Effect of Flooding Duration and Nitrogen Fertilization on Yield and Protein Content of Three Forage Species

G. C. Sigua,* M. Williams, J. Grabowski, C. Chase, and M. Kongchum

ABSTRACT

Increasing the availability and improving the quality of surface water in south Florida by temporarily flooding previously drained pastureland is one of the goals of Northern Everglades Restoration Initiative. Bahiagrass (*Paspalum notatum* Fluegge) is one of the most important forage grasses in the region and although tolerant to short- term flooding, bahiagrass is classified as a facultative upland (FACU+) species that suggest dry matter production and plant persistence might be reduced under periods of extended waterlogging. A 2-yr greenhouse study was conducted in 2008 and 2009 to determine the effect of flooding duration on dry matter yield (DMY) and crude protein content (CPC) of bahiagrass compared to two flooding tolerant forages, limpograss (*Hemarthria altissima* Poir), and maidencane (*Panicum hematomon* Schult) and to determine if N fertilization could be used to mitigate flooding effects. Dry matter production and CPC levels varied with flooding durations ($P \leq 0.001$) and levels of N fertilization ($P \leq 0.001$). Averaged across flooding duration and levels of N, limpograss had the greatest dry matter yield of 11.6 t ha^{-1} followed by maidencane (8.6 t ha^{-1}) and bahiagrass (8.5 t ha^{-1}) while bahiagrass had the highest CPC of 6.9% followed by maidencane (6.0%) and limpograss (3.7%). The overall yield response of the three forage species: bahiagrass ($R^2 = 0.95$); limpograss ($R^2 = 0.93$**); and maidencane ($R^2 = 0.99$**) were linearly related to increasing levels of N fertilization. Crude protein contents of three forage species: bahiagrass ($R^2 = 0.97$**), limpograss ($R^2 = 0.99$**), and maidencane ($R^2 = 0.87$**) were also linearly related to increasing levels of N fertilization. Averaged across forage species, dry matter yield of forages fertilized with 200 kg N ha^{-1} with no flooding were statistically comparable with plants that were fertilized with 200 kg N ha^{-1}and flooded for 84 d. Our results support our hypothesis that the negative impact of flooding could be mitigated by N fertilization.**

Increasing the availability and improving the quality of surface water in south Florida by temporarily flooding previously drained pastureland is one of the goals of Northern Everglades Restoration Initiative (Dahm et al., 1995; Shukla, 2004). However, flooding can have catastrophic impacts on the productivity of grassland pastures in south Florida, as most forage species are intolerant to excess water. In grasslands, waterlogging is frequently associated with other stresses, such as grazing, which may require specific and very different adaptive strategies and management (Naidoo and Mundree, 1993; Rubio et al., 1995). These adaptive strategies are not well understood and may still warrant extended investigations.

The negative impact of flooding on terrestrial plant could be a consequence of the slow diffusion rates of gases in water compared with in air and low solubility of O_2 in water (Jackson, 1985; Gaynard and Armstrong, 1987; Crawford, 1992; Armstrong et al., 1994; Armstrong and Drew, 2002; Voesenek et al., 2006). Plants vary in their ability to withstand flooding and this undoubtedly contributes to their ecological distribution (Crawford, 1993). There are two distinctly different strategies that plants employ to survive flooding: tolerance of anoxia and avoidance of anoxia. Anoxia tolerance involves metabolic adaptation and varies among species, plants, and tissues. Anoxia avoidance requires the development of mechanisms to deliver oxygen to the roots through internal channels. Plants that adopt this mechanism have modified patterns of growth and are not necessarily tolerant to low atmospheric oxygen levels (Jackson and Drew, 1984). Other plants possess the ability to develop a combination of mechanisms enabling them to grow under waterlogged conditions (Kozlowski, 1984). Anaerobic conditions inhibit almost immediately the transport of nutrient ions by roots (Luttge and Pitman, 1976). This may be due to insufficient energy to maintain the activity of ion pumps. Phloem unloading in the anaerobic root ceases and transport of metabolites and growth regulators between the root and shoot are therefore impeded.

Particularly little is known about the response of forage species to the combined effect of waterlogging and the addition of nutrients (e.g., N and P). In flooded rice (*Oryza sativa* L.), positive growth responses to soil nutrient enrichment have been found (Carter et al., 1986). This suggests that fertilizer application may reverse the detrimental processes associated with flooding; relatively few studies and reports are available on the ability of N fertilizer to counteract the deleterious effects of waterlogging on terrestrial plants (Drew et al., 1979; Hodgson, 1982). The use of N fertilizer before flooding may alleviate N

G.C. Sigua and C. Chase, U.S. Department of Agriculture–Agricultural Research Service, Brooksville, FL 34601; M. Williams and J. Grabowski, U.S. Department of Agriculture–Natural Resources Conservation Service, Brooksville, FL 34601; M. Kongchum., Dep. of Agronomy and Environmental Management, Louisiana State Univ., Baton Rouge, LA 70803.
Received 15 Nov., 2011 *Corresponding author (gilbert.sigua@ars.usda.gov).

Published in Agron. J. 104:791–798 (2012)
Posted online 29 Mar. 2012.
doi:10.2134/agronj2011.0364

Abbreviations: DMY, dry matter yield; PMC, Plant Material Center.

deficiency because waterlogging causes a significant decrease in N content and rate of N accumulation in plants due to reduced root activity. Net assimilation rates and photosynthetic rates decline in plants experiencing root anaerobiosis, in part due to stomatal closure, and in part due to biochemical modifications (Trought and Drew, 1980; Jackson and Drew, 1984).

