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The Effect of Nitrogen Level and Form on the Growth and Development of Vetiver Grass (*Chrysopogon zizanioides*)

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ABSTRACT

Vetiver grass (*Chrysopogon zizanioides*) is a warm season perennial grass grown as a phytoremediation tool and recently proposed as a plant material source for biofuel production. However, limited information exists on Vetiver grass fertility management practices in cropping systems. Therefore, the effects of nitrogen (N) level and N-form on Vetiver growth and development were investigated. Plant slips of *C. zizanioides* Sunshine were greenhouse-grown using nutrient solution culture. In the first experiment, N-level treatments were 26.3, 52.5, 105, 210 and 410 mg $N \cdot L^{-1}$ at a ratio of 3:1 nitrate-N: ammonium-N. In a second experiment, the ratio of NH_4:NO_3 was changed from 0:100, 25:75, 50:50, 75:25, and 100:0 while keeping a total N level of 210 mg $N \cdot L^{-1}$. At 12 weeks after transplanting (WAT), plant height, tiller number, accumulated shoot, root, and total fresh weights and dry weights, total leaf number, and chlorophyll content were recorded. Leaf number decreased linearly ($P \leq 0.10$) from 11.2 per plant at 26.3 mg $N \cdot L^{-1}$ to 9.7 per plant at 210 mg $N \cdot L^{-1}$ while leaf chlorophyll content increased, then decreased quadratically ($P \leq 0.10$) over the same N level treatment range. Shoot dry weight and total plant dry weight increased, then decreased ($P \leq 0.10$, respectively) as N level was increased in nutrient solution. In the second experiment, plant height decreased linearly ($P \leq 0.05$) from a mean of 100.9 cm per plant at 1:100 NH_4:NO_3 ratio to 90.5 cm per plant at 100:1 NH_4:NO_3 ratio treatment. Linear increases were observed for tiller number ($P \leq 0.10$) and leaf number ($P \leq 0.001$) per plant as the ratio of NH_4:NO_3 was changed from 1:100 to 100:0. Limited effects of form were identified for plant biomass parameters. Results demonstrate that Vetiver grass may successfully be cultured under lower N fertility and has a tolerance to ammonium-N in managed cropping systems.

Key words: chlorophyll, hydroponics, macronutrient, micronutrient, SPAD

INTRODUCTION

Vetiver grass (*Chrysopogon zizanioides* L. Roberty) is a perennial aromatic grass native to southern India. Vetiver cultivation is possible in all regions with a warm and moist climate, and it is intensively cultivated in many semitropical areas including China, Indonesia, Angola, Somalia, Congo, Brazil, Guatemala, Haiti, and the southern U.S. (Peyron, 1989). Vetiver grass can tolerate extreme climatic variations such as prolonged drought, flooding and temperatures between -14ºC to 55ºC. In China, it has survived at -22ºC and in the U.S. state of Georgia it was able to tolerate temperatures to -10ºC (Troung, 1996). Vetiver is highly tolerant to saline conditions, having a critical salt toxicity level of ECse = 8 $dS \cdot m^{-1}$ (Troung, 1996). Two genotypes of Vetiver have been identified; namely a seeded north Indian type and a sterile, or very low fertility south Indian type. Sunshine Vetiver is a domesticated South India type which is non-fertile, has received a low risk score for the potential to become invasive by the USDA-Natural Resources Conservation Service, and requires asexual propagation (Joy, 2009). Vetiver grass is currently grown in 100 different countries as a result of World Bank extension efforts (Slinger, 1997).

Vetiver grass has a wide range of ecological and economic applications (Wilde et al., 2005). Applications of Vetiver include use in agriculture land and slope stabilization, soil erosion control, and incorporation in ornamental landscape plantings (Maffei, 2002). It has be inter-cropped as a contour fence with sugar cane (*Saccahrum officinalis*), maize (*Zea mays*), and tea (*Camellia sinennsis*) to conserve soil moisture and restrict soil runoff (Daffron, 1993). Recently Vetiver grass has been proposed as a plant material source for biofuel production (Boucard, 2005). Vetiver grass holds annual biomass production potential of 100 to 120 $tonnes \cdot ha^{-1}$ (Troung and Smeal, 2003) and has an energy value of 16.3 $MJ \cdot kg^{-1}$ compared to petroleum (41.9 $MJ \cdot kg^{-1}$), coal (27.9-30.2 $MJ \cdot kg^{-1}$), dry wood (19.8 $MJ \cdot kg^{-1}$), and sugarcane bagasse (9.3 $MJ \cdot kg^{-1}$; Grimshaw, 2008). It also has potential as a carbon sequester and Grimshaw (2008) reported that Vetiver grass can sequester 4.5 times more carbon per year than a fast growing poplar tree (*Populus* sp.) per unit area.

