N:P ratios in 2003. Standard errors are not presented due to graphic difficulty in their interpretation. Due to normality of data and homogeneity of residuals from the ANOVA, log transformation was not necessary (see appendix II for SAS code).

Table 3.12. Tests of fixed effects on total, above-ground, and below-ground tissue phosphorus concentration of mesocosms of *Typha latifolia* and *Juncus effusus* in 2003. Please refer to appendix II for SAS code.

Measured Biomass	Effect	d.f.	F Value	P Level
Total Mesocosm	Species	1	503.07	<0.0001
Total Mesocosm	[Ammonia]	3	26.7	<0.0001
Total Mesocosm	Species x [Ammonia]	3	6.06	0.0014
Total Mesocosm	N:P Ratio	2	0.03	0.9671
Total Mesocosm	N:P Ratio x Species	2	5.02	0.0104
Total Mesocosm	N:P Ratio x [Ammonia]	6	0.4	0.8769
Total Mesocosm	Species x N:P Ratio x [Ammonia]	6	0.78	0.5876
Above-ground Mesocosm	Species	1	539.76	<0.0001
Above-ground Mesocosm	[Ammonia]	3	18.14	<0.0001
Above-ground Mesocosm	Species x [Ammonia]	3	3.51	0.0222
Above-ground Mesocosm	N:P Ratio	2	0.07	0.9302
Above-ground Mesocosm	N:P Ratio x Species	2	3.51	0.0378
Above-ground Mesocosm	N:P Ratio x [Ammonia]	6	0.27	0.9486
Above-ground Mesocosm	Species x N:P Ratio x [Ammonia]	6	0.55	0.7703
Below-ground Mesocosm	Species	1	151.6	<0.0001
Below-ground Mesocosm	[Ammonia]	3	31.37	<0.0001
Below-ground Mesocosm	Species x [Ammonia]	3	10.15	<0.0001
Below-ground Mesocosm	N:P Ratio	2	0.57	0.5708
Below-ground Mesocosm	N:P Ratio x Species	2	4.27	0.0197
Below-ground Mesocosm	N:P Ratio x [Ammonia]	6	0.64	0.6983
Below-ground Mesocosm	Species x N:P Ratio x [Ammonia]	6	0.83	0.5559

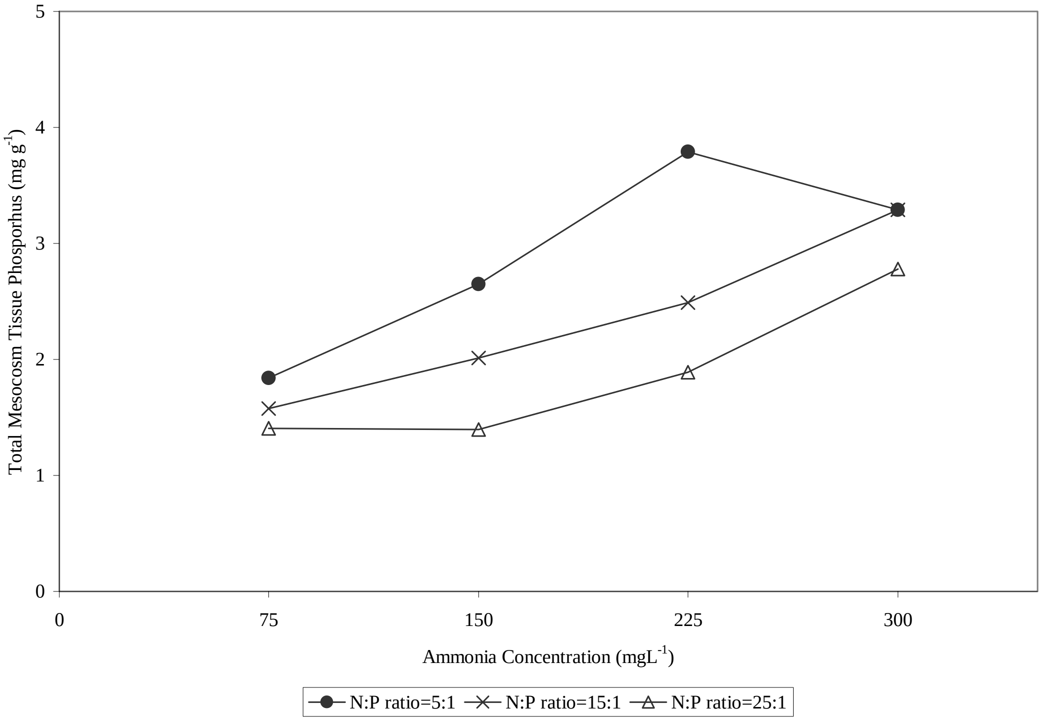

Figure 3.32. Total mesocosm tissue phosphorus concentration along a gradient of ammonia concentration treatments and at three N:P ratios in 2003. Above- and below-ground total phosphorus concentrations are not shown. Standard errors are not presented due to graphic difficulty in their interpretation. Due to normality of data and homogeneity of residuals from the ANOVA, log transformation was not necessary (see appendix II for SAS code).

This indicates a link between the ammonia concentration treatment applied and the ability of the biomass to absorb phosphorus. Other researchers have found using non-wetland species (*Allium sp.*) that at high ammonia concentrations, P uptake is inhibited, and have cited this as a potential cause of low biomass production (Abbes *at al.* 1995). The findings here suggest otherwise: since tissue nitrogen levels are so high, the amount of phosphorus needed by those tissues to supply a "normal" ratio is elevated as well. This finding shows some level of control by the plants over the tissue level N:P ratio. The P-enriched 5:1 N:P level treatment experiences an observable decline in total P absorption in the highest ammonia treatment level, indicating that some blockage in P assimilation occurs at toxic ammonia levels. Ammonia toxicity tends to decrease membrane transport efficiencies as well as decrease the functioning of enzymes or of mRNA related to nutrient uptake and storage; the blockage in P uptake may be due to this effect. Phosphorus deficiencies have been noted at phytotoxic nutrient concentrations by Abbes *et al.* (1995). Finding and using mutants which overexpress genes encoding high-affinity phosphate receptors may be one means by which this deficiency may be overcome, although for the time being mutant strains overexpressing phosphate transporters have been reported in *Arabidopsis* alone (Smith 2002). Figures for above- and below-ground mesocosm phosphorus tissue concentration are not shown, but show the same overall trends as for total mesocosm phosphorus tissue concentration (Table 3.12).

Total *Typha* tissue phosphorus concentration is affected by ammonia treatment alone (Table 3.13). Mean tissue phosphorus concentrations increased from a low of 1.6 ±0.3 mg g^{-1} at the 75 mgL^{-1} ammonia treatment to 3.5±0.8 mg g^{-1} at the 300 mgL^{-1}

Table 3.13. Tests of fixed effects on total, above-ground, and below-ground tissue phosphorus concentration of *Typha latifolia* in 2003. Please refer to appendix II for SAS code.

Measured Biomass	**Effect**	**d.f.**	**F Value**	**P Level**
Total *Typha latifolia*	[Ammonia]	3	33.94	<0.0001
Total *Typha latifolia*	N:P Ratio	2	2.75	0.0838
Total *Typha latifolia*	N:P Ratio x [Ammonia]	6	1.15	0.3636
Above-ground *Typha latifolia*	[Ammonia]	3	24.72	<0.0001
Above-ground *Typha latifolia*	N:P Ratio	2	3.8	0.0367
Above-ground *Typha latifolia*	N:P Ratio x [Ammonia]	6	1.02	0.4374
Below-ground *Typha latifolia*	[Ammonia]	3	37.51	<0.0001
Below-ground *Typha latifolia*	N:P Ratio	2	0.89	0.4219
Below-ground *Typha latifolia*	N:P Ratio x [Ammonia]	6	1	0.4492

ammonia treatment (Figure 3.33), and observably increased from a low of 2.0±0.3 mg g^{-1} at 25:1 N:P ratio treatment to 3.6±0.7 mg g^{-1} at the 5:1 N:P ratio treatment. The overall trend in *Typha* tissue phosphorus concentration is similar to that of mesocosm tissue phosphorus, with the greatest slope evident in the 25:1 N:P ratio treatment. This corresponds to the P-limited N:P ratio treatment, and implies that plants will continue to accumulate phosphorus if P-limited even under toxic ammonia concentrations. Again, figures for above- and below-ground *Typha* phosphorus tissue concentration are not shown and trends correcpond to total *Typha* phosphorus tissue concentration (Table 3.13). It is of interest to note that the N:P ratio affects above-ground phosphorus concentration and that the below-ground tissue phosphorus concentration is significantly affected by ammonia alone.

Total *Juncus effusus* tissue phosphorus concentration is significantly affected by ammonia treatment alone (Table 3.14). Mean tissue phosphorus concentrations increased obsrvably from a low of 1.8±0.3 mg g^{-1} at 25:1 N:P ratio treatment to 2.5±0.1 mg g^{-1} at the 5:1 N:P ratio treatment (Figure 3.34). The overall trend in *Juncus* tissue phosphorus is similar to that of the mesocosms on the whole, with the 25:1 N:P ratio exhibiting the greatest slope, and the 5:1 N:P ratio declining significantly between the 225 mgL^{-1} and the 300 mgL^{-1} ammonia treatments. Figures for *Juncus* above- and below-ground tissue phosphorus concentrations are not shown, but correspond well to overall *Juncus* tissue phosphorus concentrations (Table 3.14). Of interest to note is the significant effect of the N:P ratio in determining phosphorus concentration in below-ground biomass, an opposite trend to that of *Typha*.

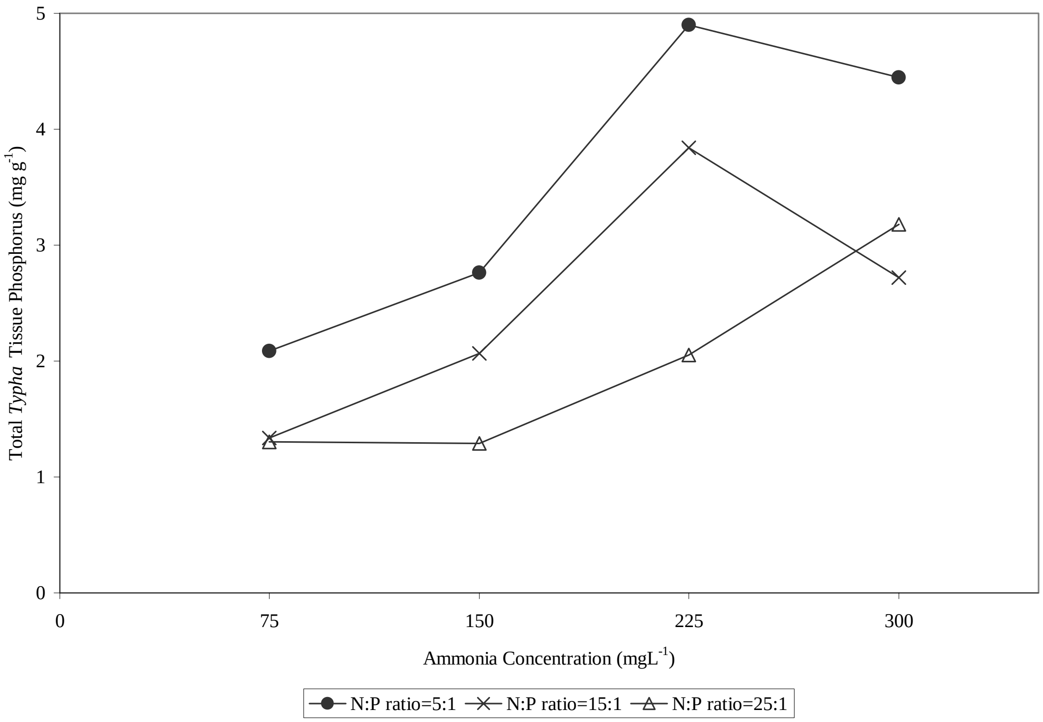

Figure 3.33. Total *Typha latifolia* tissue phosphorus concentration along a gradient of ammonia concentration treatments and at three N:P ratios in 2003. Above- and below-ground total phosphorus concentrations are not shown. Standard errors are not presented due to graphic difficulty in their interpretation. Due to normality of data and homogeneity of residuals from the ANOVA, log transformation was not necessary (see appendix II for SAS code).

Table 3.14. Tests of fixed effects on total, above-ground, and below-ground tissue phosphorus concentration of *Juncus effusus* in 2003. Please refer to appendix II for SAS code.

Measured Biomass	**Effect**	**d.f.**	**F Value**	**P Level**
Total *Juncus effusus*	[Ammonia]	3	4.49	0.0123
Total *Juncus effusus*	N:P Ratio	2	2.38	0.1144
Total *Juncus effusus*	N:P Ratio x [Ammonia]	6	0.21	0.9703
Above-ground *Juncus effusus*	[Ammonia]	3	5.1	0.0072
Above-ground *Juncus effusus*	N:P Ratio	2	0.96	0.3961
Above-ground *Juncus effusus*	N:P Ratio x [Ammonia]	6	0.16	0.9858
Below-ground *Juncus effusus*	[Ammonia]	3	5.2	0.0066
Below-ground *Juncus effusus*	N:P Ratio	2	3.83	0.0359
Below-ground *Juncus effusus*	N:P Ratio x [Ammonia]	6	0.49	0.8127

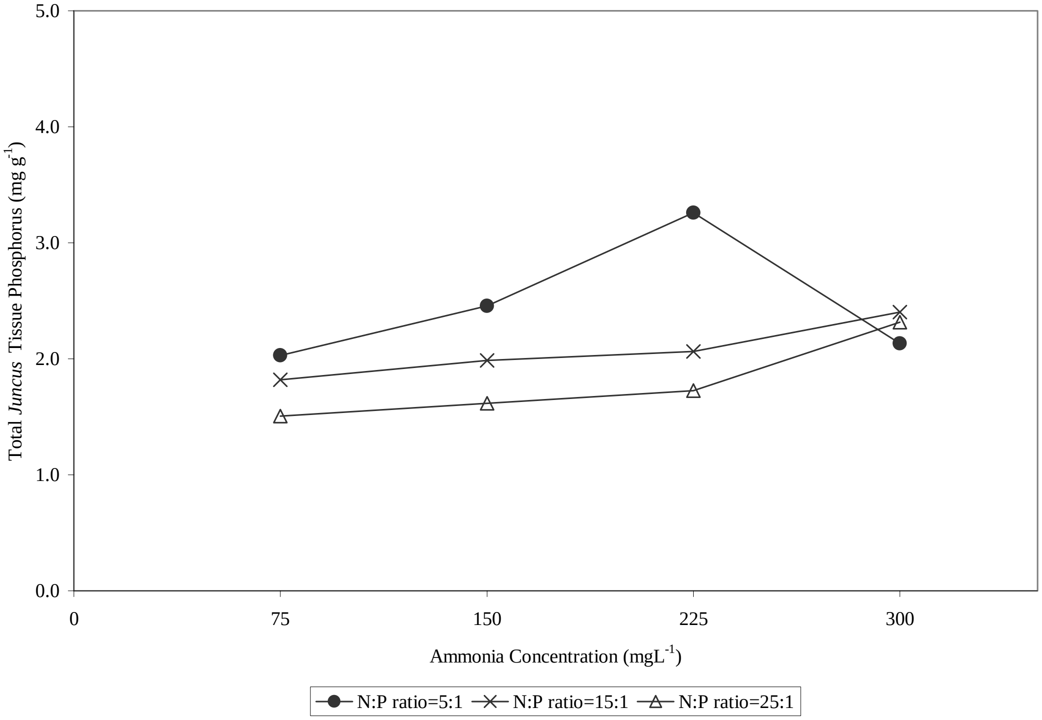

Figure 3.34. Total *Juncus effusus* tissue phosphorus concentration along a gradient of ammonia concentration treatments and at three N:P ratios in 2003. Above- and below-ground total phosphorus concentrations. Standard errors are not presented due to graphic difficulty in their interpretation. Due to normality of data and homogeneity of residuals from the ANOVA, log transformation was not necessary (see appendix II for SAS code).