The cow-calf (*Bos taurus*) industry in subtropical United States especially in Gulf Coast states and other parts of the world depends almost totally on grazed pastures. Establishment of complete and uniform stand of bahiagrass in a short time period is important economically. Failure to obtain a good stand of forage species means loss of not only the initial investment costs, but also production and its cash value (Chambliss, 1999). In grasslands, waterlogging is frequently associated with other stresses, such as grazing, which may require specific and very different adaptive strategies and management. Our hypothesis in this study is that forage species will be negatively affected by extended flooding and N application could offset the detrimental effect of flooding on yield and crude protein content. The objectives of this study were to determine under greenhouse conditions: (i) the effect of flooding duration on DMY and crude protein content (CPC) of three important forage species (bahiagrass, limpograss, and maidencane) that are widely used in the Gulf States region and (ii) if detrimental impact of flooding can be mitigated by N fertilization.

MATERIALS AND METHODS

Soil and Plants Preparation

Soils needed for this study were obtained from a Blichton soil series (loamy, siliceous, semiactive, hyperthermic Arenic Plinthic Paleauquults) at a depth of 0 to 20 cm using a backhoe from a pasture at the USDA-ARS Subtropical Agriculture Research Station (STARS), Brooksville, FL, in 2008 and 2009. The soil was air dried outside on an impervious surface at USDA-NRCS Plant Material Center (PMC), Brooksville, FL. Before drying, 8 to 10 random samples were collected to determine selected physical and initial chemical properties (Table 1). Blichton soils are poorly drained soils, typical of south Florida with water table at a depth of less than 25 cm for cumulative periods of 1 to 4 mo during most years. These soils, like many of the soils in south Florida, have argillic and spodic horizons.

The two introduced species of subtropical grasses, bahiagrass cultivar Tifton-9 and limpograss cultivar Floralta, and one native grass, maidencane cultivar Citrus, were used in the study. All of the plant material was excavated from established stands (>5 yr old) at either STARS or the PMC. Table 2 shows some selected plant characteristics and growth requirements for bahiagrass, limpograss, and maidencane. Approximately 15 by 10 cm plugs consisting of crowns, rhizomes, and roots of each forage species (trimmed off to approximately 10-cm stubble height) were transplanted from the field 12 wk before the initiation of the study each year. The plugs were planted into 15 by 60 cm planting columns that have been filled to within approximately 15 cm of the surface with the air dried, screened (1 by 1 cm screen) soils from the A horizon of Blichton series. The planting columns were sealed at the bottom to control water movement through the column. A hole was drilled at the side of the column and fitted with a drain tube with a stopcock to allow draining and sampling of the soil solution.

Table 1. Selected physical and chemical properties of soils used in the study.

Soil properties	Unit	Value
Particle size		
Sand	g kg^{-1}	868
Silt	g kg^{-1}	75
Clay	g kg^{-1}	57
Hydraulic conductivity	cm h^{-1}	7.0
Bulk density	kg m^{-3}	1.4
CEC	cmol kg^{-1}	11.2
Ca	mg kg^{-1}	2.9
Mg	mg kg^{-1}	0.5
Na	mg kg^{-1}	0.1
K	mg kg^{-1}	0.1
pH		5.5

Table 2. Selected plant characteristics of the different forage species used in the study.

Plant characteristics	Bahiagrass	Limpograss	Maidencane
1. Duration	perennial	perennial	perennial
2. Growth habit	graminoid	graminoid	graminoid
3. Anaerobic tolerance	low	medium	high
4. Drought tolerance	high	low	none
5. Fertility requirement	high	high	medium
6. Moisture use	low	high	high
7. pH, minimum	4.5	5.5	4.7
8. pH, maximum	6.5	7.5	8.6
9. Salinity tolerance	low	none	none
10. Shade tolerance	intermediate	intolerant	intermediate

Greenhouse and Experimental Treatments

Immediately after planting, the columns with open drain tubes were moved into the greenhouse (22°/32°C, 69% direct light) at the PMC and allowed to recover and grow during the 12 wk pretrial period. During the first 4 wk of the pretrial period, the plants were fertilized with a soluble complete fertilizer equivalent to 23 kg total of N, P, and K, and then fertilization discontinued. During the remainder of the adjustment periods (12 wk), the plants were watered as needed to maintain the soil moisture approximately at field capacity.

Treatments were replicated five times using a 3 × 5 × 3 split-split plot arrangement in completely randomized block design. Forage types were the main treatment effect while flooding duration and N levels were the subplots features of a 2-yr greenhouse study conducted in 2008 and 2009, respectively. All columns received 40 kg ha^{-1} of P as triple superphosphate granular fertilizer and appropriate N treatments consisted of 0, 100, and 200 kg ha^{-1} of N as NH_4NO_3 were applied to the appropriate columns for each forage species. The soil flooding duration was consisted of 0, 14, 28, 56, and 84 d to mimic flooding occurrences in south Florida that may be associated with the need to store rainfall on pastureland during summer in Florida. Flooding treatments were staggered such that termination of all flooding duration times coincided with the maximum flooding time of 84 d. For plants not receiving flood treatment, soil moisture was maintained at soil field capacity limit. Until a flooding treatment was started, all drain tubes remained open and the treatments were watered.

Dry Matter Yield and Tissue Analysis

All treatments were destructively sampled at the end of maximum flooding time treatment of 84 d. Freshly cut aboveground growth was oven-dried at 60°C for 24 h at the USDA-ARS Laboratory in Brooksville, FL. Plant samples were ground to pass through a 1-mm mesh screen in a Wiley mill. Ground forage was analyzed for total Kjeldahl N concentration (Gallagher et al., 1976) at the University of Florida Analytical Research Laboratory, Gainesville, FL. All the needed protocols and measurements for dry matter yield and tissue analysis were performed in similar fashion during the 2008 and 2009 cropping/harvest season, respectively.