The role of using green plants is a promising biological technique for phytoremediation (Schroder et al., 2002). Vetiver has unique morphological and physiological characteristics that make it an excellent phytoremediation tool (Troung, 2000). In fact, Vetiver has proved to be a more successful phytoremediation plant species than Bermudagrass (*Cynodon dactylon*), Bahiagrass (*Paspalum notatum*), Rhodes grass (*Chloris guyana*), tall wheatgrass (*Thynopyron elongatum*), marine couch (*Sporobolus virginicus*) and samphire (*Sarcocrina* spp.; Sinha et al., 2007).

Nitrogen (N) is an essential element and has a major influence on a number of plant responses including pigmentation, shoot and root growth, cold and drought tolerance, wear tolerance, thatch accumulation and recovery potential (Carrow et al., 1987). An awareness of differences in response to N between economically important grass species underlies much of the present day grassland management and experiments suggest that differences between species in response to N may be considerable (Lovvorn, 1945). Trenholm et al. (1998) reported that N also affects the development of tillers in grasses. Over fertilization of N can cause grasses to become susceptible to environmental and biological stresses like summer drought, winter desiccation, and heat and cold damage (Dunn et al., 1995). Although most managed crops have recommended fertilizer rates for targeted optimal yield and performance, information on N rate application for Vetiver grass is limited (Joy, 2009). Therefore, this current study was undertaken to investigate the optimal N fertilization rate and form for improved growth of Vetiver grass.

MATERIALS AND METHODS

Experimental Design

Two experiments were carried out in a controlled greenhouse environment (22°C day/14°C night set points) under natural lighting conditions at Illinois State University, Normal, IL (Lat. 40° 30'N) between February and May, 2009. The first study investigated the effect of nitrogen (N) fertilization level and the second study investigated the effect of N fertilization form on Vetiver grass growth and quality. The experimental design was a randomized complete block design with four replications for each experiment. Generally, Vetiver grass propagules are available in bundles of rooted slips bearing 3-5 tillers each. For the two experiments, approximately 200 mature plant slips of Sunshine Vetiver grass bearing 3-5 tillers each were purchased from Florida Vetiver Systems LLC (Maitland, FL). Mature plant slip shoots were cut back to 30 cm and roots were trimmed to 20 cm to make them uniform in size at the time of planting. Plastic 38 L containers (Sterilite® Lapis Blue; Townsend, MA) with lids having five 4 cm diameter holes equally spaced 16 cm apart were used for growing plant slips. Each container was filled with 34 L of a modified half strength nutrient solution (Hoagland and Arnon, 1950).

In the N fertilization level study, plants were grown at increasing N treatment concentrations of 26.3, 52.5, 105, 210, 420 mg $N \cdot L^{-1}$. The two dominant N forms were balanced in all of the N treatments to achieve a ratio of 25% NH_4^+:75% NO_3^-. In the N fertilization form study, the total N level was held constant at 210 mg $N \cdot L^{-1}$ and plants were grown under increasing NH_4^+ at 0, 25, 50, 75 and 100% of total N. Elemental concentrations of nutrient solutions were ($mg \cdot L^{-1}$): phosphorus (P) (89.9); potassium (K) (132); calcium (Ca) (80); magnesium (Mg) (24); iron (Fe) (0.5); boron (B) (0.125); molybdenum (Mo) (0.0025); manganese (Mn) (0.125); and zinc (Zn) (0.0125). Solutions were aerated with an air blower (Model VB-007S, Sweetwater, Ft. Collins, CO) connected to two air stones in each container. Nutrient solutions were replaced every two weeks throughout the experiment to refresh the solution to the initial nutrient concentrations.