The N:P Ratio as a Predictive Tool

These analyses allow for interpretation of the vegetation N:P ratio in *Typha* and *Juncus.* Figure 3.35 illustrates the differences between mean vegetation N:P ratios in *Typha* and *Juncus* and allows comparison of each to the tissue N:P ratios predicted by Redfield (1958) and Koerselman and Meuleman (1996); the tissue N:P levels of both species tissues falls within the predicted N:P ratio for all values except very high levels of nitrogen. At high levels of nitrogen, *Juncus* has lower N:P ratios, and *Typha* has higher N:P ratios. Due to the capacity of the vegetation to uptake nutrients preferentially, the N:P ratio generally stays between the co-limited ratio of Koerselman and Meuleman (14:1; 1996) and the co-limited ratio of Redfield (7.2:1, 1958). Of interest is that, if one were to predict nutrient limitation by the Korselman and Meuleman ratio, biomass would be slightly N-limited, whereas the Redfield ratio would predict slight P limitation. Since the plants themselves show affinities for different nutrients at different concentrations, the total N:P ratio of the biomass should fall within these ratios if in fact the biomass was co-limited by N and P. Koerselman and Meuleman (1996) demonstrate that vegetation N:P ratios of <14:1 show N limitation and >16:1 show P limitation in wetland plants. Redfield (1958) suggests a vegetation N:P ratio of 7.2:1 by weight. Despite administering experimental N:P ratios of 5:1 and 25:1, Figure 3.36 clearly shows that measured vegetation N:P ratios did not reach such levels. In the case of the high nitrogen 5:1 N:P ratio treatment, the vegetation N:P ratio does appear to approach the actual treatment ratio. Only in high nitrogen *Typha* tissue were measured N:P ratios less than the Redfield ratio, and in very low nitrogen were measured vegetation N:P ratios more than the Koerselman-Meuleman co-limited ratio (Figure 3.37). The response of *Typha* to

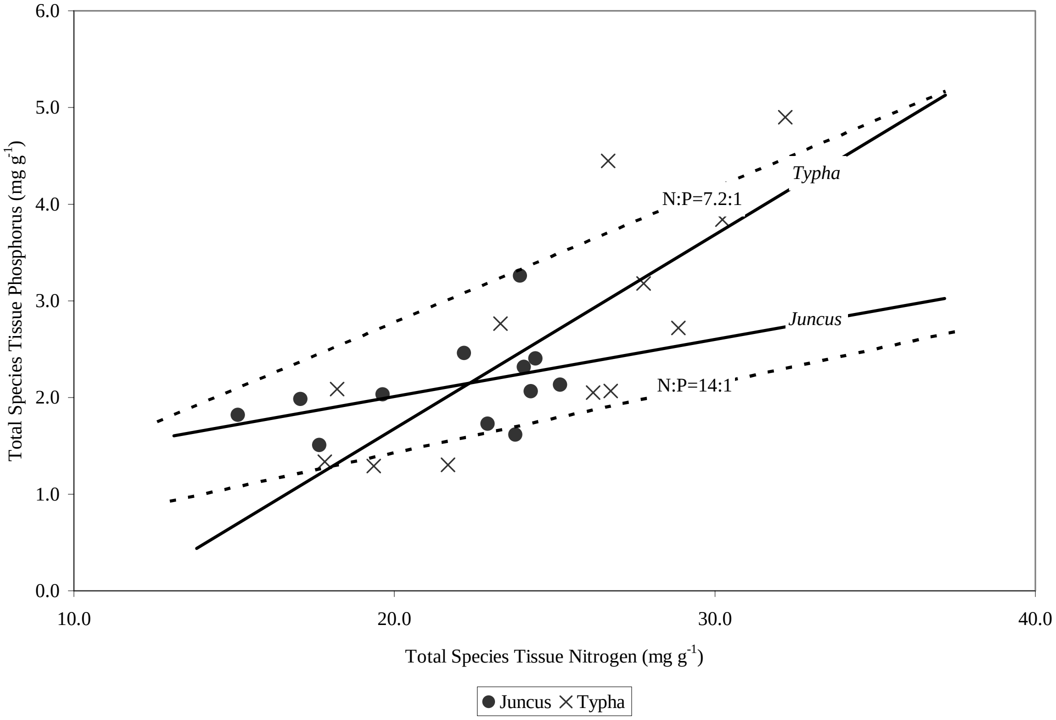

Figure 3.35. The vegetation N:P ratio of total *Juncus effusus* tissue and total *Typha latifolia* tissue presented as tissue nitrogen versus tissue phosphorus at all experimental N:P ratios and all ammonia concentration treatments in 2003. N:P ratios of 7.2:1 (Redfield 1958) and 14:1 (Koerselmen and Meuleman 1996) are provided for comparison and to show possible limitations. Standard errors are not presented due to graphic difficulty in their interpretation. Due to normality of data and homogeneity of residuals from the ANOVA, log transformation was not necessary (see appendix II for SAS code).

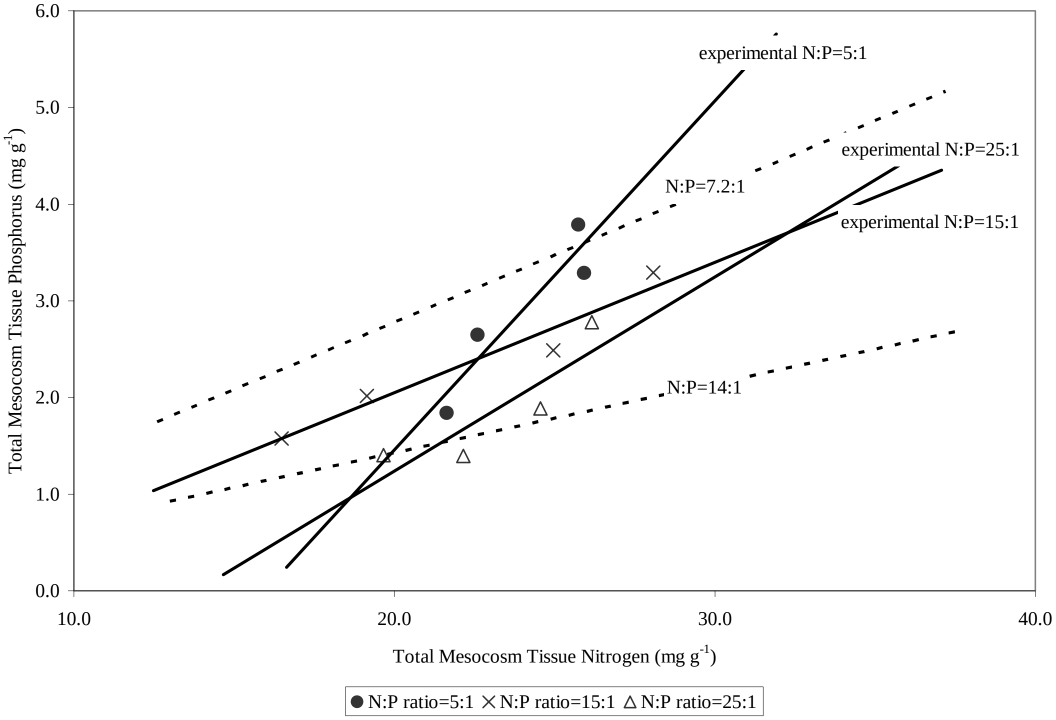

Figure 3.36. The vegetation N:P ratio of each experimental N:P ratio presented as tissue nitrogen versus tissue phosphorus at all ammonia concentration treatments and for mesocosms of *Juncus effusus* and *Typha latifolia* in 2003. N:P ratios of 7.2:1 (Redfield 1958) and 14:1 (Koerselmen and Meuleman 1996) are provided for comparison and to show possible limitations. Standard errors are not presented due to graphic difficulty in their interpretation. Due to normality of data and homogeneity of residuals from the ANOVA, log transformation was not necessary (see appendix II for SAS code).

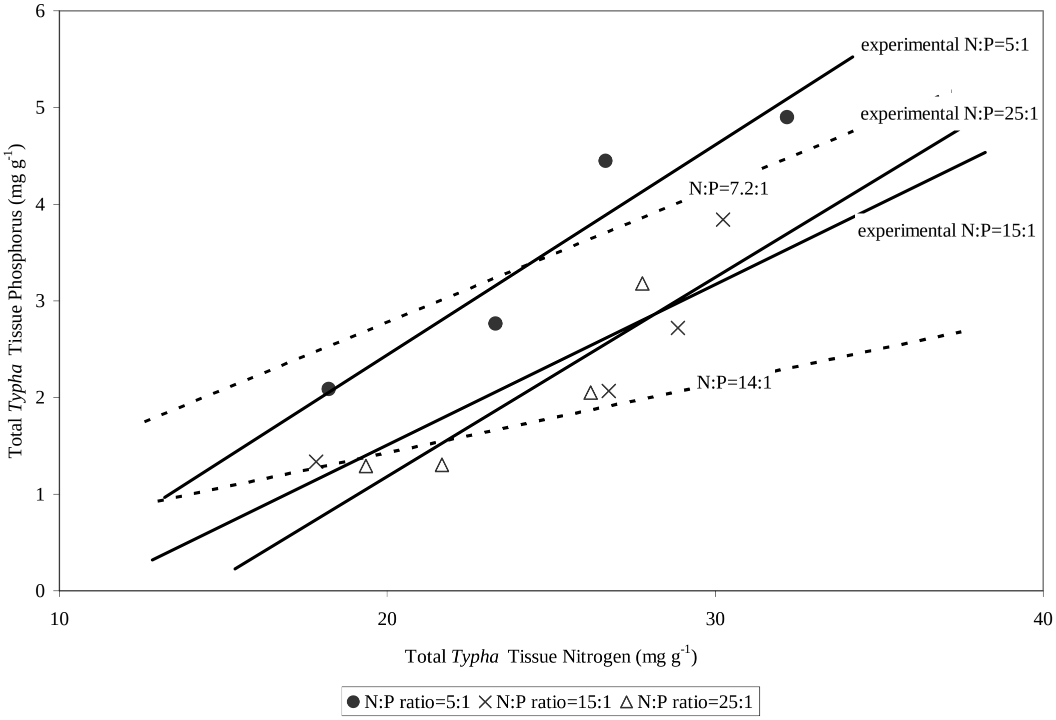

Figure 3.37. The vegetation N:P ratio of each experimental N:P ratio presented as tissue nitrogen versus tissue phosphorus at all ammonia concentration treatments and for *Typha latifolia* in 2003. N:P ratios of 7.2:1 (Redfield 1958) and 14:1 (Koerselmen and Meuleman 1996) are provided for comparison and to show possible limitations. Standard errors are not presented due to graphic difficulty in their interpretation. Due to normality of data and homogeneity of residuals from the ANOVA, log transformation was not necessary (see appendix II for SAS code).

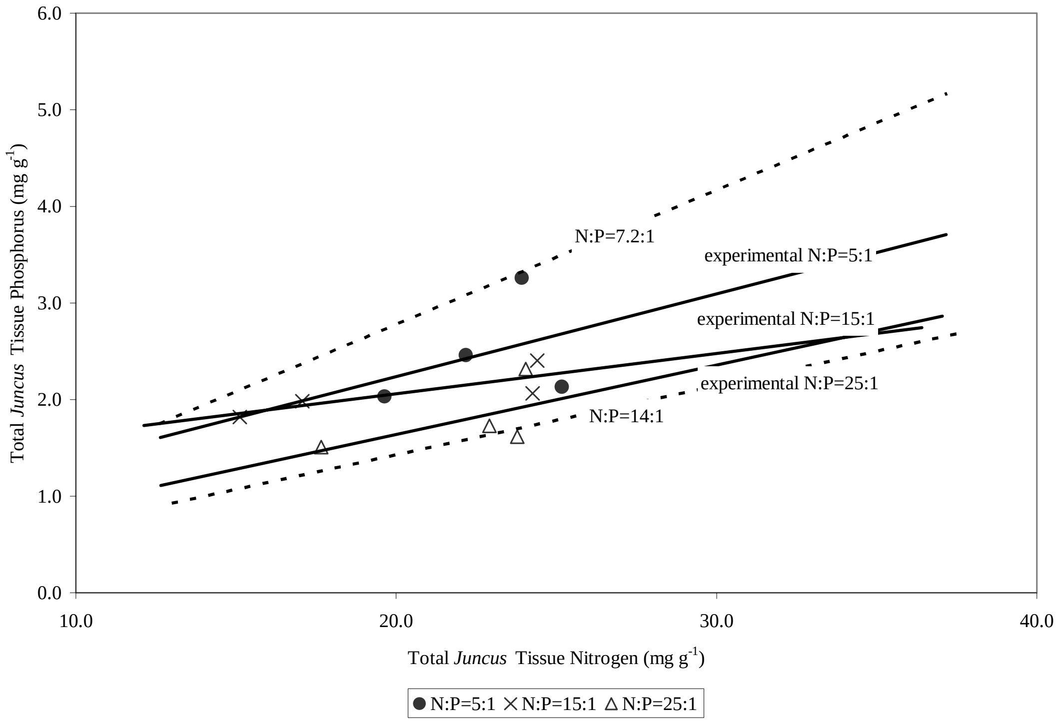

Figure 3.38. The vegetation N:P ratio of each experimental N:P ratio presented as tissue nitrogen versus tissue phosphorus at all ammonia concentration treatments and for *Juncus effusus* in 2003. N:P ratios of 7.2:1 (Redfield 1958) and 14:1 (Koerselmen and Meuleman 1996) are provided for comparison and to show possible limitations. Standard errors are not presented due to graphic difficulty in their interpretation. Due to normality of data and homogeneity of residuals from the ANOVA, log transformation was not necessary (see appendix 2 for SAS code).

elevated ammonia concentrations even in high phosphorus concentrations is to uptake more phosphorus than necessary. An N:P ratio of less than 5:1 with high tissue nitrogen may be an artifact of phytotoxicity in *Typha* and requires further study. In *Juncus*, vegetation N:P ratios never fell outside predicted ratios (Figure 3.38), although extrapolation of the lines for each N:P treatment could potentially reach such levels. In age-symmetric and age-asymmetric mixtures of *Typha* and *Schenoplectus tabernaemontanai* (K.C. Gmel) Palla in treatment wetlands, Svengsouk and Mitsch (2000) found N:P ratios likewise typically bounded by ratios of 7.2:1 and 14:1. When nitrogen and phosphorus were added in experimental concentrations of 3:1, only *Schenoplectus* tissue exhibited an N:P ratio of less than 7.2:1. Here, mesocosm N:P ratios tend to fall within the bounds of “normal” (taken here to mean lower nitrogen levels) natural system ratios, perhaps due to the fact that adjustments to such low ratios were not made. This finding is unsupported by other authors (Koerselman and Meuleman 1996, Gusewell *et al.* 2003): the tissue N:P ratio is more indicative of the nutrient requirements of the species than of relative availability rates of these nutrients (Tilman 1997).

The effects of nutrient supply rates on long-term community composition and biomass production may differ from the findings reported here for short-term fertilization (Tilman 1982, Chapin *et al.* 1997). Long-term changes in community composition may be based on changes in levels of competition following removal of nutrient deficiencies, as predicted by Tilman (1982). This is especially evident considering the extremely high nutrient concentrations used in this experiment. Most experiments regarding short- and long-term nutrient fertilization studies have been field-based in locations of oligotrophic growth (see Koerselman and Meuleman 1996 for a synopsis of fertilization experiments with acceptable treatment design). Systems such as dune slacks, bogs, fens, and northern-latitude freshwater wetlands tend to have higher species diversity, lower nutrient availability, and subsequent lower production when compared to natural systems such as tidal marshes or artificial systems such as treatment wetlands. McJannet *et al.* (1995) indicate that no differences exist N:P ratios in plant of "fertile" and "infertile" ecosystems, "fertility" was defined by a nitrogen soil concentrations in excess of 10 ppm, which is significantly lower than concentrations addressed in this study. Although many researchers have investigated the effects of nutrient additions, few report actual nutrient concentrations achieved (which is often due to fertilization with slow-release type fertilizers; McJannet *et al.* 1995, Gusewell *et al.* 2003) and few have observed the effects of nutrient additions along gradients (but see Clarke and Balwin 2002, Romero *et al.* 1999) as applied to the N:P ratio. Measurement of the N:P ratios in plant communities in ATWs instead of in artificially manipulated greenhouse experiments may provide increased interpretation of the role of the N:P ratio in determining biomass composition at high nitrogen concentrations.