Redox Potential Measurements

Reduction-oxidation (redox) potential of the soil solution was determined in 2008 (22 June–4 September) and in 2009 (23 June–5 September) to monitor anaerobic condition of soils under varying soil wetness. The 2-yr (2008–2009) average of redox potentials was reported in this paper. Soil redox potential measurements have been used to characterize the intensity of reduction and oxidation and relate this to biological processes occurring in flooded soils. Measurement equipment consisted of three pieces of equipment: platinum electrode, reference electrode, and voltmeter. The platinum electrode and the reference electrode (calomel: Ag/AgCl) were both buried into the soil column (7–10-cm depths) to be in contact with the soil solution. Wires from both the platinum electrode and reference electrode were connected to a voltmeter. The redox potential (Eh) is calculated by adding a value of +245 to the reading from the voltmeter. Our measurement of redox potentials was based on methods published by Patrick et al. (1996).

Statistical Analysis

The effects of flooding and N application on yield and crude protein contents of three forage species in 2008 and 2009, respectively, were analyzed statistically following the PROC GLM procedures (SAS, 2000). Where the *F* test indicated a significant ($P \leq 0.05$) effect, means were separated, following the method of Duncan Multiple Range test, using appropriate mean squares (SAS, 2000). For *F* test results that were highly significant, means were separated using LSD test (SAS, 2000).

RESULTS AND DISCUSSION

Effects on Dry Matter Yield

Dry matter yield was affected ($P \leq 0.001$) by the interaction effect of forage species, flooding duration, and levels of N (Table 3). The greatest dry matter yield for bahiagrass of 11.8 t ha^{-1} was from the control tube (0 d flooding) that was fertilized with 200 kg N ha^{-1} while the least amount of dry matter yield was from tube that was flooded for 84 d and fertilized with 0 kg N ha^{-1} (Fig. 1). The least amount of dry matter yield for limpograss (5.2 t ha^{-1}) was from the control tube with no N application (0 kg N ha^{-1}) while the greatest dry matter yield (19.3 t ha^{-1}) was from tube that was flooded for 28 d with 200 kg N ha^{-1} (Fig. 1). The greatest amount of dry matter yield for maidencane of 4.2 t ha^{-1} was from the control tube with no N fertilization while the greatest dry matter yield of 15.8 t ha^{-1} was from tube that was flooded for 84 d with 200 kg N ha^{-1} (Table 3). These results are suggesting that dry matter yield of the three forage species was not affected by flooding duration, but varied significantly with nitrogen fertilization. Our results support our hypothesis that detrimental impact of flooding could be mitigated by N fertilization. Results disclosed an overwhelming effect of N application on dry matter yield. This claim was exhibited by a much higher dry matter yield under any flooding duration of plants that received higher N application as opposed to those plants without N fertilization (Fig. 1).

Table 3. Crude protein content of bahiagrass, limpograss, and maidencane as affected by different flooding duration and different levels of N fertilization.

Flooding duration	Nitrogen	Bahiagrass	Limpograss	Maidencane
d	kg N ha^{-1}		%	
0	0	5.63 ± 0.19c†	3.02 ± 0.34cd	3.94 ± 0.48d
	100	4.82 ± 0.52c	4.04 ± 0.76abcd	7.22 ± 1.41bc
	200	5.10 ± 0.32c	5.10 ± 0.52ab	8.16 ± 1.03b
14	0	5.43 ± 0.89c	2.85 ± 0.26cd	4.59 ± 0.51cd
	100	5.46 ± 0.39c	4.55 ± 0.72abc	8.96 ± 0.71ab
	200	5.48 ± 0.43c	5.21 ± 0.76ab	7.21 ± 0.68bc
28	0	6.10 ± 0.87c	2.62 ± 0.27d	3.54 ± 0.25d
	100	9.70 ± 1.24a	3.56 ± 0.24bcd	9.12 ± 1.10ab
	200	4.74 ± 1.32c	5.48 ± 0.57a	11.13 ± 2.26a
56	0	6.41 ± 0.85c	2.52 ± 0.47d	2.98 ± 0.2.27d
	100	10.06 ± 1.19a	3.76 ± 0.28bcd	5.05 ± 0.78cd
	200	9.47 ± 0.78a	4.07 ± 0.39abcd	8.14 ± 0.79b
84	0	9.18 ± 0.87ab	3.04 ± 0.46cd	2.34 ± 0.26d
	100	8.92 ± 0.67ab	2.76 ± 0.11d	4.60 ± 0.16cd
	200	6.79 ± 0.56bc	3.62 ± 0.94bcd	4.79 ± 0.14cd

Dry matter yield crude protein content

Sources of variation	F values	
Plant (P)	43.04‡	91.11‡
Flooding (F)	1.62ns§	13.40‡
Nitrogen (N)	148.03‡	90.35‡
P × F	5.93***	2.65**
P × N	2.0**	7.82‡
P × F × N	2.07***	2.38**

** Significant at $p \leq 0.01$.

*** Significant at $p \leq 0.001$.

† Means in column under each subheading followed by the same letter(s) are not significantly different from each other at $p \leq 0.05$.

‡ Significant at $p \leq 0.0001$.

§ ns, not significant.

Dry matter yield of forage species were relatively comparable across flooding duration (Fig. 2). These forages can be identified as tolerant to waterlogging. The main effect of flooding on plant functioning can be mimicked by exposing roots to subambient partial pressures of O_2, indicating that O_2 shortage is one of the most important constraints during flooding (Blom and Voesenek, 1996). Quite crucial to the interpretation of our results is the limited amount of oxygen that may be present following flooding. Oxygen level was indirectly measured using the reduction-oxidation potentials (redox). Redox potential is an electrical measurement that shows the tendency of a soil solution to transfer electrons to or from a reference electrode. Measurement of soil redox can estimate whether the soil aerobic or in anaerobic condition. Figure 3 shows the different levels of redox potential readings from soils that were flooded from 0 to 84 d. Redox potentials (Eh values, mv) of the