Data Collection & Statistical Analysis

After twelve weeks of hydroponic culture, plant height, number of tillers per plant, total number of leaves per plant, and treatment-wise average chlorophyll content of mature leaves using a Minolta SPAD-502 Chlorophyll Meter (Model 2900; Konica Minolta, Japan) were recorded. Plants were then harvested and shoot and root tissues were washed, towel dried, and separated and fresh weights were recorded. Plant tissues were then dried in a forced-air oven at 60°C. When the tissues achieved a constant mass, dry weights were recorded. All data collected were analyzed by the GLM procedure of SAS 9.2 (Cary, NC) to perform analysis of variance and regression analysis to determine relationships between dependent variables and N treatments.

RESULTS AND DISCUSSION

Experiment 1: Nitrogen Fertilization Level Study

Plant height of Vetiver was within previously reported ranges (Xia and Bing, 2000). Increasing N fertilization level influenced Vetiver plant height (P=0.029). Plant height reached a maximum of 105.0 cm after 12 weeks after transplanting under the 210 mg

$N \cdot L^{-1}$ treatment (Table 1). After 14 months of growth, Xia and Bing (2003) reported 'Sunshine' Vetiver plant height ranged from 160 cm to 170 cm. However, in the current study, no significant trends were observed for plant height in response to increasing N treatments from 26.3 to 420 mg $N \cdot L^{-1}$ (Table 1). Non-significant plant height over time of Vetiver was also reported by research performed by Muensangk (2000) which investigated ecotype differences. Our results indicate that Vetiver can be cultured under N fertility as low as 26.3 mg $N \cdot L^{-1}$ and still attain previously reported average plant height.

The number of tillers per plant was also within previously reported ranges. Increasing N fertilization level influenced the number of tillers per Vetiver plant ($P \leq 0.001$). Twelve weeks after transplanting, the maximum tiller number was found to be 11.2 at 26.3 mg N·L-1 (Table 1). Xia and Bing (2003) reported tiller number per plant ranged from 7 to 59 for twelve different Vetiver ecotypes. Although no significant trends were observed in our current study, the number of tillers decreased from 11.2 at 26.3 mg $N \cdot L^{-1}$ to 9.7 at 420 mg $N \cdot L^{-1}$. Abbaszadeh et al. (2009) reported that N level also influenced tiller number in balm (*Mellissa officinalis*) and that tiller number per plant decreased as the N level increased.

Increasing N fertilization level influenced Vetiver leaf number per plant ($P=0.10$) and chlorophyll leaf content ($P=0.10$). Average leaf number per plant decreased linearly ($P \leq 0.10$) from 56.0 under 26.3 mg $N \cdot L^{-1}$ to 46.9 under 420 mg $N \cdot L^{-1}$ (Table 1). Chlorophyll content displayed a quadratic trend ($P \leq 0.10$), first increasing then decreasing with increasing N rate concentrations and showed a maximum of 52.7 $g \cdot m^{-2}$ at 105 mg $N \cdot L^{-1}$ (Table 1). Buah and Mwinkaara (2009) reported a linear increase in chlorophyll SPAD value content at increasing N levels from 0, 40, 80 and 120 kg N· ha-1 in sorghum (*Sorghum* sp.). Differences in regression trends between Buah and Mwinkaara (2009) and our current study may be attributed to differences in plant genus and level of N fertilization.

Increasing N fertilization level influenced shoot fresh weight (SFWT; $P=0.099$), shoot dry weight (SDWT; $P=0.077$), root dry weight (RDWT; $P=0.084$), and total plant dry weight (TDWT; $P=0.096$), but not root fresh weight (RFWT; $P=0.205$) and total plant fresh weight (TFWT; $P=0.228$). Shoot fresh weight and SDWT were maximum at 52.5 mg $N \cdot L^{-1}$ (Table 2). Bradshaw et al. (1964) showed that among seven grasses, yields were significantly varied as the N level concentration increased and matgrass (*Nardus stricta*) produced maximum yield at only 27 ppm ($mg \cdot L^{-1}$) N. Vetiver appears to be another grass species which produces maximum yield under lower total N fertilization. However, Vetiver root dry weight and TDWT were maximum at 210 mg $N \cdot L^{-1}$. Shoot dry weight increased, then decreased quadratically ($P \leq 0.10$) in response to increasing N levels (Table 2). Total plant dry weight also increased, then decreased quadratically ($P \leq 0.10$) in response to increasing N levels (Table 2). Muir et al. (2001) reported that total plant biomass of switchgrass (*Panicum virgatum*) increased, then decreased quadratically as total N fertilization was increased from 0 kg $N \cdot ha^{-1}$ to 224 kg $N \cdot ha^{-1}$.