Tissue N:P ratios for each species were compared with those suggested by Koerselman and Meuleman (1996) and Redfield (1958) (Figures 3.35, 3.37, 3.38). The effect of species on determining tissue N and P concentrations, and thus the vegetation N:P ratio, is significant. This contradicts the work of some researchers. In field experiments, N:P ratios tend to differ more within species (given different sites or different nutrient concentrations) than between species at a given site (Koerselman and Meuleman 1996, Gusewell *et al.* 2002), or between growth forms rather than between species (Aerts *et al.* 1999). On the other hand, in a study of 41 wetland species, McJannet *et al.* (1995) found significant differences in N:P ratios between species (n=5 samples of each species). Evidently, additional work is necessary to characterize the N:P ratio between species.

A major component of the N:P tool is its potential for characterizing competitive relationships between species. The "resource ratio model" (Tilman 1982, 1985, 1997) predicts that the concentration of a nutrient in plant biomass might indicate the ability of the species to compete for this nutrient. Thus, in elevated N conditions, the resource ratio model predicts that species with the higher N:P ratio will exhibit a competitive advantage because strong competitors have low concentrations of a limiting nutrient (Tilman 1982). The measured average N:P ratio of *Juncus* was 12.0:1; the measured average N:P of *Typha* was 9.1:1. As treatment N levels increased, *Juncus* sequestered less N in the biomass (Figure 3.20) than *Typha* (Figure 3.17), and slightly less P (Figure 3.34) than *Typha* (Figure 3.33). Thus, despite increases in ammonia concentration, the N:P ratio in *Juncus* remained at a level more suitable for growth as evidenced in Figure 3.32.

If N and P biomass sequestering differs between species, as shown here, a real potential for more precise ATW planting schemes should be investigated. Especially in regards to the fact that high levels of ammonia tend to be limiting to plant biomass in these systems due to phytotoxic effect, those species which tend to accumulate lower amounts of N in tissue may play an important role in designing ATWs to handle high N-loading rates. Gusewell *et al.* (2003) state that species with higher N:P ratios would respond in the long term to additions of nitrogen, while species with lower N:P ratios would respond to additions of phosphorus, and that these predictions are based on the work of Tilman's (1982) resource ratio model. In point of fact, it appears that in extremely high ammonia, P-limited systems, species exhibiting a higher N:P ratio (*Juncus*) would have higher biomass than species exhibiting a low N:P ratio. Perhaps since N levels are so high in ATWs, those species which are able to exclude toxic ammonia-N from their tissues have a competitive advantage through stress tolerance.

Additionally, tissue N concentrations must be compared with total N removed by each treatment condition. Of interest is that, despite higher N loading in the 300 mgL^{-1} treatment, less total N was removed due to lower biomass (Figure 3.23). By finding the optimal N level or by amending the N:P level, managers could achieve removal of the highest amount of N over time. Planting to achieve such function would require schemes that focus on stress tolerators such as *Juncus* in high ammonia concentrations or highly competitive species such as *Typha* under more ideal ammonia concentrations. The 15:1 N:P level accumulated the least total tissue nitrogen in the experiment; this finding is surprising considering this ratio is "optimal" and "co-limiting" based on the work of other researchers (Korselman and Meuleman 1996). Total tissue N over the course of the experiment was highest in the 5:1 level, where, due to an overabundance of P, macrophytes should not have been able to utilize interstitial nitrogen; the fact that high tissue N resulted under these conditions needs additional study.

CONCLUSIONS

Utility of the N:P ratio as a predictive tool in high-ammonia ATWs may be recommended under the conditions predicted by the model of Koerselman and Meuleman (1996). Optimal predictive power is achieved by use of a larger system, here the mesocosm, *in situ* the ATW itself; differences in the N:P ratios based on the species themselves can likewise be recommended based on the data presented, with trends in the data possibly indicating differences in N and P usage in *Juncus* and in *Typha*.

The fact that differences in N and P uptake are based on species is not in agreement with other authors, and represents an important distinction in creating ATWs. Koerselman and Meuleman (1996) found significant differences in the N:P ratio alone, not in the respective differences in the masses of N and P in the biomass tissue. McJannet *et al.* (1995) likewise found statistically significant differences in N:P ratio alone, as opposed to differences in the masses of N and P within 41 wetland species. Other authors only supply information regarding the results of tests on the N:P ratio (versus the Redfield ratio or t-tests between species or sites) without providing information regarding the individual terms of N uptake and P uptake sequestered in the biomass (Gusewell *et al.* 2003, Romero *et al.* 2003, Svengsouk and Mitsch 2000, Tilman 1996). This is primarily due to a lack of gradient-based, controlled bench-scale analyses on the subject. The aforementioned authors used primarily large-scale *in situ* testing strategies on actual functioning ATWs. While large scale applications of the N:P ratio test may prove worthwhile in the long run in determining means to effect higher denitrification rates in ATWs, issues with species specific N and P loading rates should be addressed prior to planting treatment wetlands in experiments such as this.

Certainly, these systems differ from natural systems with lower amounts of nitrogen, and the results of fertilizing them with levels on orders of magnitude higher than natural systems will elicit changes in vegetation dynamics atypical of most field studies. At the same time, however, vegetation uptake of N and P must occur in both types of systems for growth of plant material to occur. Differences in the way ammonia-N forces physiologic change in these species should be further addressed, and may provide additional insight as to how the N:P ratio may be used to as a management tool in ATWs to improve treatment efficiency.

CHAPTER FOUR

SYNTHESIS

High ammonia concentrations are one of a number of potential limits to vegetation colonization of ATWs. Additional limits include substrate stability, herbivory, water depth, and the potential effects of other phytotoxic compounds such as sulfates, nitrates, or soluble metals such as iron, copper, or aluminum (Surrency 1993, Cronk 1996, Clarke 1999). However, the exceptionally high levels of nitrogen, especially those found in ammonia form, have been cited or inferred frequently as the most limiting phytotoxic element in ATWs (Wang 1991, Surrency 1993, Hammer and Knight 1994, Dijk and Eck 1995, Cronk 1996, Kadlec and Knight 1996, Verhoeven *et al.* 1996, Hill *et al.* 1997, Bobbink *et al.* 1998, Humenik *et al.* 1999, Aerts and Bobbink 1999, Clarke 1999, Clarke and Baldwin 2002). Graded tests of ammonia toxicity have dominated the literature on this subject. It is important to note, however, that nitrogen plays an important role as a plant nutrient as well, and therefore the fate of ammonia in ATWs may be of multiple paths (Mitsch and Gosselink 2003): it may be transformed to nitrates, nitrogen gases or bacterial biomass, it may be transformed to plant biomass, or it may exit the system as ammonia. Experimental ratios of the percentage ammonia removed by the former processes are about 60% removal by bacterial action, and about 40% removal by plant uptake (Howes *et al.* 1981, Gumbricht 1993, Bachand and Horne 2000b).

Therefore, while it is important to achieve optimal spatial distribution of redoxic conditions for removal of nitrogen via the nitrification-denitrification pathway (and plant biomass plays important roles in providing oxygen to the root zone and in providing a substrate upon which the reaction may occur), it is likewise important to maintain optimal growth of macrophytes in constructed wetlands. In natural systems, plant growth tends to be limited by nitrogen, phosphorus, and to a lesser extent, potassium (Mitsch and Gosselink 1993, Taiz and Zeiger 2002). Nitrogen is rarely limiting in ATWs, and ammonia concentrations ranging to 500 mgL^{-1} have been reported (Hammer 1992). Potassium is likewise seldom limiting due to its prevalence in mineral soils. To increase biomass production, then, it would seem ideal to increase phosphorus concentrations to approach ideal co-limited natural ratios, such as those proposed by Redfield (1958) or Koerselman and Meuleman (1996). This formed the basis of the exploration conducted in this thesis.

Although, in this study, differences in biomass production along an ammonia gradient with N:P ratio manipulation failed to produce reliably significant results, certain conclusions regarding the effects of high ammonia treatments and the N:P ratio on biomass may be made from the studies conducted. In the initial study, where no modification of the N:P ratio was conducted, convex curves indicating the response of both mesocosms and individual species to increasing ammonia concentrations indicates that ammonia has a fertilization effect at low to moderate concentrations, and an inhibitory effect at high concentrations (Figures 2.3, 2.6, and 2.9). The maximum of total biomass production for a mesocosm of *Juncus* and *Typha* planted together occurred at between 75 mgL^{-1} and 150 mgL^{-1}, with the patterns of treatment effect of above- and below-ground biomass being similar. Additions of phosphate to create co-limited systems did not result in a statistically significant change in biomass production due to the low sample size of N:P ratio treatments within ammonia treatments (n=3), but served to illustrate a pattern of highest production at phytotoxic nitrogen concentrations at an N:P level of 15:1 (Figures 3.2, 3.5 and 3.8). By planting these species in mesocosms, a better idea of field-level ATW functioning was achieved by noting effects of the system as a whole; an ammonia regime of less than 150 mgL^{-1} is suggested due to impaired system functioning above that level.

When applied to mesocosms of *Typha* and *Juncus* planted together, ammonia concentrations forced a shift in biomass towards dominance by *Juncus* at all treatment concentrations. This indication seems novel; dry-matter production by *Typha* tends to be significantly higher than other species. In fact, in most cases, *Typha* tends to be assumed as a highly competitive species (Grace and Wetzel 1981, Tilman 1987, Svengsouk and Mitsch 2000). Despite the warnings of ATW planting literature not to plant monocultures of cattails (SCS 1991, Kadlec and Knight 1996), here *Typha* was seriously out-produced by *Juncus* at all treatment concentrations. The fact that *Juncus* was less inhibited at higher concentrations is in accordance with other researchers (Hill *et al.* 1997, Clarke 1999, Emery *et al.* 2001., Clarke and Baldwin 2002); the interactive effects of phytotoxic gradients of ammonia and N:P ratios on species planted in polyculture have yet to be tested in great detail. Work on polycultures in treatment wetlands has focused on the effects of additions of single levels of N, P and co-limiting N and P, or on the effects of asymmetric-density plantings on the dominance of *Typha* (Svengsouk and Mitsch 2000). These findings may serve as a bench-level test of the trade-off between competitive performance and stress tolerance noted elsewhere (Grace 1981, Clarke 1999, Emery *et al.* 2001, Clarke and Baldwin 2002); in cases where stress tolerance is essential in supporting biomass production, species which are highly competitive will perform poorly. With regard to management issues, planting *Juncus* in ATWs known to have high nitrogen loading may be preferable for sustained biomass production.

Statistically significant results for biomass production or tissue nitrogen concentration were shown only in a few instances by altering the N:P ratio for either species. There is some indication based on the pattern of biomass production that *Juncus* and *Typha* will both produce more biomass when N:P ratios are adjusted to co-limitation, especially under higher ammonia treatments (Figure 3.2). However, there is also evidence that tissue nitrogen concentrations will decline if a 15:1 N:P ratio is used (Figure 3.14).

The fact that non-significant results were shown for alterations in the N:P ratio in these species is of special import due to the significance effect of species on both N uptake and P uptake. *Juncus* stores less nitrogen in the tissue, and therefore has a higher N:P ratio at 12.0 than *Typha* at 9.1. This is evident in the morphology of the two species as well; with a large rootstock, *Typha* stores nutrients in the tissue for times of resource shortage, whereas *Juncus* does not. Although other authors have noted that differences in tissue N:P ratios do not indicate dominance, these studies have tended to occur in low-nutrient environments. In ATWs that are dominated by high nitrogen concentrations, those species which can sequester less N in the tissue may tolerate concentrations which other species which cannot. The management implication in ATWs receiving high N inputs may be to plant species without large rootstocks (such as *Juncus*, with its smaller corm), thereby minimizing storage and accumulation of phytotoxic levels of tissue nitrogen.

Typically, N:P ratios tend to differ between species, whereas N uptake and P uptake are dependant on differences in major phenotypic groups or ecological boundaries (McJannet *et al.* 1995, Gusewell *et al.* 2003). In this study, N:P ratios did differ based on species. Nitrogen and phosphorus uptake was dependant on species as well, despite the ecological and phenotypic type similarity of the species used. Both species occur in shallow water as emergent wetland clonal graminoids. In systems of high stress, perhaps it is the ability to exclude a phytotoxic chemical than to uptake a limiting one that sustains growth. It would be of interest to test the N:P ratios and N and P uptake parameters of field-scale ATWs to determine whether this trend is consistent.

The N:P ratio has been used in the past to predict nutrient limitations as according to Koerselman and Meuleman (1996); N:P ratios over 16:1 indicate P limitation, and under 14:1 indicate N limitation. In this case, most N:P ratios fell under 14:1, indicating N limitation. The implications of this are twofold. Because N:P ratios were below 14:1 for most treatments, even in experimental abundance of N (at N:P ratios of 25:1), a problem with nitrogen uptake is to blame. This physiologic response indicates an impairment of the mechanism for nitrogen uptake at phytotoxic N concentrations. The down-regulation of ammonia-N uptake in this case is significant in that this is the first occasion in which it is noted; there is an upper limit to the amount of nitrogen able to be fixed in the biomass regardless of availability of other co-limiting nutrients. Secondly, fertilization effects of phosphorus do not alleviate phytotoxic N regimes, because of limitations in the amount of nitrogen that can be moved through plant biomass. This infers that the phytotoxicity of ammonia-N to plants is due to absolute concentrations and is not able to be manipulated by experimental methods, such as by increasing the availability of phosphorus. Implications of these findings are such that managers should limit the concentration of ammonia-N in the system at all times, and that potential phytotoxic effects are independent of concentrations of co-limiting nutrients.

The utility of the N:P ratio in determining nutrient limitations in ATWs is therefore suspect due to high nitrogen concentrations endemic to such systems. Koerselman and Meuleman (1996) and Gusewell *et al.* (2003) indicate potential limitations to the use of the N:P ratio in determining nutrient availability in systems due to differences in within-species N:P ratios. Tables 3.6 and 3.12 show this species effect on tissue nitrogen concentration and tissue phosphorus concentration, respectively. Measured tissue nitrogen and tissue phosphorus concentrations corresponded primarily with ammonia concentration and species, and to a lesser extent the experimental N:P ratios. Individual species N uptake and P uptake differ significantly, as do differences in the overall N:P ratio between species. This implies that in field-scale research, biomass from a particular ATW may not be pooled to determine environmental limitations. In management implications, however, the absolute concentration of N probably affects biomass to a greater degree than nutrient limitations.

Tests of the ecological and environmental aspects of agricultural treatment wetlands are a new trend in biological research. While appropriate engineering of the structural and fluid properties of ATWs is of great importance, a major “black box” in ATW design is the impact of plants to the system. The effects of plants towards increasing nitrification rates (and subsequently denitrification rates due to coupled reactions) by increasing substrate oxidation are well known, but management decisions necessary to maintain and increase biomass production in species found in ATWs must be based on research as to the limiting conditions imposed by chemical parameters of ATWs. The adverse effects of high ammonia levels are but a single example of the difficulties imposed by extremely eutrophic settings; eutrophication on a fertilization level in natural systems tends to inhibit processes seen as environmentally valuable. Field scale experimentation with the elevated ammonia concentrations typical of ATWs should be the next step in understanding the effects of high levels of nutrients on the biomass and functioning of wetland macrophytes.