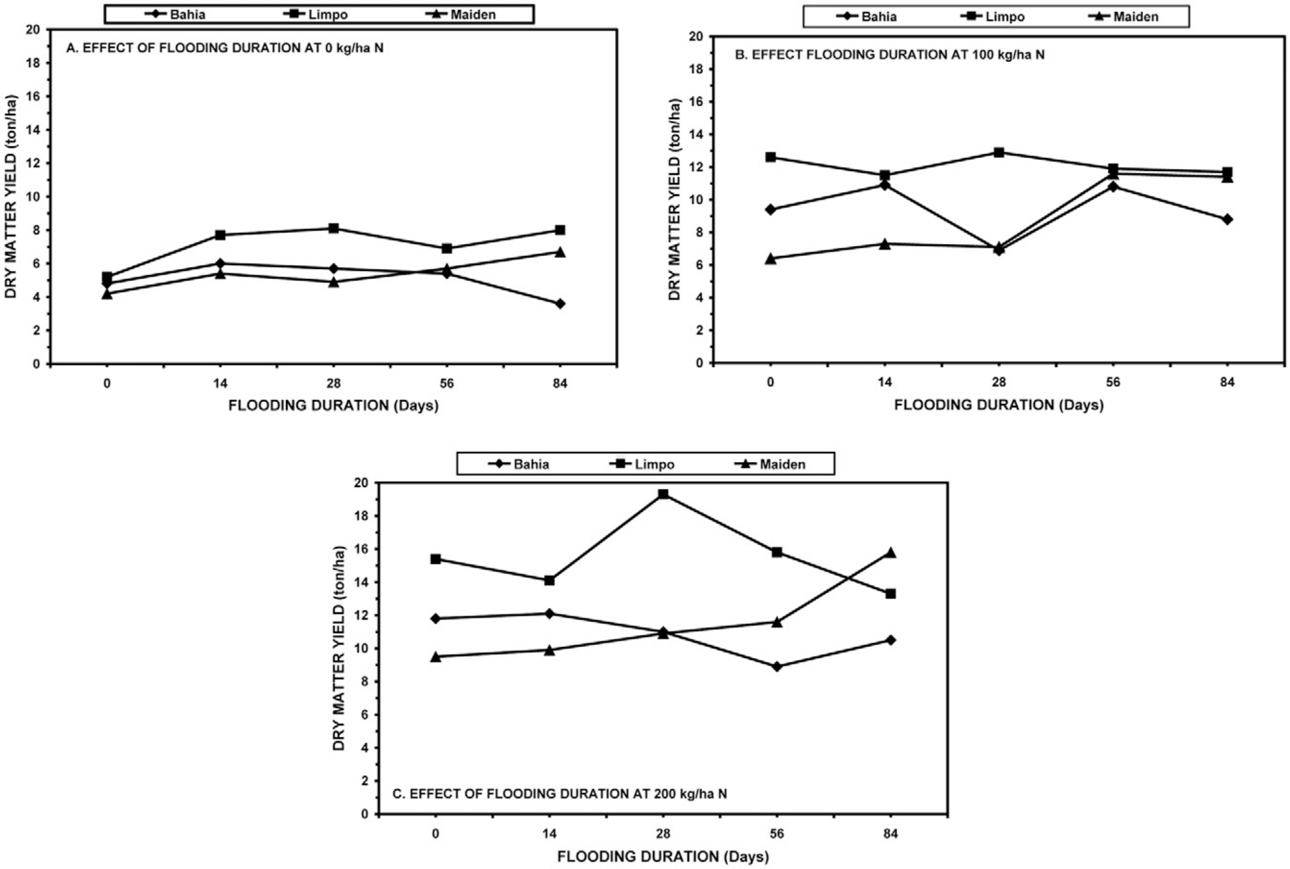

Fig. 1. **Dry matter yield of three forage species as affected by flooding duration at different levels of N fertilization (A: 0 kg N ha^{-1}; B: 100 kg N ha^{-1}; C: 200 kg N ha^{-1}).**

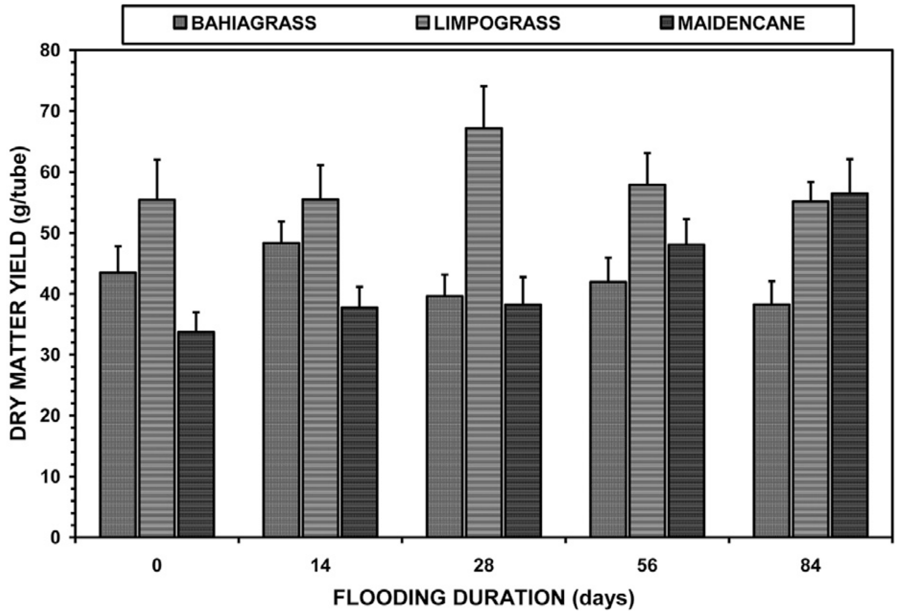

Fig. 2. Dry matter yield of three forage species as affected by different flooding duration.

soils that were flooded from 0 to 84 d ranged from 400mv to −150mv. Except for the nonflooded (control) soils, all the soils attained the stage of anaerobic conditions between 8 and 10 d and became fully anaerobic thereafter. An Eh reading below 0mv would mean limited supply of oxygen in the soil (Fig. 3). It is generally accepted that energy deficit is one of the most severe problems encountered by plants, especially for facultative upland species like bahiagrass when subjected to flooding. Oxygen is the terminal acceptor of electrons in the oxidative phosphorylation that indirectly provides the plant with adenosine triphosphate (ATP) (Voesenek et al., 2006).