Experiment 2: Nitrogen Fertilization Form Study

Plant height of Vetiver in this experiment was also within previously reported ranges (Xia and Bing, 2000) and similar to experiment 1 (Table 1). Changing the ratio of $NH_4^+:NO_3^-$ influenced Vetiver plant height ($P=0.001$). Plant height reached a maximum of 103.4 cm 12 weeks after transplanting under the 50:50 $NH_4^+:NO_3^-$ (Table 3). Plant height decreased linearly (P≤0.05) as the ratio of NH_4^+:NO_3^- was changed from 0:100 to 100:0 (Table 3). Increasing ammonium-N fertilization has been reported to decrease plant height in many different plant species (Brown et al., 2010; Muniz et al., 2009; Kim et al., 2006). This is because carbon used for plant growth is reallocated and utilized in the detoxification of absorbed ammonium (Mills and Jones, Jr., 1996).

The number of tillers per plant was also within previously reported ranges (Xia and Bing, 2000) and similar to experiment 1 (Table 1). Changing the ratio of NH_4^+:NO_3^- influenced Vetiver tiller number (P=0.001) and tiller number increased linearly (P≤0.10) from 8.2 tiller per plant at 0% NH_4^+ to 10.4 tillers per plant at 100% NH_4^+ 12 WAT (Table 3). Nitrogen form has been demonstrated to affect tillering in grasses (Assuero and Tognetti, 2010), however maximum tillering has been reported in grasses fertilized with a 50:50 NH_4^+:NO_3^-. Leaf number per plant also increased linearly (P≤0.01) in response to increasing NH_4^+ in solution (Table 3). A similar trend was reported by McInenly et al. (2010) who showed that aboveground biomass of rough fescue (*Festuca campestris*) was increased when foliar N was supplied as 100% ammonium as opposed to 100% nitrate. Cholorphyll leaf content, as measured by SPAD, did not significantly change in response to changing the ratio of NH_4^+:NO_3^- in solution culture (Table 3).

Changing the ratio of NH_4^+:NO_3^- influenced RFWT (P=0.10) but did not influenced SFWT (P=0.537), TFWT (P=0.359), SDWT (P=0.566), RDWT (P=0.184), or TDWT (P=0.636). Twelve weeks after transplanting, RFWT decreased linearly (P≤0.10) in response to increasing NH_4^+ in solution (Table 4). When N was supplied as NH_4^+, biomass allocation to roots decreased in thickspike wheatgrass (*Agropyron dasystachyum*; Li and Redmann, 1992). Similar results were also reported in creeping bentgrass (*Agrostis palustri*s) where root weight was found to be highest at a 1:3 ratio of NH_4^+:NO_3^- (Glinski et al., 1990). Total fresh shoot and root yields of napiergrass (*Pennisetum purpureum*), a tropical grassland species, did not differ when N fertilization treatment were changed from solely nitrate to solely ammonium (Rahman et al., 2010). Bowler and Press (1996) reported that total dry weight of colonial bentgrass (*Agrostis capillaris*) was not affected by N form. The response of Vetiver grass growth to the ratio of NH_4^+:NO_3^- from 0:100 to 100:0 follows reported trends in other grass species.

Vetiver grass growth and biomass parameters showed varied responses and were mostly non-significant in both the N fertilization level and N fertilization form experiments. This lack of significance may indicate that Sunshine Vetiver has a higher amount of genetic variability than its cultivar classification. According to Grimshaw (2009), Sunshine is a Vetiver cultivar but is also referred to as an upland ecotype. Igbokwe et al. (1991) reported the extent of the fertilizer response among four Vetiver accessions depended on factors such as seasonal variation and genetic variability and Muensangk (2000) reported non-significant trends over time for several growth factors among Vetiver ecotypes. A similar situation has been identified in switchgrass, where two main ecotypes differ in ploidy, morphology, growth pattern, and climatic adaptation (Zalapa et al., 2011). Adams and Daffron (1997) reported that Monto Vetiver from Australia and Sunshine Vetiver from North America have no morphological and physiological differences and Joy (2009) lists Sunshine as genetically similar to the Vetiver cultivars Boucard, Fort Polk, Haiti, Huffman, Monto, and Vallonia. An ecotypic Sunshine Vetiver population may not respond uniformly to N fertility treatments due to genetic variations. Therefore, further refinement of the current Sunshine population and its related cultivars may be needed prior to large scale production consideration. The same recommendations are being proposed for switchgrass in order to produce significant heterotic increases in biomass yield in managed cropping systems (Zalapa et al., 2011).