APPENDIX I

SAS CODE FOR CHAPTER II STATISTICAL ANALYSES

```
quit;
options ls=80 ps=50 center nodate pageno=1;
title1 2002 data;
data total;
input column$ row$ ammonia$ species$ type$   biomass;
datalines;
A    1    300  TY   a    24.2
A    1    300  TY   b    1.2
A    1    300  TY   tot  25.4
A    1    300  JU   a    157.4
A    1    300  JU   b    193.6
A    1    300  JU   tot  351
A    1    300  tot  tot  376.4
A    1    300  tot  b    194.8
A    1    300  tot  a    181.6
A    2    150  TY   a    96
A    2    150  TY   b    90
A    2    150  TY   tot  186
A    2    150  JU   a    154.2
A    2    150  JU   b    92.3
A    2    150  JU   tot  246.5
A    2    150  tot  tot  432.5
A    2    150  tot  b    182.3
A    2    150  tot  a    250.2
A    3    225  TY   a    105
A    3    225  TY   b    68.6
A    3    225  TY   tot  173.6
A    3    225  JU   a    137.3
A    3    225  JU   b    86.8
A    3    225  JU   tot  224.1
A    3    225  tot  tot  397.7
A    3    225  tot  b    155.4
A    3    225  tot  a    242.3
A    4    0    TY   a    8.3
A    4    0    TY   b    33.5
A    4    0    TY   tot  41.8
A    4    0    JU   a    78.5
A    4    0    JU   b    85.6
A    4    0    JU   tot  164.1
A    4    0    tot  tot  205.9
A    4    0    tot  b    119.1
A    4    0    tot  a    86.8
A    5    75   TY   a    131.2
```

A	5	75	TY	b	156.9
A	5	75	TY	tot	288.1
A	5	75	JU	a	201
A	5	75	JU	b	120.7
A	5	75	JU	tot	321.7
A	5	75	tot	tot	609.8
A	5	75	tot	b	277.6
A	5	75	tot	a	332.2
A	6	225	TY	a	132.6
A	6	225	TY	b	96.6
A	6	225	TY	tot	229.2
A	6	225	JU	a	185.2
A	6	225	JU	b	228.3
A	6	225	JU	tot	413.5
A	6	225	tot	tot	642.7
A	6	225	tot	b	324.9
A	6	225	tot	a	317.8
A	7	75	TY	a	85.2
A	7	75	TY	b	85.9
A	7	75	TY	tot	171.1
A	7	75	JU	a	312.8
A	7	75	JU	b	199.9
A	7	75	JU	tot	512.7
A	7	75	tot	tot	683.8
A	7	75	tot	b	285.8
A	7	75	tot	a	398
A	8	300	TY	a	40.4
A	8	300	TY	b	9.4
A	8	300	TY	tot	49.8
A	8	300	JU	a	208.1
A	8	300	JU	b	143.1
A	8	300	JU	tot	351.2
A	8	300	tot	tot	401
A	8	300	tot	b	152.5
A	8	300	tot	a	248.5
A	9	0	TY	a	52.2
A	9	0	TY	b	88
A	9	0	TY	tot	140.2
A	9	0	JU	a	156
A	9	0	JU	b	139.3
A	9	0	JU	tot	295.3
A	9	0	tot	tot	435.5
A	9	0	tot	b	227.3
A	9	0	tot	a	208.2
A	10	150	TY	a	149.4
A	10	150	TY	b	152.8
A	10	150	TY	tot	302.2

```
A    10   150  JU   a    214.3
A    10   150  JU   b    248.6
A    10   150  JU   tot  462.9
A    10   150  tot  tot  765.1
A    10   150  tot  b    401.4
A    10   150  tot  a    363.7
B    1    150  TY   a    152.8
B    1    150  TY   b    78.7
B    1    150  TY   tot  231.5
B    1    150  JU   a    126.4
B    1    150  JU   b    79.9
B    1    150  JU   tot  206.3
B    1    150  tot  tot  437.8
B    1    150  tot  b    158.6
B    1    150  tot  a    279.2
B    2    75   TY   a    65.1
B    2    75   TY   b    118.8
B    2    75   TY   tot  183.9
B    2    75   JU   a    212.5
B    2    75   JU   b    133.8
B    2    75   JU   tot  346.3
B    2    75   tot  tot  530.2
B    2    75   tot  b    252.6
B    2    75   tot  a    277.6
B    3    0    TY   a    16.4
B    3    0    TY   b    73.3
B    3    0    TY   tot  89.7
B    3    0    JU   a    79.9
B    3    0    JU   b    80.5
B    3    0    JU   tot  160.4
B    3    0    tot  tot  250.1
B    3    0    tot  b    153.8
B    3    0    tot  a    96.3
B    4    300  TY   a    75.5
B    4    300  TY   b    34.7
B    4    300  TY   tot  110.2
B    4    300  JU   a    113
B    4    300  JU   b    62.5
B    4    300  JU   tot  175.5
B    4    300  tot  tot  285.7
B    4    300  tot  b    97.2
B    4    300  tot  a    188.5
B    5    225  TY   a    91.9
B    5    225  TY   b    32.7
B    5    225  TY   tot  124.6
B    5    225  JU   a    179.2
B    5    225  JU   b    73.7
```

B	5	225	JU	tot	252.9
B	5	225	tot	tot	377.5
B	5	225	tot	b	106.4
B	5	225	tot	a	271.1
B	6	300	TY	a	34.8
B	6	300	TY	b	16.9
B	6	300	TY	tot	51.7
B	6	300	JU	a	232.1
B	6	300	JU	b	92.2
B	6	300	JU	tot	324.3
B	6	300	tot	tot	376
B	6	300	tot	b	109.1
B	6	300	tot	a	266.9
B	7	0	TY	a	9.7
B	7	0	TY	b	73.5
B	7	0	TY	tot	83.2
B	7	0	JU	a	65.2
B	7	0	JU	b	91.5
B	7	0	JU	tot	156.7
B	7	0	tot	tot	239.9
B	7	0	tot	b	165
B	7	0	tot	a	74.9
B	8	150	TY	a	148
B	8	150	TY	b	126.3
B	8	150	TY	tot	274.3
B	8	150	JU	a	197.1
B	8	150	JU	b	107.3
B	8	150	JU	tot	304.4
B	8	150	tot	tot	578.7
B	8	150	tot	b	233.6
B	8	150	tot	a	345.1
B	9	75	TY	a	43.3
B	9	75	TY	b	76.8
B	9	75	TY	tot	120.1
B	9	75	JU	a	237.6
B	9	75	JU	b	131.7
B	9	75	JU	tot	369.3
B	9	75	tot	tot	489.4
B	9	75	tot	b	208.5
B	9	75	tot	a	280.9
B	10	225	TY	a	136.7
B	10	225	TY	b	55
B	10	225	TY	tot	191.7
B	10	225	JU	a	148.4
B	10	225	JU	b	90.2
B	10	225	JU	tot	238.6
B	10	225	tot	tot	430.3

B	10	225	tot	b	145.2
B	10	225	tot	a	285.1
C	1	75	TY	a	138.1
C	1	75	TY	b	164.5
C	1	75	TY	tot	302.6
C	1	75	JU	a	122.6
C	1	75	JU	b	46.9
C	1	75	JU	tot	169.5
C	1	75	tot	tot	472.1
C	1	75	tot	b	211.4
C	1	75	tot	a	260.7
C	2	300	TY	a	16.8
C	2	300	TY	b	9.8
C	2	300	TY	tot	26.6
C	2	300	JU	a	178.2
C	2	300	JU	b	66.4
C	2	300	JU	tot	244.6
C	2	300	tot	tot	271.2
C	2	300	tot	b	76.2
C	2	300	tot	a	195
C	3	150	TY	a	101.4
C	3	150	TY	b	106.1
C	3	150	TY	tot	207.5
C	3	150	JU	a	185.2
C	3	150	JU	b	62
C	3	150	JU	tot	247.2
C	3	150	tot	tot	454.7
C	3	150	tot	b	168.1
C	3	150	tot	a	286.6
C	4	225	TY	a	107.7
C	4	225	TY	b	41
C	4	225	TY	tot	148.7
C	4	225	JU	a	182.1
C	4	225	JU	b	65.6
C	4	225	JU	tot	247.7
C	4	225	tot	tot	396.4
C	4	225	tot	b	106.6
C	4	225	tot	a	289.8
C	5	0	TY	a	4.1
C	5	0	TY	b	21.5
C	5	0	TY	tot	25.6
C	5	0	JU	a	75.5
C	5	0	JU	b	70
C	5	0	JU	tot	145.5
C	5	0	tot	tot	171.1
C	5	0	tot	b	91.5
C	5	0	tot	a	79.6

C	6	75	TY	a	81.6
C	6	75	TY	b	89.2
C	6	75	TY	tot	170.8
C	6	75	JU	a	259
C	6	75	JU	b	123.8
C	6	75	JU	tot	382.8
C	6	75	tot	tot	553.6
C	6	75	tot	b	213
C	6	75	tot	a	340.6
C	7	150	TY	a	98.1
C	7	150	TY	b	61
C	7	150	TY	tot	159.1
C	7	150	JU	a	215.9
C	7	150	JU	b	102.4
C	7	150	JU	tot	318.3
C	7	150	tot	tot	477.4
C	7	150	tot	b	163.4
C	7	150	tot	a	314
C	8	225	TY	a	80.4
C	8	225	TY	b	53.8
C	8	225	TY	tot	134.2
C	8	225	JU	a	190
C	8	225	JU	b	81.2
C	8	225	JU	tot	271.2
C	8	225	tot	tot	405.4
C	8	225	tot	b	135
C	8	225	tot	a	270.4
C	9	300	TY	a	25.8
C	9	300	TY	b	69.3
C	9	300	TY	tot	95.1
C	9	300	JU	a	166.7
C	9	300	JU	b	6.9
C	9	300	JU	tot	173.6
C	9	300	tot	tot	268.7
C	9	300	tot	b	76.2
C	9	300	tot	a	192.5
C	10	0	TY	a	29
C	10	0	TY	b	94.7
C	10	0	TY	tot	123.7
C	10	0	JU	a	62.7
C	10	0	JU	b	75.4
C	10	0	JU	tot	138.1
C	10	0	tot	tot	261.8
C	10	0	tot	b	170.1
C	10	0	tot	a	91.7
D	1	225	TY	a	41.8
D	1	225	TY	b	9.3

D	1	225	TY	tot	51.1
D	1	225	JU	a	159.9
D	1	225	JU	b	55.4
D	1	225	JU	tot	215.3
D	1	225	tot	tot	266.4
D	1	225	tot	b	64.7
D	1	225	tot	a	201.7
D	2	0	TY	a	21.1
D	2	0	TY	b	21.7
D	2	0	TY	tot	42.8
D	2	0	JU	a	63.9
D	2	0	JU	b	74.8
D	2	0	JU	tot	138.7
D	2	0	tot	tot	181.5
D	2	0	tot	b	96.5
D	2	0	tot	a	85
D	3	75	TY	a	52.5
D	3	75	TY	b	59.5
D	3	75	TY	tot	112
D	3	75	JU	a	224.5
D	3	75	JU	b	64.4
D	3	75	JU	tot	288.9
D	3	75	tot	tot	400.9
D	3	75	tot	b	123.9
D	3	75	tot	a	277
D	4	150	TY	a	106
D	4	150	TY	b	91.7
D	4	150	TY	tot	197.7
D	4	150	JU	a	137.4
D	4	150	JU	b	95.9
D	4	150	JU	tot	233.3
D	4	150	tot	tot	431
D	4	150	tot	b	187.6
D	4	150	tot	a	243.4
D	5	300	TY	a	6.6
D	5	300	TY	b	1.2
D	5	300	TY	tot	7.8
D	5	300	JU	a	201.2
D	5	300	JU	b	82.7
D	5	300	JU	tot	283.9
D	5	300	tot	tot	291.7
D	5	300	tot	b	83.9
D	5	300	tot	a	207.8
D	6	150	TY	a	113
D	6	150	TY	b	112.5
D	6	150	TY	tot	225.5
D	6	150	JU	a	120.9

D	6	150	JU	b	100.3
D	6	150	JU	tot	221.2
D	6	150	tot	tot	446.7
D	6	150	tot	b	212.8
D	6	150	tot	a	233.9
D	7	300	TY	a	16.9
D	7	300	TY	b	5.2
D	7	300	TY	tot	22.1
D	7	300	JU	a	185.9
D	7	300	JU	b	83.7
D	7	300	JU	tot	269.6
D	7	300	tot	tot	291.7
D	7	300	tot	b	88.9
D	7	300	tot	a	202.8
D	8	0	TY	a	32.3
D	8	0	TY	b	34.7
D	8	0	TY	tot	67
D	8	0	JU	a	30.1
D	8	0	JU	b	41.7
D	8	0	JU	tot	71.8
D	8	0	tot	tot	138.8
D	8	0	tot	b	76.4
D	8	0	tot	a	62.4
D	9	225	TY	a	76.7
D	9	225	TY	b	49.1
D	9	225	TY	tot	125.8
D	9	225	JU	a	189.2
D	9	225	JU	b	65.3
D	9	225	JU	tot	254.5
D	9	225	tot	tot	380.3
D	9	225	tot	b	114.4
D	9	225	tot	a	265.9
D	10	75	TY	a	6.8
D	10	75	TY	b	5.3
D	10	75	TY	tot	12.1
D	10	75	JU	a	296.8
D	10	75	JU	b	197.9
D	10	75	JU	tot	494.7
D	10	75	tot	tot	506.8
D	10	75	tot	b	203.2
D	10	75	tot	a	303.6
E	1	0	TY	a	10
E	1	0	TY	b	38.9
E	1	0	TY	tot	48.9
E	1	0	JU	a	63.4
E	1	0	JU	b	83.3
E	1	0	JU	tot	146.7