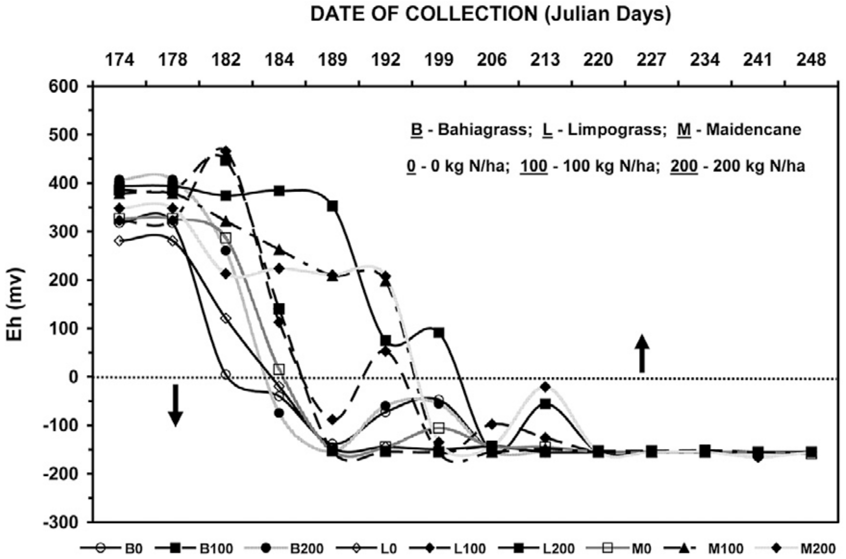

Fig. 3. Average levels of reduction-oxidation (Eh) readings during the flooding experiment.

It is important to note that the dry matter yield of the three forage species did not vary significantly with flooding duration (Table 3). While extended period of flooding did not affect total biomass of bahiagrass, limpograss, maidencane, waterlogging slightly promoted their growth. For plants to be classified as tolerant to waterlogging they must maintain their growth rate under an extended period of flooding condition (Heathcote et al., 1987; Naidoo and Naidoo, 1992). Insausti et al. (2001) reported that flooding enhanced stomatal conductance, leaf water potential, and net photosynthesis, especially under conditions leading to high air-vapor pressure deficits because flooded plants experienced fewer water deficits during periods of high atmospheric evaporative demand (e.g., greenhouse condition). Although flooding typically causes a reduction in the abundance of flood-sensitive plant species, it can also promote biomass growth in flood-tolerant species to exploit resources that otherwise would be shared with nontolerant competitors (Crawford et al., 1989; Insausti et al., 1999).

Recent studies in grasslands of Argentina found that native grasses present slight tolerant responses to flooding because of aerenchyma tissue formation and increase in plant height (Rubio et al., 1995; Loreti and Oesterheld, 1996). Aerenchyma formation and leaf elongation are important for the recovery of contact with aerial environment and allow oxygen transport to the submerged tissues of native grasses (Laan et al., 1990; Van der Samn et al., 1991). The three forage species in our study may have the capacity for regulating leaf water and C relations under highly changing atmospheric conditions. Voesenek et al. (2006) suggested that hormonal effects were involved in growth response for plants under waterlogged conditions because photosynthesis rates could be enhanced by increased leaf temperature at higher air vapor pressure deficit in most C_4 grasses (e.g., bahiagrass) while differences in photosynthetic activities between flooded and control plants may be accounted for by the differences in stomatal conductance. Our visual observations during the experiment suggest that the aboveground biomass of forage species under waterlogged conditions were similar with nonwaterlogged forage species.

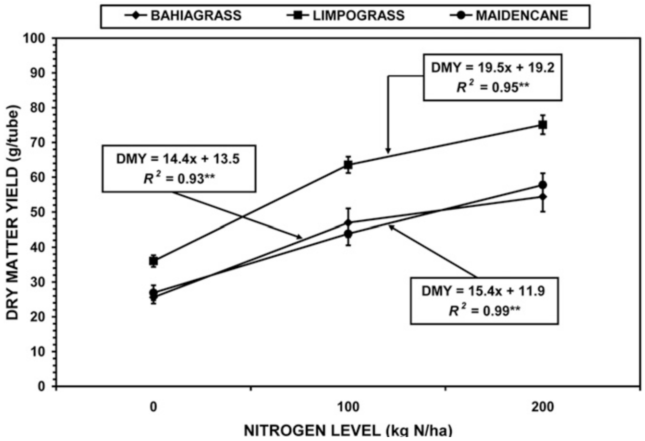

Fig. 4. Regression models describing the relationship of dry matter yield of three forage species with increasing levels of N fertilization.

The effect of N fertilization on dry matter yield is shown in Fig. 4. The amount of dry matter yield for each forage specie increased linearly with increasing amount of N fertilization. Figure 4 shows the relationships between dry matter yield and levels of N fertilization. The different regression models that described the relationship of dry matter yield with levels of N (x) are given below:

$$\mathrm{DMY}_{\mathrm{Bahiagrass}} = 14.4x + 13.5; \; R^2 = 0.93^{**} \quad [1]$$

$$\mathrm{DMY}_{\mathrm{Limpograss}} = 19.5x + 19.2; \; R^2 = 0.95^{**} \quad [2]$$

$$\mathrm{DMY}_{\mathrm{Maidencane}} = 15.4x + 11.9; \; R^2 = 0.99^{**} \quad [3]$$

Our results show that the three forage species have the ability to tolerate waterlogging. This is a tool not only to survive in such an environment, but also to respond to growth stimulating factors, such as N fertilization. We can assume that soil N availability was quite similar in both waterlogged and nonwaterlogged treatments. Nitrogen fertilization indicated a positive result on dry matter yield of the three forage species (Fig. 1, Fig. 4). Differences in dry matter yield among the three forage species as affected by N fertilization can be considered an adaptation to improve their nutrient uptake efficiency (Atkinson, 1973; Aerts et al., 1991). Results are suggesting that waterlogging does not produce detrimental effect either in the growth of these forage species or in their response capacity to stimulating growth factors, such as N fertilization.