CONCLUSION

Due to its unique physical and morphological characteristics, its tolerance to environmental extremes and low risk of becoming an invasive plant species, Vetiver grass may have production potential in the U.S. as a plant material source for biofuel production or carbon sequestration. Plant height and total plant fresh weight were not significantly different between 26.3 and 420 $mg \cdot L^{-1}$ N treatments. Therefore, our results indicate that Vetiver grass may successfully be cultured under lower N fertility. Vetiver also showed tolerance to NH_4^+-N. Although plant height decreased as NH_4^+-N increased, results were still within reported ranges for Vetiver cultivation. No significant differences between 0% and 100% NH_4^+-N, at a luxuriant total N level, for total plant fresh weight were observed. These results can be used to better predict the optimal N fertilization rates for investigating Vetiver grass culture under managed field conditions for large scale production. However, despite the wide range of environmental adaptation and non-fertile reproductive characteristics of Sunshine Vetiver, caution should be exercised when introducing a non-native plant species over a wide geographic area. Additional research is also needed to confirm if Sunshine Vetiver grass is actually an ecotype which warrants further genetic refinement through breeding to produce the uniformity of a true cultivar.

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Table 1. Mean values[z] of Vetiver grass plant height, tiller number, leaf number, and chlorophyll content 12 weeks after transplanting (WAT) into a modified Hoagland's nutrient solution with nitrogen levels (N LEVEL) varying from 26.3 $mg \cdot L^{-1}$ to 420 $mg \cdot L^{-1}$ with ratio of 1:3 NH_4:NO_3.

N LEVEL ($mg \cdot L^{-1}$)	Plant height[y] (cm)	Tiller Number	Leaf Number	Chlorophyll[x] ($mg \cdot m^{-2}$)
26.3	100.1 ± 17.6	11.2 ± 4.8	56.00 ± 6.13	48.42 ± 3.42
52.5	100.4 ± 13.4	10.5 ± 4.9	49.25 ± 2.93	48.62 ± 1.37
105	102.4 ± 13.0	10.7 ± 5.7	53.05 ± 11.68	52.72 ± 1.69
210	105.0 ± 8.7	10.4 ± 3.3	47.25 ± 5.20	48.52 ± 2.89
420	99.8 ± 12.2	9.7 ± 3.9	46.95 ± 10.35	48.37 ± 2.85
Contrast[w]				
Linear	NS	NS	*	NS
Quadratic	NS	NS	NS	*

[z] Mean of 5 plants per treatment replication ± standard deviation.

[y] Plant height was measured from base of the crown to leaf tip in centimeters (cm).

[x] Mean chlorophyll content of mature leaves measured in $mg \cdot m^{-2}$ using a hand-held SPAD meter.

[w] Significance for linear and quadratic orthogonal contrasts for N LEVELS.

NS, *, **, *** Non-significant or significance at P≤0.10, P≤0.05, P≤0.01, respectively.

Table 2. Mean values[z] of Vetiver grass plant biomass 12 weeks after transplanting into a modified Hoagland's nutrient solution with nitrogen levels (N LEVEL) varying from 26.3 $mg \cdot L^{-1}$ to 420 $mg \cdot L^{-1}$ with a ratio of 1:3 NH_4:NO_3.

N LEVEL	Plant biomass[y] (g)					
($mg \cdot L^{-1}$)	SFWT	RFWT	TFWT	SDWT	RDWT	TDWT
26.3	387.5 ± 75.0	233.9 ± 50.1	621.4 ± 119.9	77.2 ± 14.0	35.3 ± 5.5	112.5 ± 18.5
52.5	406.4 ± 62.0	215.8 ± 14.2	622.1 ± 101.2	85.2 ± 14.9	34.7 ± 2.3	119.9 ± 16.6
105	391.3 ± 73.5	264.1 ± 69.6	655.5 ± 141.5	82.1 ± 12.9	37.2 ± 6.3	119.3 ± 18.9
210	388.7 ± 21.4	263.8 ± 24.7	652.5 ± 19.6	82.9 ± 5.3	37.7 ± 1.9	120.6 ± 5.4
420	345.7 ± 12.4	246.9 ± 27.7	592.7 ± 33.9	71.5 ± 2.8	34.1 ± 2.0	105.6 ± 3.8
Contrast[x]						
Linear	NS	NS	NS	NS	NS	NS
Quadratic	NS	NS	NS	*	NS	*

[z] Mean of 5 plants per treatment replication ± standard deviation. Abbreviations: SFWT=shoot fresh weight, RFWT=root fresh weight, TFWT=total plant fresh weight, SDWT=shoot dry weight, RDWT=root dry weight, and TDWT=total plant dry weight.