E	1	0	tot	tot	195.6
E	1	0	tot	b	122.2
E	1	0	tot	a	73.4
E	2	225	TY	a	0.3
E	2	225	TY	b	2.6
E	2	225	TY	tot	2.9
E	2	225	JU	a	224.1
E	2	225	JU	b	95.2
E	2	225	JU	tot	319.3
E	2	225	tot	tot	322.2
E	2	225	tot	b	97.8
E	2	225	tot	a	224.4
E	3	300	TY	a	40.5
E	3	300	TY	b	28.9
E	3	300	TY	tot	69.4
E	3	300	JU	a	172.1
E	3	300	JU	b	131.4
E	3	300	JU	tot	303.5
E	3	300	tot	tot	372.9
E	3	300	tot	b	160.3
E	3	300	tot	a	212.6
E	4	75	TY	a	21.9
E	4	75	TY	b	15.8
E	4	75	TY	tot	37.7
E	4	75	JU	a	274
E	4	75	JU	b	41.5
E	4	75	JU	tot	315.5
E	4	75	tot	tot	353.2
E	4	75	tot	b	57.3
E	4	75	tot	a	295.9
E	5	150	TY	a	125.4
E	5	150	TY	b	136.7
E	5	150	TY	tot	262.1
E	5	150	JU	a	104.7
E	5	150	JU	b	39.7
E	5	150	JU	tot	144.4
E	5	150	tot	tot	406.5
E	5	150	tot	b	176.4
E	5	150	tot	a	230.1
E	6	0	TY	a	18.8
E	6	0	TY	b	126.9
E	6	0	TY	tot	145.7
E	6	0	JU	a	32.2
E	6	0	JU	b	36.2
E	6	0	JU	tot	68.4
E	6	0	tot	tot	214.1
E	6	0	tot	b	163.1

```
E    6    0    tot  a    51
E    7    225  TY   a    35.4
E    7    225  TY   b    18.1
E    7    225  TY   tot  53.5
E    7    225  JU   a    240.1
E    7    225  JU   b    120.3
E    7    225  JU   tot  360.4
E    7    225  tot  tot  413.9
E    7    225  tot  b    138.4
E    7    225  tot  a    275.5
E    8    75   TY   a    161.8
E    8    75   TY   b    161.4
E    8    75   TY   tot  323.2
E    8    75   JU   a    89.8
E    8    75   JU   b    69.5
E    8    75   JU   tot  159.3
E    8    75   tot  tot  482.5
E    8    75   tot  b    230.9
E    8    75   tot  a    251.6
E    9    150  TY   a    70.5
E    9    150  TY   b    111.9
E    9    150  TY   tot  182.4
E    9    150  JU   a    147.2
E    9    150  JU   b    47.9
E    9    150  JU   tot  195.1
E    9    150  tot  tot  377.5
E    9    150  tot  b    159.8
E    9    150  tot  a    217.7
E    10   300  TY   a    24.6
E    10   300  TY   b    3.3
E    10   300  TY   tot  27.9
E    10   300  JU   a    101.8
E    10   300  JU   b    123.1
E    10   300  JU   tot  224.9
E    10   300  tot  tot  252.8
E    10   300  tot  b    126.4
E    10   300  tot  a    126.4
run;
title2 biomass ANOVAs in 2002;
title3 total biomass for both species in 2002 ANOVA;
data total_biomass;
set total;
if species="tot" then delete;
if type="a" then delete;
if type="b" then delete;
run;
proc mixed data=total_biomass;
```

```
class ammonia column species;
model biomass=ammonia|species column/ddfm=kr outp=resids;
*lsmeans ammonia|species / pdiff adjust=tukey;
run;
title4 checks for normality;
proc univariate data=total_biomass normal plot;
var biomass;
run;
          *test shows a Shapiro-Wilk's Test (as S-W test
from now on)
          of 0.97, indicating normality;
proc plot data=resids vpercent=50;
plot resid*pred / vref=0;
quit;
          *shows decent and acceptable homogeneity of
resids;
***********************************************************
******************************;
title3 aboveground biomass for both species in 2002 ANOVA;
data total_above;
set total;
if species="tot" then delete;
if type="tot" then delete;
if type="b" then delete;
run;
proc mixed data=total_above;
class ammonia column species;
model biomass=ammonia|species column/ddfm=kr outp=resids;
*lsmeans ammonia|species / pdiff adjust=tukey;
run;
title4 checks for normality;
proc univariate data=total_above normal plot;
var biomass;
run;
          *test shows a S-W test of 0.96, indicating
normality;
proc plot data=resids vpercent=50;
plot resid*pred / vref=0;
quit;
          *shows decent and acceptable homogeneity of
resids;
***********************************************************
******************************;
title3 belowground biomass for both species in 2002 ANOVA;
data total_below;
set total;
if species="tot" then delete;
```

```
if type="tot" then delete;
if type="a" then delete;
run;
proc mixed data=total_below;
class ammonia column species;
model biomass=ammonia|species column/ddfm=kr outp=resids;
lsmeans ammonia|species / pdiff adjust=tukey;
run;
title4 checks for normality;
proc univariate data=total_below normal plot;
var biomass;
run;
          *test shows a S-W test of 0.96, indicating
normality;
proc plot data=resids vpercent=50;
plot resid*pred / vref=0;
quit;
          *shows decent and acceptable homogeneity of
resids;
***********************************************************
*****************************;
title3 total biomass for TYPHA in 2002 ANOVA;
data typha_total;
set total;
if species="tot" then delete;
if species="JU" then delete;
if type="b" then delete;
if type="a" then delete;
run;
proc mixed data=typha_total;
class ammonia column;
model biomass=ammonia column/ddfm=kr outp=resids;
*lsmeans ammonia / pdiff adjust=tukey;
run;
title4 checks for normality;
proc univariate data=typha_total normal plot;
var biomass;
run;
          *test shows a S-W test of 0.94, indicating
normality;
proc plot data=resids vpercent=50;
plot resid*pred / vref=0;
quit;
          *shows decent and acceptable homogeneity of
resids;
***********************************************************
****************************;
```

```
title3 aboveground biomass for TYPHA in 2002 ANOVA;
data typha_above;
set total;
if species="tot" then delete;
if species="JU" then delete;
if type="tot" then delete;
if type="b" then delete;
run;
proc mixed data=typha_above;
class ammonia column;
model biomass=ammonia column/ddfm=kr outp=resids;
*lsmeans ammonia / pdiff adjust=tukey;
run;
title4 checks for normality;
proc univariate data=typha_above normal plot;
var biomass;
run;
          *test shows a S-W test of 0.92, indicating
normality;
proc plot data=resids vpercent=50;
plot resid*pred / vref=0;
quit;
          *shows decent and acceptable homogeneity of
resids;
***********************************************************
******************************;
title3 belowground biomass for TYPHA in 2002 ANOVA;
data typha_below;
set total;
if species="tot" then delete;
if species="JU" then delete;
if type="tot" then delete;
if type="a" then delete;
run;
proc mixed data=typha_below;
class ammonia column;
model biomass=ammonia column/ddfm=kr outp=resids;
*lsmeans ammonia / pdiff adjust=tukey;
run;
title4 checks for normality;
proc univariate data=typha_below normal plot;
var biomass;
run;
          *test shows a S-W test of 0.94, indicating
normality;
proc plot data=resids vpercent=50;
plot resid*pred / vref=0;
```

```
quit;
          *shows decent and acceptable homogeneity of
resids;
***********************************************************
******************************;
title3 total biomass for JUNCUS in 2002 ANOVA;
data juncus_total;
set total;
if species="tot" then delete;
if species="TY" then delete;
if type="b" then delete;
if type="a" then delete;
run;proc mixed data=juncus_total;
class ammonia column;
model biomass=ammonia column/ddfm=kr outp=resids;
*lsmeans ammonia / pdiff adjust=tukey;
run;
title4 checks for normality;
proc univariate data=juncus_total normal plot;
var biomass;
run;
          *test shows a S-W test of 0.97, indicating
normality;
proc plot data=resids vpercent=50;
plot resid*pred / vref=0;
quit;
          *shows decent and acceptable homogeneity of
resids;
***********************************************************
******************************;
title3 aboveground biomass for JUNCUS in 2002 ANOVA;
data juncus_above;
set total;
if species="tot" then delete;
if species="TY" then delete;
if type="tot" then delete;
if type="b" then delete;
run;
proc mixed data=juncus_above;
class ammonia column;
model biomass=ammonia column/ddfm=kr outp=resids;
*lsmeans ammonia / pdiff adjust=tukey;
run;
title4 checks for normality;
proc univariate data=juncus_above normal plot;
var biomass;
run;
```

```
          *test shows a S-W test of 0.98, indicating
normality;
proc plot data=resids vpercent=50;
plot resid*pred / vref=0;
quit;
          *shows decent and acceptable homogeneity of
resids;
***********************************************************
******************************;
title3 belowground biomass for JUNCUS in 2002 ANOVA;
data juncus_below;
set total;
if species="tot" then delete;
if species="TY" then delete;
if type="tot" then delete;
if type="a" then delete;
run;proc mixed data=juncus_below;
class ammonia column;
model biomass=ammonia column/ddfm=kr outp=resids;
*lsmeans ammonia / pdiff adjust=tukey;
run;
title4 checks for normality;
proc univariate data=total_below normal plot;
var biomass;
run;
          *test shows a S-W test of 0.96, indicating
normality;
proc plot data=resids vpercent=50;
plot resid*pred / vref=0;
quit;
          *shows decent and acceptable homogeneity of
resids;


***********************************************************
**********************
Here ends the biomass ANOVA
series...............................................
***********************************************************
*********************;


title2 root-to-shoot ratio ANOVAs in 2002;
data rs_ratio;
input column$ row$ ammonia$ species$ r_s;
log_r_s=log(r_s);
datalines;
```

A	4	0	t	4.036144578
A	9	0	t	1.685823755
B	3	0	t	4.469512195
B	7	0	t	7.577319588
C	5	0	t	5.243902439
C	10	0	t	3.265517241
D	2	0	t	1.028436019
D	8	0	t	1.074303406
E	1	0	t	3.89
E	6	0	t	6.75
A	5	75	t	1.195884146
A	7	75	t	1.008215962
B	2	75	t	1.824884793
B	9	75	t	1.773672055
C	1	75	t	1.191165822
C	6	75	t	1.093137255
D	3	75	t	1.133333333
D	10	75	t	0.779411765
E	4	75	t	0.721461187
E	8	75	t	0.997527812
A	2	150	t	0.9375
A	10	150	t	1.022757697
B	1	150	t	0.515052356
B	8	150	t	0.853378378
C	3	150	t	1.046351085
C	7	150	t	0.621814475
D	4	150	t	0.86509434
D	6	150	t	0.995575221
E	5	150	t	1.090111643
E	9	150	t	1.587234043
A	3	225	t	0.653333333
A	6	225	t	0.728506787
B	5	225	t	0.355821545
B	10	225	t	0.402340892
C	4	225	t	0.380687094
C	8	225	t	0.669154229
D	1	225	t	0.222488038
D	9	225	t	0.640156454
E	2	225	t	7.789095267
E	7	225	t	0.511299435
A	1	300	t	0.049586777
A	8	300	t	0.232673267
B	4	300	t	0.459602649
B	6	300	t	0.485632184
C	2	300	t	0.583333333
C	9	300	t	2.686046512
D	5	300	t	0.181818182

D	7	300	t	0.307692308
E	3	300	t	0.713580247
E	10	300	t	0.134146341
A	4	0	j	1.09044586
A	9	0	j	0.892948718
B	3	0	j	1.007509387
B	7	0	j	1.403374233
C	5	0	j	0.927152318
C	10	0	j	1.202551834
D	2	0	j	1.17057903
D	8	0	j	1.38538206
E	1	0	j	1.313880126
E	6	0	j	1.124223602
A	5	75	j	0.600497512
A	7	75	j	0.639066496
B	2	75	j	0.629647059
B	9	75	j	0.554292929
C	1	75	j	0.382544861
C	6	75	j	0.477992278
D	3	75	j	0.286859688
D	10	75	j	0.666778976
E	4	75	j	0.151459854
E	8	75	j	0.773942094
A	2	150	j	0.598573281
A	10	150	j	1.160055996
B	1	150	j	0.632120253
B	8	150	j	0.544393709
C	3	150	j	0.334773218
C	7	150	j	0.474293654
D	4	150	j	0.697962154
D	6	150	j	0.829611249
E	5	150	j	0.379178606
E	9	150	j	0.325407609
A	3	225	j	0.63219228
A	6	225	j	1.232721382
B	5	225	j	0.411272321
B	10	225	j	0.607816712
C	4	225	j	0.360241625
C	8	225	j	0.427368421
D	1	225	j	0.346466542
D	9	225	j	0.345137421
E	2	225	j	0.424810353
E	7	225	j	0.501041233
A	1	300	j	1.229987294
A	8	300	j	0.687650168
B	4	300	j	0.553097345
B	6	300	j	0.397242568

```
C    2    300  j    0.372615039
C    9    300  j    0.041391722
D    5    300  j    0.411033797
D    7    300  j    0.450242066
E    3    300  j    0.763509587
E    10   300  j    1.209233792
run;
title3 root-to-shoot ratios for mesocosms in 2002 ANOVA;
proc mixed data=rs_ratio;
class ammonia column species;
model log_r_s=ammonia|species column / ddfm=kr outp=resids;
*lsmeans ammonia|species / pdiff adjust=tukey;
run;
title4 checks for normality;
proc univariate data=rs_ratio normal plot;
where species='t';
var log_r_s;
run;
          *test shows a S-W test of 0.97, indicating
acceptable normality;
proc plot data=resids vpercent=50;
plot resid*pred / vref=0;
quit;
          *shows decent and acceptable homogenity of resids
after log-transform;
***********************************************************
******************************;
title3 root-to-shoot ratios for TYPHA in 2002 ANOVA;
proc mixed data=rs_ratio;
where species='t';
class ammonia column;
model log_r_s=ammonia column/ ddfm=kr outp=resids;
*lsmeans ammonia / pdiff adjust=tukey;
run;
title4 checks for normality;
proc univariate data=rs_ratio normal plot;
where species='t';
var log_r_s;
run;
          *test shows a S-W test of 0.97, indicating
acceptable normality;
proc plot data=resids vpercent=50;
plot resid*pred / vref=0;
quit;
          *shows decent and acceptable homogeneity of
resids;
```

```
***********************************************************
*****************************;
title3 root-to-shoot ratio for JUNCUS in 2002 ANOVA;
proc mixed data=rs_ratio;
where species='j';
class ammonia column;
model r_s=ammonia column/ ddfm=kr outp=resids;
*lsmeans ammonia / pdiff adjust=tukey;
run;
title4 checks for normality;
proc univariate data=rs_ratio normal plot;
where species='j';
var r_s;
run;
          *test shows a S-W test of 0.93, indicating
normality;
proc plot data=resids vpercent=50;
plot resid*pred / vref=0;
quit;
          *shows decent and acceptable homogeneity of
resids;
quit;
```

APPENDIX II

SAS CODE FOR 2003 EXPERIMENT

```
quit;
options ls=80 ps=50 center nodate pageno=1;
title1 2003 data;
data total;
input column$ row$ ammonia$ n_p$ species$ type$ biomass
n_conc p_conc;
log_biomass=log(biomass);
log_log_biomass=log(log_biomass);
log_n_conc=log(n_conc);
log_p_conc=log(p_conc);
n_mass=n_conc*biomass;
Datalines;
A    1    300  5    ju   a    24.962    1.8905865 130.4
A    1    300  5    ju   b    26.082    1.8905865 48.8
A    1    300  5    ju   tot  25.522    1.8905865 179.2
A    1    300  5    tot  a    25.4485   2.9186226 160.7
A    1    300  5    tot  b    26.165    2.9186226 59.5
A    1    300  5    tot  tot  25.80675  2.9186226 220.2
A    1    300  5    ty   a    25.935    3.946658  30.3
A    1    300  5    ty   b    26.248    3.946658  10.7
A    1    300  5    ty   tot  26.0915   3.946658  41
A    2    300  15   ju   a    26.307    2.0807142 150.8
A    2    300  15   ju   b    26.387    2.0807142 49.4
A    2    300  15   ju   tot  26.347    2.0807142 200.2
A    2    300  15   tot  a    28.3145   3.2900522 167.9
A    2    300  15   tot  b    27.8415   3.2900522 55.2
A    2    300  15   tot  tot  28.078    3.2900522 223.1
A    2    300  15   ty   a    30.322    4.4993902 17.1
A    2    300  15   ty   b    29.296    4.4993905 5.8
A    2    300  15   ty   tot  29.809    4.4993905 22.9
A    3    300  25   ju   a    24.308    1.6025775 162.7
A    3    300  25   ju   b    24.786    1.6025475 60.3
A    3    300  25   ju   tot  24.547    1.6027475 223
A    3    300  25   tot  a    26.2175   2.4452625 176.2
A    3    300  25   tot  b    26.156    2.4224525 66
A    3    300  25   tot  tot  26.18675  2.4222625 242.2
A    3    300  25   ty   a    28.127    3.23305   13.5
A    3    300  25   ty   b    27.526    3.423305  5.7
A    3    300  25   ty   tot  27.8265   3.2423305 19.2
A    4    300  5    ju   a    24.748    1.9672195 154.5
A    4    300  5    ju   b    24.82     1.9672195 41.9
A    4    300  5    ju   tot  24.784    1.9672195 196.4
A    4    300  5    tot  a    24.523    2.6243495 195.2
A    4    300  5    tot  b    24.3965   2.6243495 57.6
A    4    300  5    tot  tot  24.45975  2.6243495 252.8
A    4    300  5    ty   a    24.298    3.2814795 40.7
A    4    300  5    ty   b    23.973    3.2814795 15.7
A    4    300  5    ty   tot  24.1355   3.2814795 56.4
A    5    300  15   ju   a    24.256    1.9063435 147.3
```