Despite potential environmental effect of higher N fertilization, fertilizer applications have improved the tolerance of forage grasses to waterlogging. Application of N at higher rate (200 kg N ha^{-1}) had resulted to higher dry matter yield and this could be associated with higher nutrient requirements (especially N) for bahiagrass, limpograss, and maidencane (Table 2). The concern for potential losses of applied N could be diminished because of the effect of plant density. While plant density of bahiagrass, limpograss, and maidencane with higher rates of N application resulted in much higher plant density when compared with the control plants, higher plant density can decrease light penetration and potentially reduce algal activity and thus maintain the pH near neutral conditions. Near neutral pH conditions among these forage species under flooded condition would not result to ammonia volatilization. When the floodwater and soil are alkaline (more OH$^-$), more ammonium ions react with OH^{-1}, resulting in the production of unionized ammonia. Thus, for volatilization to occur, ammonia should be in aqueous phase in the water column.

Effects on Crude Protein Content

The CPC varied widely ($P \leq 0.001$) and was significantly affected by the interaction effects of forage species, flooding duration, and levels of N (Table 3). Crude protein contents of three forage species were also significantly affected by the main treatment effects and interaction effects of flooding duration ($P \leq 0.001$) and levels of nitrogen ($P \leq 0.001$). Interaction of forage species, flooding duration, and levels of nitrogen on CPC are shown in Table 3. The greatest amount of CPC in bahiagrass (10.1%) was from the control tube (0 d flooding) with 200 kg N ha^{-1} (Table 3). The highest amount of CPC for limpograss (5.2%) was from plants flooded for 14 d with 200 kg N ha^{-1}. The greatest amount of CPC for maidencane (11.1%) was from plants flooded for 28 d with 200 kg N ha^{-1} (Fig. 5).

It appears that forage plants that were fertilized with higher amounts of N have significantly higher amounts of CPC when compared to those plants with no or less amount of applied N under any flooding duration. These results again support the hypothesis of the study on the offsetting effect of N on the detrimental effect of flooding on CPC. The use of N fertilizer before flooding may alleviate N deficiency because waterlogging causes a significant decrease in N content and rate of N accumulation in plants due to reduced root activity. Net assimilation rates and photosynthetic rates decline in plants experiencing root anaerobiosis, in part due to stomatal closure, and in part due to biochemical modifications (Trought and Drew, 1980; Jackson and Drew, 1984).

The effect of N fertilization on CPC is shown in Fig. 6. The amount of CPC for each forage specie increased linearly with increasing amount of N fertilization (Fig. 6). These results are likely to be a forage species response. The individual genetic composition and metabolic behavior for each species of forage would have an influence on these results. Differences in CPC among the three forage species as affected by N fertilization can be considered an adaptation to improve their nutrient uptake efficiency, and possibly may have had affected crude protein formation (Atkinson, 1973; Aerts et al., 1991). Nitrogen fertilization of pasture forages generally increases digestibility. Early reports (Hart et al., 1965; Mathias et al., 1973; Horn and Taliaferro, 1974; Barth et al., 1982) claimed that on bermudagrass pastures, CPC of the forage increased with each increment in N fertilization up to 504 kg ha^{-1}. The different regression

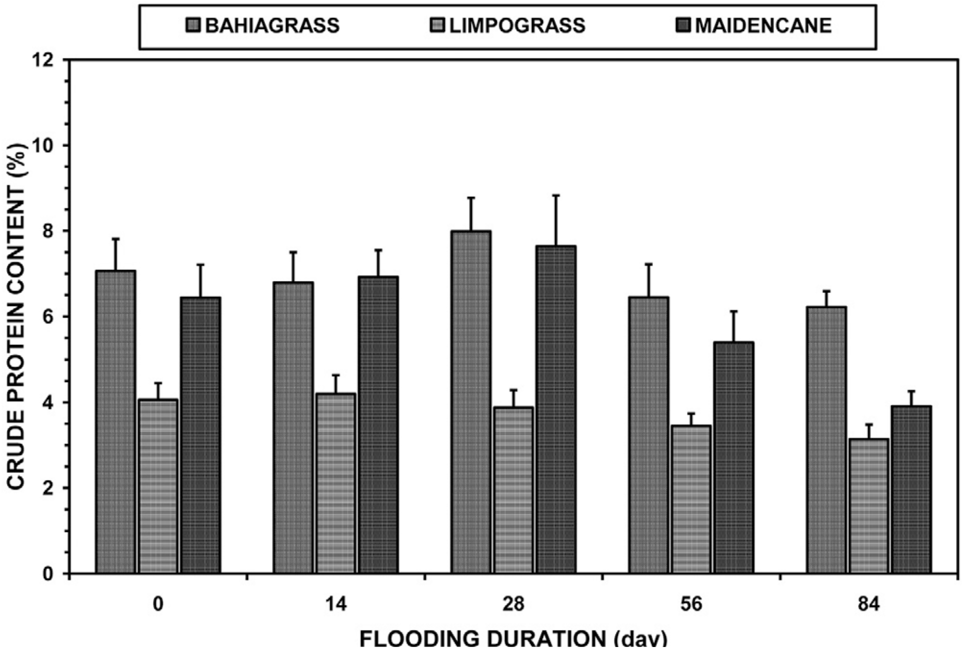

Fig. 5. Crude protein contents of three forage species as affected by flooding duration.