[y] Mean plant biomass measured in grams (g).

[x] Significance for linear and quadratic orthogonal contrasts for N LEVELS.

NS, *, **, *** Non-significant or significance at P≤0.10, P≤0.05, P≤0.01, respectively.

Table 3. Mean values[z] of Vetiver grass plant height, tiller number, leaf number, and chlorophyll content 12 weeks after transplanting (WAT) into a modified Hoagland's nutrient solution with varying NH_4:NO_3 ratios of 0:100, 25:75, 50:50, 75:25, 100:0 while keeping total N level constant at 210 mg $N \cdot L^{-1}$.

RATIO of NH_4:NO_3	Plant height[y] (cm)	Tiller number	Leaf Number	Chlorophyll[x] ($mg \cdot m_{-2}$)
0:100	100.9 ± 14.1	8.2 ± 3.2	36.2 ± 2.6	45.4 ± 3.6
25:75	102.5 ± 11.3	9.2 ± 4.2	40.6 ± 5.1	46.0 ± 2.9
50:50	103.4 ± 9.8	9.1 ± 3.8	42.1 ± 8.2	48.9 ± 4.2
75:25	100.6 ± 9.3	9.5 ± 3.7	42.3 ± 3.1	48.8 ± 3.3
100:0	90.5 ± 14.5	10.4 ± 4.2	44.9 ± 3.3	47.1 ± 4.3
Contrast[w]				
Linear	**	*	***	NS
Quadratic	***	NS	**	NS

[z] Mean of 5 plants per treatment replication ± standard deviation.

[y] Plant height was measured from base of the crown to leaf tip in centimeters (cm).

[x] Mean chlorophyll content of mature leaves measured in $mg \cdot m^{-2}$ using a hand-held SPAD meter.

[w] Significance for linear and quadratic orthogonal contrasts for N RATIOS.

NS, *, **, *** Non-significant or significance at P≤0.10, P≤0.05, P≤0.01, respectively.

Table 4. Mean values[z] of Vetiver grass plant biomass 12 weeks after transplanting into a modified Hoagland's nutrient solution with varying NH_4:NO_3 ratios of 0:100, 25:75, 50:50, 75:25, 100:0 while keeping total N rate constant at 210 mg $N \cdot L^{-1}$.

RATIO of	Plant biomass[y] (g)					
NH_4:NO_3	SFWT	RFWT	TFWT	SDWT	RDWT	TDWT
0:100	357.3 ± 45.2	208.5 ± 25.5	565.7 ± 69.3	75.9 ± 6.7	32.5 ± 0.9	108.3 ± 7.6
25:75	383.9 ± 53.6	236.2 ± 66.8	620.2 ± 109.8	84.9 ± 13.5	34.2 ± 5.9	119.1 ± 17.6
50:50	418.7 ± 68.6	204.1 ± 47.8	622.8 ± 112.2	88.3 ± 11.9	31.6 ± 6.1	119.9 ± 17.1
75:25	351.5 ± 35.1	202.2 ± 73.8	553.7 ± 108.7	75.9 ± 5.9	31.1 ± 6.7	107.1 ± 10.0
100:0	349.8 ± 43.4	161.3 ± 9.2	511.1 ± 45.6	82.6 ± 13.4	29.2 ± 1.0	111.8 ± 12.9
Contrast[x]						
Linear	NS	*	NS	NS	NS	NS
Quadratic	NS	NS	NS	NS	NS	NS

[z] Mean of 5 plants per treatment replication ± standard deviation. Abbreviations: SFWT=shoot fresh weight, RFWT=root fresh weight, TFWT=total plant fresh weight, SDWT=shoot dry weight, RDWT=root dry weight, and TDWT=total plant dry weight.

[y] Mean plant biomass measured in grams (g).

[x] Significance for linear and quadratic orthogonal contrasts for N RATIOS.

NS, *, **, *** Non-significant or significance at P≤0.10, P≤0.05, P≤0.01, respectively.