A	5	300	15	ju	b	25.073	1.9063435	65.3
A	5	300	15	ju	tot	24.6645	1.9063435	212.6
A	5	300	15	tot	a	.	.	168.4
A	5	300	15	tot	b	.	.	86
A	5	300	15	tot	tot	.	.	254.4
A	5	300	15	ty	a	.	.	21.1
A	5	300	15	ty	b	.	.	20.7
A	5	300	15	ty	tot	.	.	41.8
A	6	300	25	ju	a	22.976	3.0310325	140.8
A	6	300	25	ju	b	24.111	3.0310825	58.7
A	6	300	25	ju	tot	23.5435	3.0313825	199.5
A	6	300	25	tot	a	25.302	3.1684375	157.5
A	6	300	25	tot	b	26.969	3.1364375	65
A	6	300	25	tot	tot	26.1355	3.1684375	222.5
A	6	300	25	ty	a	27.628	3.2423305	16.7
A	6	300	25	ty	b	29.827	3.2423305	6.3
A	6	300	25	ty	tot	28.7275	3.2423305	23
A	7	300	5	ju	a	25.741	2.5417935	111.7
A	7	300	5	ju	b	24.698	2.5417935	44.3
A	7	300	5	ju	tot	25.2195	2.5417935	156
A	7	300	5	tot	a	27.7335	4.3266215	133.3
A	7	300	5	tot	b	27.247	4.3266215	46.2
A	7	300	5	tot	tot	27.49025	4.3266375	179.5
A	7	300	5	ty	a	29.726	6.111449	21.6
A	7	300	5	ty	b	29.796	6.1114495	1.9
A	7	300	5	ty	tot	29.761	6.1114425	23.5
A	8	300	15	ju	a	22.977	3.2256225	128.2
A	8	300	15	ju	b	21.442	3.2256722	53.4
A	8	300	15	ju	tot	22.2095	3.2256725	181.6
A	8	300	15	tot	a	24.748	2.556412	160.9
A	8	300	15	tot	b	.	.	70
A	8	300	15	tot	tot	.	.	230.9
A	8	300	15	ty	a	26.519	1.8871575	32.7
A	8	300	15	ty	b	.	.	16.6
A	8	300	15	ty	tot	13.2595	0.9435587	49.3
A	9	300	25	ju	a	.	.	135.5
A	9	300	25	ju	b	.	.	57.7
A	9	300	25	ju	tot	.	.	193.2
A	9	300	25	tot	a	.	.	158.8
A	9	300	25	tot	b	.	.	62.7
A	9	300	25	tot	tot	.	.	221.5
A	9	300	25	ty	a	27.583	3.0560635	23.3
A	9	300	25	ty	b	25.947	3.0560637	5
A	9	300	25	ty	tot	26.765	3.0560637	28.3
B	1	225	5	ju	a	24.034	3.4510027	176
B	1	225	5	ju	b	25.457	3.4510027	49.2
B	1	225	5	ju	tot	24.7455	3.4510027	225.2
B	1	225	5	tot	a	27.3305	3.6756203	207.1
B	1	225	5	tot	b	27.6575	3.6756203	60.1
B	1	225	5	tot	tot	27.494	3.6756203	267.2
B	1	225	5	ty	a	30.627	3.900238	31.1
B	1	225	5	ty	b	29.858	3.900238	10.9
B	1	225	5	ty	tot	30.2425	3.900238	42
B	2	225	15	ju	a	25.206	1.912815	173.1

B	2	225	15	ju	b	24.367	1.9128145	56.9
B	2	225	15	ju	tot	24.7865	1.9128145	230
B	2	225	15	tot	a	25.2395	2.489521	221
B	2	225	15	tot	b	24.687	2.489521	76.5
B	2	225	15	tot	tot	24.96325	2.489521	297.5
B	2	225	15	ty	a	25.273	3.0662225	47.9
B	2	225	15	ty	b	25.007	3.0662225	19.6
B	2	225	15	ty	tot	25.14	3.0662725	67.5
B	3	225	25	ju	a	23.732	1.485091	147.2
B	3	225	25	ju	b	23.294	1.485091	40.6
B	3	225	25	ju	tot	23.513	1.485091	187.8
B	3	225	25	tot	a	25.5495	1.413439	210.5
B	3	225	25	tot	b	26.0535	1.413439	56.6
B	3	225	25	tot	tot	25.8015	1.413439	267.1
B	3	225	25	ty	a	27.367	1.341787	63.3
B	3	225	25	ty	b	28.813	1.341787	16
B	3	225	25	ty	tot	28.09	1.341787	79.3
B	4	225	5	ju	a	22.277	3.066412	148.2
B	4	225	5	ju	b	23.907	3.066241	54.2
B	4	225	5	ju	tot	23.092	3.066241	202.4
B	4	225	5	tot	a	23.569	3.902149	204.4
B	4	225	5	tot	b	24.4045	3.902149	69.9
B	4	225	5	tot	tot	23.98675	3.902149	274.3
B	4	225	5	ty	a	24.861	4.738056	56.2
B	4	225	5	ty	b	24.902	4.7380565	15.7
B	4	225	5	ty	tot	24.8815	4.7380565	71.9
B	5	225	15	ju	a	23.643	2.2152815	154.5
B	5	225	15	ju	b	23.84	2.2152815	68.5
B	5	225	15	ju	tot	23.7415	2.2152815	223
B	5	225	15	tot	a	.	.	199.3
B	5	225	15	tot	b	.	.	90
B	5	225	15	tot	tot	.	.	289.3
B	5	225	15	ty	a	.	.	44.8
B	5	225	15	ty	b	.	.	21.5
B	5	225	15	ty	tot	.	.	66.3
B	6	225	25	ju	a	21.292	1.940391	171.7
B	6	225	25	ju	b	20.466	1.9403915	61.9
B	6	225	25	ju	tot	20.879	1.9403915	233.6
B	6	225	25	tot	a	21.9785	1.8030022	204.6
B	6	225	25	tot	b	20.9655	1.8030021	77.4
B	6	225	25	tot	tot	21.472	1.8030021	282
B	6	225	25	ty	a	22.665	1.665613	32.9
B	6	225	25	ty	b	21.465	1.665613	15.5
B	6	225	25	ty	tot	22.065	1.665613	48.4
B	7	225	5	ju	a	.	.	150.3
B	7	225	5	ju	b	.	.	58.4
B	7	225	5	ju	tot	.	.	208.7
B	7	225	5	tot	a	.	.	204.5
B	7	225	5	tot	b	.	.	79.6
B	7	225	5	tot	tot	.	.	284.1
B	7	225	5	ty	a	39.805	6.0588082	54.2
B	7	225	5	ty	b	43.098	6.0588082	21.2
B	7	225	5	ty	tot	41.4515	6.0588082	75.4
B	8	225	15	ju	a	.	.	165.2

B	8	225	15	ju	b	.	.	79.6
B	8	225	15	ju	tot	.	.	244.8
B	8	225	15	tot	a	.	.	209
B	8	225	15	tot	b	.	.	94.4
B	8	225	15	tot	tot	.	.	303.4
B	8	225	15	ty	a	34.325	4.6163167	43.8
B	8	225	15	ty	b	36.341	4.6163167	14.8
B	8	225	15	ty	tot	35.333	4.6163167	58.6
B	9	225	25	ju	a	24.55	1.7548872	154.5
B	9	225	25	ju	b	24.146	1.7548872	85.6
B	9	225	25	ju	tot	24.348	1.7548872	240.1
B	9	225	25	tot	a	25.8425	2.4533627	174.8
B	9	225	25	tot	b	26.9565	2.4533627	96.3
B	9	225	25	tot	tot	26.3995	2.4533627	271.1
B	9	225	25	ty	a	27.135	3.1518382	20.3
B	9	225	25	ty	b	29.767	3.1518382	10.7
B	9	225	25	ty	tot	28.451	3.1518382	31
C	1	150	5	ju	a	19.791	2.6988897	181.3
C	1	150	5	ju	b	20.576	2.6988897	48.8
C	1	150	5	ju	tot	20.1835	2.6988897	230.1
C	1	150	5	tot	a	23.106	3.1970976	267.4
C	1	150	5	tot	b	26.5915	3.1970976	81.5
C	1	150	5	tot	tot	24.84875	3.1970976	348.9
C	1	150	5	ty	a	26.421	3.6953055	86.1
C	1	150	5	ty	b	32.607	3.6953055	32.7
C	1	150	5	ty	tot	29.514	3.6953055	118.8
C	2	150	15	ju	a	10.747	2.127868	177.9
C	2	150	15	ju	b	11.452	2.1278682	48.1
C	2	150	15	ju	tot	11.0995	2.1278682	226
C	2	150	15	tot	a	16.731	2.0677111	251
C	2	150	15	tot	b	16.553	2.0677111	84.7
C	2	150	15	tot	tot	16.642	2.0677111	335.7
C	2	150	15	ty	a	22.715	2.007554	73.1
C	2	150	15	ty	b	21.654	2.007554	36.6
C	2	150	15	ty	tot	22.1845	2.007554	109.7
C	3	150	25	ju	a	27.115	1.568104	190.1
C	3	150	25	ju	b	27.065	1.5681042	53.8
C	3	150	25	ju	tot	27.09	1.5681042	243.9
C	3	150	25	tot	a	24.027	1.5448716	261
C	3	150	25	tot	b	24.5085	1.5448716	85.7
C	3	150	25	tot	tot	24.26775	1.5448716	346.7
C	3	150	25	ty	a	20.939	1.521639	70.9
C	3	150	25	ty	b	21.952	1.521639	31.9
C	3	150	25	ty	tot	21.4455	1.521639	102.8
C	4	150	5	ju	a	22.208	2.303088	154.5
C	4	150	5	ju	b	23.251	2.3030887	58.4
C	4	150	5	ju	tot	22.7295	2.3030887	212.9
C	4	150	5	tot	a	.	.	232.7
C	4	150	5	tot	b	.	.	113.2
C	4	150	5	tot	tot	.	.	345.9
C	4	150	5	ty	a	.	.	78.2
C	4	150	5	ty	b	.	.	54.8
C	4	150	5	ty	tot	.	.	133
C	5	150	15	ju	a	.	.	172.2

C	5	150	15	ju	b	.	.	49.0
C	5	150	15	ju	tot	.	.	221.2
C	5	150	15	tot	a	.	.	216.4
C	5	150	15	tot	b	.	.	72.9
C	5	150	15	tot	tot	.	.	289.3
C	5	150	15	ty	a	35.744	2.1147337	44.2
C	5	150	15	ty	b	39.911	2.1147337	23.9
C	5	150	15	ty	tot	37.8275	2.1147337	68.1
C	6	150	25	ju	a	21.557	1.8419985	188.7
C	6	150	25	ju	b	21.346	1.8419985	60.3
C	6	150	25	ju	tot	21.4515	1.8419985	249
C	6	150	25	tot	a	.	.	253.9
C	6	150	25	tot	b	.	.	103
C	6	150	25	tot	tot	.	.	356.9
C	6	150	25	ty	a	.	.	65.2
C	6	150	25	ty	b	.	.	42.7
C	6	150	25	ty	tot	.	.	107.9
C	7	150	5	ju	a	23.714	2.372604	193.2
C	7	150	5	ju	b	23.485	2.3726042	51.2
C	7	150	5	ju	tot	23.5995	2.3726045	244.4
C	7	150	5	tot	a	20.354	2.1007837	245.4
C	7	150	5	tot	b	20.3445	2.1007843	74.8
C	7	150	5	tot	tot	20.34925	2.1007843	320.2
C	7	150	5	ty	a	16.994	1.8289645	52.2
C	7	150	5	ty	b	17.204	1.8289645	23.6
C	7	150	5	ty	tot	17.099	1.8289645	75.8
C	8	150	15	ju	a	23.191	1.842798	213.9
C	8	150	15	ju	b	22.878	1.842798	57.5
C	8	150	15	ju	tot	23.0345	1.842798	271.4
C	8	150	15	tot	a	21.0545	1.9610547	277.5
C	8	150	15	tot	b	22.226	1.9610543	90.3
C	8	150	15	tot	tot	21.64025	1.9610543	367.8
C	8	150	15	ty	a	18.918	2.0793107	63.6
C	8	150	15	ty	b	21.574	2.0793107	32.8
C	8	150	15	ty	tot	20.246	2.0793107	96.4
C	9	150	25	ju	a	22.862	1.4387587	178.7
C	9	150	25	ju	b	22.737	1.4387587	66.3
C	9	150	25	ju	tot	22.7995	1.4387587	245
C	9	150	25	tot	a	19.5745	1.2497783	235
C	9	150	25	tot	b	20.487	1.2497783	97.6
C	9	150	25	tot	tot	20.03075	1.2497783	332.6
C	9	150	25	ty	a	16.287	1.060798	56.3
C	9	150	25	ty	b	18.237	1.060798	31.3
C	9	150	25	ty	tot	17.262	1.060798	87.6
D	1	75	5	ju	a	19.251	2.0141755	143.8
D	1	75	5	ju	b	19.787	2.0141755	44.7
D	1	75	5	ju	tot	19.519	2.0141755	188.5
D	1	75	5	tot	a	21.474	1.8410576	262.1
D	1	75	5	tot	b	21.797	1.8410576	115.4
D	1	75	5	tot	tot	21.6355	1.8410576	377.5
D	1	75	5	ty	a	23.697	1.6679397	118.3
D	1	75	5	ty	b	23.807	1.6679397	70.7
D	1	75	5	ty	tot	23.752	1.6679397	189
D	2	75	15	ju	a	15.123	1.9987757	174.7

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D    2    75   15   ju   b    14.433    1.9987757 67.1
D    2    75   15   ju   tot  14.778    1.9987757 241.8
D    2    75   15   tot  a    18.519    1.8620626 258.4
D    2    75   15   tot  b    18.669    1.8620626 130.1
D    2    75   15   tot  tot  18.594    1.8620626 388.5
D    2    75   15   ty   a    21.915    1.7253495 83.7
D    2    75   15   ty   b    22.905    1.7253495 63
D    2    75   15   ty   tot  22.41     1.7253495 146.7
D    3    75   25   ju   a    19.931    1.3594965 199.7
D    3    75   25   ju   b    19.613    1.3594965 75.6
D    3    75   25   ju   tot  19.772    1.3594965 275.3
D    3    75   25   tot  a    20.26     1.5885226 273.2
D    3    75   25   tot  b    19.581    1.5885226 128.8
D    3    75   25   tot  tot  19.9205   1.5885226 402
D    3    75   25   ty   a    20.589    1.817548  73.5
D    3    75   25   ty   b    19.549    1.817548  53.2
D    3    75   25   ty   tot  20.069    1.817548  126.7
D    4    75   5    ju   a    .    .    141.2
D    4    75   5    ju   b    .    .    73.2
D    4    75   5    ju   tot  .    .    214.4
D    4    75   5    tot  a    .    .    235.3
D    4    75   5    tot  b    .    .    144.3
D    4    75   5    tot  tot  .    .    379.6
D    4    75   5    ty   a    12.451    2.508661  94.1
D    4    75   5    ty   b    12.896    2.508661  71.1
D    4    75   5    ty   tot  12.6735   2.508661  165.2
D    5    75   15   ju   a    15.769    2.0187715 100.4
D    5    75   15   ju   b    15.376    2.0187715 66.4
D    5    75   15   ju   tot  15.5725   2.0187715 166.8
D    5    75   15   tot  a    14.22     1.6215755 181.5
D    5    75   15   tot  b    14.123    1.6215755 128.2
D    5    75   15   tot  tot  14.1715   1.6215755 309.7
D    5    75   15   ty   a    12.671    1.2243795 81.1
D    5    75   15   ty   b    12.87     1.2243795 61.8
D    5    75   15   ty   tot  12.7705   1.2243795 142.9
D    6    75   25   ju   a    12.864    1.8440585 159.1
D    6    75   25   ju   b    11.986    1.8440585 75.4
D    6    75   25   ju   tot  12.425    1.8440585 234.5
D    6    75   25   tot  a    14.8415   1.4130683 236
D    6    75   25   tot  b    13.914    1.4130683 118.6
D    6    75   25   tot  tot  14.37775  1.4130683 354.6
D    6    75   25   ty   a    16.819    0.9820785 76.9
D    6    75   25   ty   b    15.842    0.9820785 43.2
D    6    75   25   ty   tot  16.3305   0.9820785 120.1
D    7    75   5    ju   a    20.45     2.0483652 163.6
D    7    75   5    ju   b    19.042    2.0483652 65.8
D    7    75   5    ju   tot  19.746    2.0483652 229.4
D    7    75   5    tot  a    .    .    262
D    7    75   5    tot  b    .    .    129.5
D    7    75   5    tot  tot  .    .    391.5
D    7    75   5    ty   a    .    .    98.4
D    7    75   5    ty   b    .    .    63.7
D    7    75   5    ty   tot  .    .    162.1
D    8    75   15   ju   a    15.147    1.4438872 221.6
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D    8    75   15   ju   b    14.856    1.4438872 99.4
D    8    75   15   ju   tot  15.0015   1.4438872 321
D    8    75   15   tot  a    17.13     1.2500068 246.8
D    8    75   15   tot  b    16.1915   1.2500068 118.8
D    8    75   15   tot  tot  16.66075  1.2500068 365.6
D    8    75   15   ty   a    19.113    1.0561265 25.2
D    8    75   15   ty   b    17.527    1.0561265 19.4
D    8    75   15   ty   tot  18.32     1.0561265 44.6
D    9    75   25   ju   a    21.08     1.316284  164.4
D    9    75   25   ju   b    20.452    1.3162847 74.6
D    9    75   25   ju   tot  20.766    1.3162847 239
D    9    75   25   tot  a    24.529    1.2143286 244.3
D    9    75   25   tot  b    24.844    1.2143286 144
D    9    75   25   tot  tot  24.6865   1.2143286 388.3
D    9    75   25   ty   a    27.978    1.1123725 79.9
D    9    75   25   ty   b    29.236    1.1123725 69.4
D    9    75   25   ty   tot  28.607    1.1123725 149.3

run;
title2 biomass ANOVAs in 2003;
title3 total biomass for both species in 2003 ANOVA;
data total_biomass;
set total;
if species="tot" then delete;
if type="a" then delete;
if type="b" then delete;
run;
proc mixed data=total_biomass;
class ammonia n_p species;
model biomass=species|ammonia|n_p/ddfm=kr outp=resids;
*lsmeans species|ammonia|n_p / pdiff adjust=tukey;
run;
title4 checks for normality;
proc univariate data=total_biomass normal plot;
var biomass;
run;
          *test shows a Shapiro-Wilk's Test (as S-W test
from now on)
               of 0.97, indicating normality;
proc plot data=resids vpercent=50;
plot resid*pred / vref=0;
quit;
          *shows decent and acceptable homogeneity of
resids;
************************************************************
*****************************;
title3 aboveground biomass for both species in 2003 ANOVA;
data total_above;
set total;
if species="tot" then delete;
if type="tot" then delete;
if type="b" then delete;
run;
proc mixed data=total_above;
```