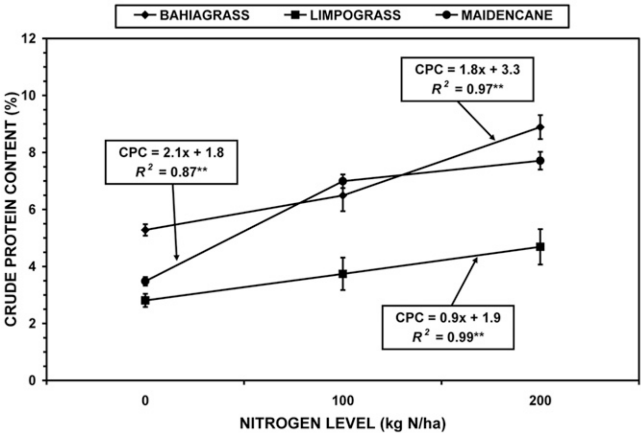

Fig. 6. Regression models describing the relationship of crude protein contents of three forage species with increasing levels of N fertilization.

models that described the relationship of CPC with levels of N (*x*) are given below:

$$\mathrm{CPC}_{\mathrm{Bahiagrass}} = 1.8x + 3.3;\ R^2 = 0.97^{**} \qquad [4]$$

$$\mathrm{CPC}_{\mathrm{Limpograss}} = 2.1x + 1.8;\ R^2 = 0.87^{**} \qquad [5]$$

$$\mathrm{CPC}_{\mathrm{Maidencane}} = 0.9x + 1.9;\ R^2 = 0.99^{**} \qquad [6]$$

Our results were similar to the early findings of Rubio et al. (1995) who reported that the nutrient demand of waterlogging-tolerant plants is supposed to be high under soil anoxia because plants are able to maintain or even increase their biomass production and nutrient uptake efficiency. Rubio et al. (1995) observed a reduction in root/shoot ratio of two grasses (*Paspalum dilatatum* and *Danthonia montevidensis*) caused by waterlogging did not have a cost in terms of capacity for nutrient uptake. As observed, CPC of forage species was positively affected by N fertilization despite of anoxic environment and these results could be somehow explained by sufficient nutrients that compensate for the negative effect of waterlogging (Fig. 6). Earlier findings of Chapin (1980) and Struik and Bray (1970) showed that in nutrient-rich environments, root systems can satisfy plant nutrient requirements resulting in normal metabolic activities of plants including crude protein formation.

An early published report of Sigua and Hudnall (1992) confirmed the importance of gypsum and N fertilization on protein contents of four specie of wetland vegetation under saline environment. Their results disclosed highly significant protein content responses to gypsum and N additions. The beneficial effect of N fertilization on crude protein content of the three forage specie suggest the positive impact of N on keeping a good quality of forage even under waterlogged condition. Increased growth, yield, and protein content were observed from the fertilized plants.

SUMMARY AND CONCLUSION

To determine the potential ecological impact of periodic flooding on plant growth and protein content, three species of subtropical grasses: Tifton 9 bahiagrass; Floralta limpograss; and "Citrus" maidencane were evaluated under differing flooding durations and levels of N fertilization under greenhouse conditions in 2008 and 2009, respectively. The overall results and observations in this study could be briefly summarized as follows:

1. Results disclosed that yield and crude protein content (averaged across years) varied significantly with flooding durations and levels of N fertilization;
2. The individual yield response and crude protein content of each forage species (averaged across years) was linearly related to increasing levels of N application; and
3. Our results support our hypothesis that detrimental impact of flooding could be mitigated by N fertilization. Since fertilizer applications have improved the tolerance of forage grasses to waterlogging, the market value of environmental service of water storage on south Florida pastureland should be adjusted to include additional fertilizer costs.

Our results will help to increase insight into processes acting in plant communities under changing hydrological conditions could have had valuable application to many areas all over the world that were suffering from flooding. Knowledge of adaptive responses of different forages to flooding and the potential off-setting effect of N fertilization would be valuable tool in restoring damaged areas as a result of changing hydrological conditions in Florida and other Gulf state region.

REFERENCES

Aerts, R., R.G.A. Boot, and P.J.M.van der Aart. 1991. The relation between above- and belowground biomass allocation patterns and competitive ability. Oecologia 87:551–559. doi:10.1007/BF00320419

Armstrong, W., R. Brandle, and M.B. Jackson. 1994. Mechanisms of flood tolerance in plants. Acta Botanica Neerlandica 43: 307–358.

Armstrong, W., and M.C. Drew. 2002. Root growth and metabolism under oxygen deficiency. In: Y. Waisel, A. Eshel, and U. Kafkfi, editors, Plant roots: The hidden half. Marcel Dekker, New York. p. 761–792.

Atkinson, D. 1973. Some general effects of phosphorus deficiency on growth and development. New Phytol 72:101–111. doi:10.1111/j.1469-8137.1973.tb02014.x

Barth, K.M., J.B. McLaren, H.A. Fribourg, and L.A. Carver. 1982. Crude protein content of forage consumed by steers grazing nitrogen-fertilized. Bermuda grass and Orchard Grass-Ladino clover pastures. J. Anim. Sci. 55:1008–1014.

Blom, C.W.P.M., and L.A.C.J. Voesenek. 1996. Flooding: The survival strategies of plants. Tree. 11(7): 290–295.

Carter, M.F., P.L.G. Vlek, and J.T. Touchton. 1986. Agronomic evaluation of new urea forms for flooded rice. Soil Sci. Soc. Am. J. 50:1055–1060. doi:10.2136/sssaj1986.03615995005000040044x

Chambliss, C.G. 1999. Bahiagrass. In: C.G. Chambliss, editor, Florida forage handbook. Univ. of Florida, Gainesville. p. 17–23.

Chapin, F.S. 1980. The mineral nutrition of wild plants. Annual reviews of ecology and systematics 11:233–260. doi:10.1146/annurev.es.11.110180.001313

Crawford, R.M. 1993. Plant survival without oxygen. Biologist 40:110–114.