```
class ammonia n_p species;
model biomass=species|ammonia|n_p/ddfm=kr outp=resids;
*lsmeans ammonia n_p*ammonia/ pdiff adjust=tukey;
run;
title4 checks for normality;
proc univariate data=total_above normal plot;
var biomass;
run;
          *test shows a S-W test of 0.97, indicating
normality;
proc plot data=resids vpercent=50;
plot resid*pred / vref=0;
quit;
          *shows decent and acceptable homogeneity of
resids;
************************************************************
****************************;
title3 belowground biomass for both species in 2003 ANOVA;
data total_below;
set total;
if species="tot" then delete;
if type="a" then delete;
if type="tot" then delete;
run;
proc mixed data=total_below;
class ammonia n_p species;
model biomass=species|ammonia|n_p /ddfm=kr outp=resids;
*lsmeans ammonia n_p*ammonia/ pdiff adjust=tukey;
run;
title4 checks for normality;
proc univariate data=total_below normal plot;
var biomass;
run;
          *test shows a S-W test of 0.96, indicating
normality;
proc plot data=resids vpercent=50;
plot resid*pred / vref=0;
quit;
          *shows decent and acceptable homogeneity of
resids;
************************************************************
****************************;
title3 total biomass for TYPHA in 2003 ANOVA;
data typha_total;
set total;
if species="tot" then delete;
if species="ju" then delete;
if type="a" then delete;
if type="b" then delete;
run;
proc mixed data=typha_total;
class ammonia n_p;
model biomass=ammonia|n_p/ddfm=kr outp=resids;
*lsmeans ammonia n_p*ammonia/ pdiff adjust=tukey;
```

```
run;
title4 checks for normality;
proc univariate data=typha_total normal plot;
var biomass;
run;
          *test shows a S-W test of 0.97, indicating
normality;
proc plot data=resids vpercent=50;
plot resid*pred / vref=0;
quit;
          *shows decent homogeneity of resids;
************************************************************
****************************;
title3 aboveground biomass for TYPHA in 2003 ANOVA;
data typha_above;
set total;
if species="tot" then delete;
if species="ju" then delete;
if type="tot" then delete;
if type="b" then delete;
run;
proc mixed data=typha_above;
class ammonia n_p;
model biomass=ammonia|n_p/ddfm=kr outp=resids;
*lsmeans ammonia n_p*ammonia/ pdiff adjust=tukey;
run;
title4 checks for normality;
proc univariate data=typha_above normal plot;
var biomass;
run;
          *test shows a S-W test of 0.98, indicating
normality;
proc plot data=resids vpercent=50;
plot resid*pred / vref=0;
quit;
          *shows decent and acceptable homogeneity of
resids;
************************************************************
****************************;
title3 belowground biomass for TYPHA in 2003 ANOVA;
data typha_below;
set total;
if species="tot" then delete;
if species="ju" then delete;
if type="a" then delete;
if type="tot" then delete;
run;
proc mixed data=typha_below;
class ammonia n_p;
model biomass=ammonia|n_p/ddfm=kr outp=resids;
*lsmeans ammonia n_p*ammonia/ pdiff adjust=tukey;
run;
title4 checks for normality;
proc univariate data=typha_below normal plot;
```

```
var biomass;
run;
          *test shows a S-W test of 0.97, indicating decent
normality;
proc plot data=resids vpercent=50;
plot resid*pred / vref=0;
quit;
          *shows decent and acceptable homogeneity of
resids;
************************************************************
****************************;
title3 total biomass for JUNCUS in 2003 ANOVA;
data juncus_total;
set total;
if species="tot" then delete;
if species="ty" then delete;
if type="a" then delete;
if type="b" then delete;
run;
proc mixed data=juncus_total;
class ammonia n_p;
model biomass=ammonia|n_p/ddfm=kr outp=resids;
*lsmeans ammonia n_p*ammonia/ pdiff adjust=tukey;
run;
title4 checks for normality;
proc univariate data=juncus_total normal plot;
var biomass;
run;
          *test shows a S-W test of 0.87, indicating
normality, supported graphically;
proc plot data=resids vpercent=50;
plot resid*pred / vref=0;
quit;
          *shows decent and acceptable homogeneity of
resids;
************************************************************
****************************;
title3 aboveground biomass for JUNCUS in 2003 ANOVA;
data juncus_above;
set total;
if species="tot" then delete;
if species="ty" then delete;
if type="tot" then delete;
if type="b" then delete;
run;
proc mixed data=juncus_above;
class ammonia n_p;
model biomass=ammonia|n_p/ddfm=kr outp=resids;
*lsmeans ammonia n_p*ammonia/ pdiff adjust=tukey;
run;
title4 checks for normality;
proc univariate data=juncus_above normal plot;
var biomass;
run;
```

```
          *test shows a S-W test of 0.88, indicating
normality, supported graphically;
proc plot data=resids vpercent=50;
plot resid*pred / vref=0;
quit;
          *shows decent and acceptable homogeneity of
resids;
************************************************************
****************************;
title3 belowground biomass for JUNCUS in 2003 ANOVA;
data juncus_below;
set total;
if species="tot" then delete;
if species="ty" then delete;
if type="a" then delete;
if type="tot" then delete;
run;
proc mixed data=juncus_below;
class ammonia n_p;
model biomass=ammonia|n_p/ddfm=kr outp=resids;
*lsmeans ammonia n_p*ammonia/ pdiff adjust=tukey;
run;
title4 checks for normality;
proc univariate data=juncus_below normal plot;
var biomass;
run;
          *test shows a S-W test of 0.88, indicating
normality, supported graphically;
proc plot data=resids vpercent=50;
plot resid*pred / vref=0;
quit;
          *shows decent and acceptable homogeneity of
resids;


************************************************************
****************************
Here ends the biomass ANOVA
series............................................................
************************************************************
**************************;


title2 Tissue Nitrogen Concentration ANOVAs in 2003;
title3 total n_conc for both species in 2003 ANOVA;
data total_n_conc;
set total;
if species="tot" then delete;
if type="a" then delete;
if type="b" then delete;
run;
proc mixed data=total_n_conc;
class ammonia n_p species;
model n_conc=species|ammonia|n_p/ddfm=kr outp=resids;
```

```
*lsmeans species|ammonia|n_p / pdiff adjust=tukey;
run;
title4 checks for normality;
proc univariate data=total_n_conc normal plot;
var n_conc;
run;
          *test shows a Shapiro-Wilk's Test (as S-W test
from now on)
               of 0.88, indicating normality;
proc plot data=resids vpercent=50;
plot resid*pred / vref=0;
quit;
          *shows decent and acceptable homogeneity of
resids;
************************************************************
*****************************;
title3 aboveground n_conc for both species in 2003 ANOVA;
data total_above;
set total;
if species="tot" then delete;
if type="tot" then delete;
if type="b" then delete;
run;
proc mixed data=total_above;
class ammonia n_p species;
model n_conc=species|ammonia|n_p/ddfm=kr outp=resids;
*lsmeans ammonia n_p*ammonia/ pdiff adjust=tukey;
run;
title4 checks for normality;
proc univariate data=total_above normal plot;
var n_conc;
run;
          *test shows a S-W test of 0.86, indicating
normality;
proc plot data=resids vpercent=50;
plot resid*pred / vref=0;
quit;
          *shows decent and acceptable homogeneity of
resids;
************************************************************
*****************************;
title3 belowground n_conc for both species in 2003 ANOVA;
data total_below;
set total;
if species="tot" then delete;
if type="a" then delete;
if type="tot" then delete;
run;
proc mixed data=total_below;
class ammonia n_p species;
model n_conc=species|ammonia|n_p /ddfm=kr outp=resids;
*lsmeans ammonia n_p*ammonia/ pdiff adjust=tukey;
run;
title4 checks for normality;
```

```
proc univariate data=total_below normal plot;
var n_conc;
run;
          *test shows a S-W test of 0.86, indicating
normality;
proc plot data=resids vpercent=50;
plot resid*pred / vref=0;
quit;
          *shows decent and acceptable homogeneity of
resids;
************************************************************
*****************************;
title3 total n_conc for TYPHA in 2003 ANOVA;
data typha_total;
set total;
if species="tot" then delete;
if species="ju" then delete;
if type="a" then delete;
if type="b" then delete;
run;
proc mixed data=typha_total;
class ammonia n_p;
model n_conc=ammonia|n_p/ddfm=kr outp=resids;
*lsmeans ammonia n_p*ammonia/ pdiff adjust=tukey;
run;
title4 checks for normality;
proc univariate data=typha_total normal plot;
var n_conc;
run;
          *test shows a S-W test of 0.91, indicating
normality;
proc plot data=resids vpercent=50;
plot resid*pred / vref=0;
quit;
          *shows decent homogeneity of resids;
************************************************************
*****************************;
title3 aboveground n_conc for TYPHA in 2003 ANOVA;
data typha_above;
set total;
if species="tot" then delete;
if species="ju" then delete;
if type="tot" then delete;
if type="b" then delete;
run;
proc mixed data=typha_above;
class ammonia n_p;
model n_conc=ammonia|n_p/ddfm=kr outp=resids;
*lsmeans ammonia n_p*ammonia/ pdiff adjust=tukey;
run;
title4 checks for normality;
proc univariate data=typha_above normal plot;
var n_conc;
run;
```

```
          *test shows a S-W test of 0.91, indicating
normality;
proc plot data=resids vpercent=50;
plot resid*pred / vref=0;
quit;
          *shows decent and acceptable homogeneity of
resids;
************************************************************
****************************;
title3 belowground n_conc for TYPHA in 2003 ANOVA;
data typha_below;
set total;
if species="tot" then delete;
if species="ju" then delete;
if type="a" then delete;
if type="tot" then delete;
run;
proc mixed data=typha_below;
class ammonia n_p;
model n_conc=ammonia|n_p/ddfm=kr outp=resids;
*lsmeans ammonia n_p*ammonia/ pdiff adjust=tukey;
run;
title4 checks for normality;
proc univariate data=typha_below normal plot;
var n_conc;
run;
          *test shows a S-W test of 0.88, indicating decent
normality;
proc plot data=resids vpercent=50;
plot resid*pred / vref=0;
quit;
          *shows decent and acceptable homogeneity of
resids;
************************************************************
****************************;
title3 total n_conc for JUNCUS in 2003 ANOVA;
data juncus_total;
set total;
if species="tot" then delete;
if species="ty" then delete;
if type="a" then delete;
if type="b" then delete;
run;
proc mixed data=juncus_total;
class ammonia n_p;
model n_conc=ammonia|n_p/ddfm=kr outp=resids;
*lsmeans ammonia n_p*ammonia/ pdiff adjust=tukey;
run;
title4 checks for normality;
proc univariate data=juncus_total normal plot;
var n_conc;
run;
          *test shows a S-W test of 0.91, indicating
normality;
```

```
proc plot data=resids vpercent=50;
plot resid*pred / vref=0;
quit;
          *shows decent and acceptable homogeneity of
resids;
************************************************************
*****************************;
title3 aboveground n_conc for JUNCUS in 2003 ANOVA;
data juncus_above;
set total;
if species="tot" then delete;
if species="ty" then delete;
if type="tot" then delete;
if type="b" then delete;
run;
proc mixed data=juncus_above;
class ammonia n_p;
model n_conc=ammonia|n_p/ddfm=kr outp=resids;
*lsmeans ammonia n_p*ammonia/ pdiff adjust=tukey;
run;
title4 checks for normality;
proc univariate data=juncus_above normal plot;
var n_conc;
run;
          *test shows a S-W test of 0.91, indicating
normality;
proc plot data=resids vpercent=50;
plot resid*pred / vref=0;
quit;
          *shows decent and acceptable homogeneity of
resids;
************************************************************
*****************************;
title3 belowground n_conc for JUNCUS in 2003 ANOVA;
data juncus_below;
set total;
if species="tot" then delete;
if species="ty" then delete;
if type="a" then delete;
if type="tot" then delete;
run;
proc mixed data=juncus_below;
class ammonia n_p;
model n_conc=ammonia|n_p/ddfm=kr outp=resids;
*lsmeans ammonia n_p*ammonia/ pdiff adjust=tukey;
run;
title4 checks for normality;
proc univariate data=juncus_below normal plot;
var n_conc;
run;
          *test shows a S-W test of 0.91, indicating
normality;
proc plot data=resids vpercent=50;
plot resid*pred / vref=0;
```

```
quit;
          *shows decent and acceptable homogeneity of
resids;

************************************************************
****************************
Here ends the tissue nitrogen concentration ANOVA
series................................
************************************************************
***************************;

title2 Tissue Mass Nitrogen ANOVAs in 2003;
title3 total n_mass for both species in 2003 ANOVA;
data total_n_mass;
set total;
if species="tot" then delete;
if type="a" then delete;
if type="b" then delete;
run;
proc mixed data=total_n_mass;
class ammonia n_p species;
model n_mass=species|ammonia|n_p/ddfm=kr outp=resids;
*lsmeans species|ammonia|n_p / pdiff adjust=tukey;
run;
title4 checks for normality;
proc univariate data=total_n_mass normal plot;
var n_mass;
run;
          *test shows a Shapiro-Wilk's Test (as S-W test
from now on)
               of 0.78, indicating normality;
proc plot data=resids vpercent=50;
plot resid*pred / vref=0;
quit;
          *shows decent and acceptable homogeneity of
resids;
************************************************************
*****************************;
title3 aboveground n_mass for both species in 2003 ANOVA;
data total_above;
set total;
if species="tot" then delete;
if type="tot" then delete;
if type="b" then delete;
run;
proc mixed data=total_above;
class ammonia n_p species;
model n_mass=species|ammonia|n_p/ddfm=kr outp=resids;
*lsmeans ammonia n_p*ammonia/ pdiff adjust=tukey;
run;
title4 checks for normality;
proc univariate data=total_above normal plot;
var n_mass;
run;
```

```
          *test shows a S-W test of 0.78, indicating
normality;
proc plot data=resids vpercent=50;
plot resid*pred / vref=0;
quit;
          *shows decent and acceptable homogeneity of
resids;
************************************************************
****************************;
title3 belowground n_mass for both species in 2003 ANOVA;
data total_below;
set total;
if species="tot" then delete;
if type="a" then delete;
if type="tot" then delete;
run;
proc mixed data=total_below;
class ammonia n_p species;
model n_mass=species|ammonia|n_p /ddfm=kr outp=resids;
*lsmeans ammonia n_p*ammonia/ pdiff adjust=tukey;
run;
title4 checks for normality;
proc univariate data=total_below normal plot;
var n_mass;
run;
          *test shows a S-W test of 0.78, indicating
normality;
proc plot data=resids vpercent=50;
plot resid*pred / vref=0;
quit;
          *shows decent and acceptable homogeneity of
resids;
************************************************************
****************************;
title3 total n_mass for TYPHA in 2003 ANOVA;
data typha_total;
set total;
if species="tot" then delete;
if species="ju" then delete;
if type="a" then delete;
if type="b" then delete;
run;
proc mixed data=typha_total;
class ammonia n_p;
model n_mass=ammonia|n_p/ddfm=kr outp=resids;
*lsmeans ammonia n_p*ammonia/ pdiff adjust=tukey;
run;
title4 checks for normality;
proc univariate data=typha_total normal plot;
var n_mass;
run;
          *test shows a S-W test of 0.86, indicating
normality;
proc plot data=resids vpercent=50;
```

```
plot resid*pred / vref=0;
quit;
          *shows decent homogeneity of resids;
************************************************************
****************************;
title3 aboveground n_mass for TYPHA in 2003 ANOVA;
data typha_above;
set total;
if species="tot" then delete;
if species="ju" then delete;
if type="tot" then delete;
if type="b" then delete;
run;
proc mixed data=typha_above;
class ammonia n_p;
model n_mass=ammonia|n_p/ddfm=kr outp=resids;
*lsmeans ammonia n_p*ammonia/ pdiff adjust=tukey;
run;
title4 checks for normality;
proc univariate data=typha_above normal plot;
var n_mass;
run;
          *test shows a S-W test of 0.86, indicating
normality;
proc plot data=resids vpercent=50;
plot resid*pred / vref=0;
quit;
          *shows decent and acceptable homogeneity of
resids;
************************************************************
****************************;
title3 belowground n_mass for TYPHA in 2003 ANOVA;
data typha_below;
set total;
if species="tot" then delete;
if species="ju" then delete;
if type="a" then delete;
if type="tot" then delete;
run;
proc mixed data=typha_below;
class ammonia n_p;
model n_mass=ammonia|n_p/ddfm=kr outp=resids;
*lsmeans ammonia n_p*ammonia/ pdiff adjust=tukey;
run;
title4 checks for normality;
proc univariate data=typha_below normal plot;
var n_mass;
run;
          *test shows a S-W test of 0.86, indicating decent
normality;
proc plot data=resids vpercent=50;
plot resid*pred / vref=0;
quit;
```

```
          *shows decent and acceptable homogeneity of
resids;
************************************************************
*****************************;
title3 total n_mass for JUNCUS in 2003 ANOVA;
data juncus_total;
set total;
if species="tot" then delete;
if species="ty" then delete;
if type="a" then delete;
if type="b" then delete;
run;
proc mixed data=juncus_total;
class ammonia n_p;
model n_mass=ammonia|n_p/ddfm=kr outp=resids;
*lsmeans ammonia n_p*ammonia/ pdiff adjust=tukey;
run;
title4 checks for normality;
proc univariate data=juncus_total normal plot;
var n_mass;
run;
          *test shows a S-W test of 0.95, indicating
normality, supported graphically;
proc plot data=resids vpercent=50;
plot resid*pred / vref=0;
quit;
          *shows decent and acceptable homogeneity of
resids;
************************************************************
*****************************;
title3 aboveground n_mass for JUNCUS in 2003 ANOVA;
data juncus_above;
set total;
if species="tot" then delete;
if species="ty" then delete;
if type="tot" then delete;
if type="b" then delete;
run;
proc mixed data=juncus_above;
class ammonia n_p;
model n_mass=ammonia|n_p/ddfm=kr outp=resids;
*lsmeans ammonia n_p*ammonia/ pdiff adjust=tukey;
run;
title4 checks for normality;
proc univariate data=juncus_above normal plot;
var n_mass;
run;
          *test shows a S-W test of 0.96, indicating
normality;
proc plot data=resids vpercent=50;
plot resid*pred / vref=0;
quit;
          *shows decent and acceptable homogeneity of
resids;
```

```
************************************************************
****************************;
title3 belowground n_mass for JUNCUS in 2003 ANOVA;
data juncus_below;
set total;
if species="tot" then delete;
if species="ty" then delete;
if type="a" then delete;
if type="tot" then delete;
run;
proc mixed data=juncus_below;
class ammonia n_p;
model n_mass=ammonia|n_p/ddfm=kr outp=resids;
*lsmeans ammonia n_p*ammonia/ pdiff adjust=tukey;
run;
title4 checks for normality;
proc univariate data=juncus_below normal plot;
var n_mass;
run;
          *test shows a S-W test of 0.95, indicating
normality;
proc plot data=resids vpercent=50;
plot resid*pred / vref=0;
quit;
          *shows decent and acceptable homogeneity of
resids;

************************************************************
***************************
Here ends tissue mass nitrogen ANOVA
series...............................
************************************************************
**************************;

title2 Tissue Phosphorus Concentration ANOVAs in 2003;
title3 total p_conc for both species in 2003 ANOVA;
data total_p_conc;
set total;
if species="tot" then delete;
if type="a" then delete;
if type="b" then delete;
run;
proc mixed data=total_p_conc;
class ammonia n_p species;
model p_conc=species|ammonia|n_p/ddfm=kr outp=resids;
*lsmeans species|ammonia|n_p / pdiff adjust=tukey;
run;
title4 checks for normality;
proc univariate data=total_p_conc normal plot;
var p_conc;
run;
          *test shows a Shapiro-Wilk's Test (as S-W test
from now on)
              of 0.93, indicating normality;
```

```
proc plot data=resids vpercent=50;
plot resid*pred / vref=0;
quit;
          *shows decent and acceptable homogeneity of
resids;
************************************************************
*****************************;
title3 aboveground p_conc for both species in 2003 ANOVA;
data total_above;
set total;
if species="tot" then delete;
if type="tot" then delete;
if type="b" then delete;
run;
proc mixed data=total_above;
class ammonia n_p species;
model p_conc=species|ammonia|n_p/ddfm=kr outp=resids;
*lsmeans ammonia n_p*ammonia/ pdiff adjust=tukey;
run;
title4 checks for normality;
proc univariate data=total_above normal plot;
var p_conc;
run;
          *test shows a S-W test of 0.93, indicating
normality;
proc plot data=resids vpercent=50;
plot resid*pred / vref=0;
quit;
          *shows decent and acceptable homogeneity of
resids;
************************************************************
*****************************;
title3 belowground p_conc for both species in 2003 ANOVA;
data total_below;
set total;
if species="tot" then delete;
if type="a" then delete;
if type="tot" then delete;
run;
proc mixed data=total_below;
class ammonia n_p species;
model p_conc=species|ammonia|n_p /ddfm=kr outp=resids;
*lsmeans ammonia n_p*ammonia/ pdiff adjust=tukey;
run;
title4 checks for normality;
proc univariate data=total_below normal plot;
var p_conc;
run;
          *test shows a S-W test of 0.96, indicating
normality;
proc plot data=resids vpercent=50;
plot resid*pred / vref=0;
quit;
```

```
          *shows decent and acceptable homogeneity of
resids;
************************************************************
*****************************;
title3 total p_conc for TYPHA in 2003 ANOVA;
data typha_total;
set total;
if species="tot" then delete;
if species="ju" then delete;
if type="a" then delete;
if type="b" then delete;
run;
proc mixed data=typha_total;
class ammonia n_p;
model p_conc=ammonia|n_p/ddfm=kr outp=resids;
*lsmeans ammonia n_p*ammonia/ pdiff adjust=tukey;
run;
title4 checks for normality;
proc univariate data=typha_total normal plot;
var p_conc;
run;
          *test shows a S-W test of 0.94, indicating
normality;
proc plot data=resids vpercent=50;
plot resid*pred / vref=0;
quit;
          *shows decent homogeneity of resids;
************************************************************
*****************************;
title3 aboveground p_conc for TYPHA in 2003 ANOVA;
data typha_above;
set total;
if species="tot" then delete;
if species="ju" then delete;
if type="tot" then delete;
if type="b" then delete;
run;
proc mixed data=typha_above;
class ammonia n_p;
model p_conc=ammonia|n_p/ddfm=kr outp=resids;
*lsmeans ammonia n_p*ammonia/ pdiff adjust=tukey;
run;
title4 checks for normality;
proc univariate data=typha_above normal plot;
var p_conc;
run;
          *test shows a S-W test of 0.96, indicating
normality;
proc plot data=resids vpercent=50;
plot resid*pred / vref=0;
quit;
          *shows decent and acceptable homogeneity of
resids;
```

```
************************************************************
*****************************;
title3 belowground p_conc for TYPHA in 2003 ANOVA;
data typha_below;
set total;
if species="tot" then delete;
if species="ju" then delete;
if type="a" then delete;
if type="tot" then delete;
run;
proc mixed data=typha_below;
class ammonia n_p;
model p_conc=ammonia|n_p/ddfm=kr outp=resids;
*lsmeans ammonia n_p*ammonia/ pdiff adjust=tukey;
run;
title4 checks for normality;
proc univariate data=typha_below normal plot;
var p_conc;
run;
          *test shows a S-W test of 0.89, indicating decent
normality;
proc plot data=resids vpercent=50;
plot resid*pred / vref=0;
quit;
          *shows decent and acceptable homogeneity of
resids;
************************************************************
*****************************;
title3 total p_conc for JUNCUS in 2003 ANOVA;
data juncus_total;
set total;
if species="tot" then delete;
if species="ty" then delete;
if type="a" then delete;
if type="b" then delete;
run;
proc mixed data=juncus_total;
class ammonia n_p;
model p_conc=ammonia|n_p/ddfm=kr outp=resids;
*lsmeans ammonia n_p*ammonia/ pdiff adjust=tukey;
run;
title4 checks for normality;
proc univariate data=juncus_total normal plot;
var p_conc;
run;
          *test shows a S-W test of 0.96, indicating
normality, supported graphically;
proc plot data=resids vpercent=50;
plot resid*pred / vref=0;
quit;
          *shows decent and acceptable homogeneity of
resids;
************************************************************
*****************************;
```

```
title3 aboveground p_conc for JUNCUS in 2003 ANOVA;
data juncus_above;
set total;
if species="tot" then delete;
if species="ty" then delete;
if type="tot" then delete;
if type="b" then delete;
run;
proc mixed data=juncus_above;
class ammonia n_p;
model p_conc=ammonia|n_p/ddfm=kr outp=resids;
*lsmeans ammonia n_p*ammonia/ pdiff adjust=tukey;
run;
title4 checks for normality;
proc univariate data=juncus_above normal plot;
var p_conc;
run;
          *test shows a S-W test of 0.99, indicating
normality;
proc plot data=resids vpercent=50;
plot resid*pred / vref=0;
quit;
          *shows decent and acceptable homogeneity of
resids;
************************************************************
****************************;
title3 belowground p_conc for JUNCUS in 2003 ANOVA;
data juncus_below;
set total;
if species="tot" then delete;
if species="ty" then delete;
if type="a" then delete;
if type="tot" then delete;
run;
proc mixed data=juncus_below;
class ammonia n_p;
model p_conc=ammonia|n_p/ddfm=kr outp=resids;
*lsmeans ammonia n_p*ammonia/ pdiff adjust=tukey;
run;
title4 checks for normality;
proc univariate data=juncus_below normal plot;
var p_conc;
run;
          *test shows a S-W test of 0.95, indicating
normality;
proc plot data=resids vpercent=50;
plot resid*pred / vref=0;
quit;
          *shows decent and acceptable homogeneity of
resids;

************************************************************
***************************
```

```
Here ends tissue phosphorus concentration ANOVA
series.................................
************************************************************
**************************;

title2 root-to-shoot ratio ANOVAs in 2003;
data r_sratio;
input column$ row$ ammonia$ n_p$ species$ r_s;
log_r_s=log(r_s);
datalines;
d    1    75   5    j    0.310848401
d    2    75   15   j    0.518413598
d    3    75   25   j    0.402200489
d    4    75   5    j    0.384087006
d    5    75   15   j    0.661354582
d    6    75   25   j    0.448555957
d    7    75   5    j    0.378567852
d    8    75   15   j    0.473915776
d    9    75   25   j    0.45377129
c    1    150  5    j    0.269167126
c    2    150  15   j    0.377993528
c    3    150  25   j    0.265010352
c    4    150  5    j    0.270376616
c    5    150  15   j    0.284552846
c    6    150  25   j    0.268817204
c    7    150  5    j    0.283008943
c    8    150  15   j    0.319554849
c    9    150  25   j    0.371012871
b    1    225  5    j    0.279545455
b    2    225  15   j    0.365721997
b    3    225  25   j    0.388556221
b    4    225  5    j    0.328711727
b    5    225  15   j    0.443365696
b    6    225  25   j    0.481840194
b    7    225  5    j    0.275815217
b    8    225  15   j    0.360512522
b    9    225  25   j    0.554045307
a    1    300  5    j    0.374233129
a    2    300  15   j    0.271197411
a    3    300  25   j    0.39659803
a    4    300  5    j    0.327586207
a    5    300  15   j    0.443312967
a    6    300  25   j    0.416536661
a    7    300  5    j    0.370620774
a    8    300  15   j    0.416903409
a    9    300  25   j    0.425830258
d    1    75   5    t    0.597633136
d    2    75   15   t    0.755579171
d    3    75   25   t    0.647357724
d    4    75   5    t    0.752688172
d    5    75   15   t    0.762022195
d    6    75   25   t    0.76984127
d    7    75   5    t    0.723809524
d    8    75   15   t    0.561768531
```

```
d    9    75   25   t    0.868585732
c    1    150  5    t    0.379790941
c    2    150  15   t    0.700767263
c    3    150  25   t    0.45210728
c    4    150  5    t    0.500683995
c    5    150  15   t    0.540723982
c    6    150  25   t    0.51572327
c    7    150  5    t    0.449929478
c    8    150  15   t    0.654907975
c    9    150  25   t    0.555950266
b    1    225  5    t    0.350482315
b    2    225  15   t    0.279359431
b    3    225  25   t    0.391143911
b    4    225  5    t    0.409185804
b    5    225  15   t    0.479910714
b    6    225  25   t    0.337899543
b    7    225  5    t    0.252764613
b    8    225  15   t    0.47112462
b    9    225  25   t    0.527093596
a    1    300  5    t    0.353135314
a    2    300  15   t    0.385749386
a    3    300  25   t    0.087962963
a    4    300  5    t    0.339181287
a    5    300  15   t    0.981042654
a    6    300  25   t    0.50764526
a    7    300  5    t    0.422222222
a    8    300  15   t    0.377245509
a    9    300  25   t    0.214592275
run;
title3 root-to-shoot ratios for mesocosms in 2003 ANOVA;
proc mixed data=r_sratio;
class ammonia n_p species;
model r_s=species|ammonia|n_p/ ddfm=kr outp=resids;
*lsmeans ammonia / pdiff;
run;
title4 checks for normality;
proc univariate data=r_sratio normal plot;
var r_s;
run;
          *test shows a S-W test of 0.93, indicating
normality;
proc plot data=resids vpercent=50;
plot resid*pred / vref=0;
quit;
          *shows decent and acceptable homogeneity of
resids;
************************************************************
*****************************;
title3 root-to-shoot ratios for TYPHA in 2003 ANOVA;
proc mixed data=r_sratio;
where species='t';
class ammonia n_p;
model r_s=ammonia|n_p/ ddfm=kr outp=resids;
run;
```

```
title4 checks for normality;
proc univariate data=r_sratio normal plot;
where species='t';
var r_s;
run;
          *test shows a S-W test of 0.98, indicating
normality;
proc plot data=resids vpercent=50;
plot resid*pred / vref=0;
quit;
          *shows decent and acceptable homogeneity of
resids;
************************************************************
*****************************;
title3 root-to-shoot ratio for JUNCUS in 2003 ANOVA;
proc mixed data=r_sratio;
where species='j';
class ammonia n_p;
model r_s=ammonia|n_p/ ddfm=kr outp=resids;
run;
title4 checks for normality;
proc univariate data=r_sratio normal plot;
where species='j';
var r_s;
run;
          *test shows a S-W test of 0.93, indicating
normality;
proc plot data=resids vpercent=50;
plot resid*pred / vref=0;
quit;
          *shows decent and acceptable homogeneity of
resids;
quit;
```

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