Crawford, R.M. 1992. Oxygen availability as an ecological limit to plant distribution. Adv. Ecol. Res. 23:93–185. doi:10.1016/S0065-2504(08)60147-6

Crawford, R.M., C. Studer, and K. Studer. 1989. Deprivation indifference as a survival strategy in competition: Advantages and disadvantages of anoxia tolerance in wetland vegetation. Flora 182:189–201.

Dahm C.N., K.W. Cummins, H.M. Valett, and R.L. Colemen. 1995. An ecosystem view of the restoration of the Kissimmee River. Restor Ecol. 3: 225–238. doi:10.1111/j.1526-100X.1995.tb00172.x

Drew, M.C., E.J. Sisworo, and L.R. Saker. 1979. Alleviation of waterlogging damage to young barley plants by application of nitrate and synthetic cytokinin. New. Phytol. 82:315–329. doi:10.1111/j.1469-8137.1979.tb02657.x

Gallagher, R.N., G.O. Weldon, and F.C. Boswell. 1976. A semi-automated procedure for total nitrogen in plant and soil samples. Soil Sci. Soc. Am. Proc. 40:887–889.

Gaynard, T.J., and W. Armstrong. 1987. Some aspects of internal plant aeration in amphibious habitats. In: R.M.M. Crawford, editor, Plant life in aquatic and amphibious habitats. Blackwell Scientific Publ., Oxford, UK. p. 303–320.

Hart, R.H., G.W. Burton, and J.E. Jackson. 1965. Seasonal variation in chemical composition and yield of Coastal bermudagrass as affected by nitrogen fertilization schedule. Agron. J. 57:381–385. doi:10.2134/agronj1965.00021962005700040022x

Heathcote, C.A., M.S. Davies, and J.R. Etherington. 1987. Phenotypic flexibility of *Carex flacca* (Shreb) tolerance of soil flooding by populations from contrasting habitats. New Phytol. 105:381–392. doi:10.1111/j.1469-8137.1987.tb00875.x

Hodgson, A.S. 1982. The effects of duration, timing and chemical amelioration of short-term waterlogging during furrow irrigation of cotton in cracking gray soil. Aust. J. Agr. Res. 33:1019–1028. doi:10.1071/AR9821019

Horn, F.P., and C.M. Taliaferro. 1974. Yield, composition, and IVDMD of four bermudagrass. J. Anim. Sci. 38:224 (Abstract).

Insausti, P., E.J. Chaneton, and A. Soriano. 1999. Flooding reverted grazing effects on plant community structure in mesocosms of lowland grassland. Oikos 84:266–276. doi:10.2307/3546721

Insausti, P., A.A. Grimoldi, E.J. Chaneton, and V. Vasellati. 2001. Flooding induces a suite of adaptive plastic responses in the grass *Paspalum dilatatum*. New Phytologist 152:291–299. doi:10.1111/j.0028-646X.2001.257_1.x

Jackson, M.B. 1985. Ethylene and the responses of plants to soil waterlogging and submergence. Annual Review of Plant Physiol. 36:145–174. doi:10.1146/annurev.pp.36.060185.001045

Jackson, M.B., and M.C. Drew. 1984. Effects of flooding on growth and metabolism of herbaceous plants. In: E.T. Kozlowski, editor, Flooding and plant growth. Academic Press, Orlando, FL. p. 47–128.

Kozlowski, T.T. 1984. Plant responses to flooding. BioScience 34: 162–167. doi:10.2307/1309751

Laan P., M. Tosserama, C.W.P.M. Blom, and B.W. Veen. 1990. Internal oxygen transport in *Rumex* species and its significance for respiration under hypoxic conditions. Plant Soil 122: 39–46. doi:10.1007/BF02851908

Loreti, J., and M. Oesterheld. 1996. Intraspecific variation in the resistance to flooding and drought in populations of *Paspalum dilitatum* from different topographic positions. Oecologia 92: 279–284.

Luttge, V., and M.G. Pitman. 1976. Transport and energy. Encyclopedia Plant Physiol. New Ser. 2A:251–259.

Mathias, E.L., O.L. Bennett, and P.E. Lundberg. 1973. Effect of rates of nitrogen on yield, nitrogen use, and winter survival of Midland bermudagrass (*Cynodon dactylon*, L) in Appalachia. Agron. J. 65:65–67.

Naidoo, G., and S.G. Mundree. 1993. Relationship between morphological and physiological responses to waterlogging and salinity in *Sporobolus virginicus* (L). Oecologia 93:360–366. doi:10.1007/BF00317879

Naidoo, G., and S. Naidoo. 1992. Waterlogging responses of *Sporobolus virginicus* (L). Oecologia 90:445–450. doi:10.1007/BF00317704

Patrick, W.H., R.P. Gambrell, and S.P. Faulkner. 1996. Redox measurements of soils. In: D.L. Sparks et al., editors, Methods of soil analysis. Part 3: Chemical methods. SSSA, Madison, WI. p. 1255–1273.

Rubio, G., G. Casasola, and R.S. Lavado. 1995. Adaptation and biomass production of two grasses in response to waterlogging and soil nutrient enrichment. Oecologia 102:102–105.

SAS Institute. 2000. SAS/STAT user's guide. Release 6.03. SAS Inst., Cary, NC.

Shukla, S. 2004. Watersheds: Functions and management. Fact Sheet ABE350. Dep. Agric. and Biological Engin., Univ. of Florida, IFAS, Coop. Ext. Serv., Gainesville. http://edis.ifas.ufl.edu/AE265 (accessed 6 Sept. 2011).

Sigua, G.C., and W.H. Hudnall. 1992. Nitrogen and gypsum: Management tools for revegetation and productivity improvement of brackish marsh in southwest Louisiana. Commun. Soil Sci. Plant Anal. 23: 283–299. doi:10.1080/00103629209368588

Struik, G., and J.R. Bray. 1970. Root-shoot ratios of native forest herbs and Zea mays at different soil-moisture levels. Ecology 51:892–893. doi:10.2307/1933983

Trought, M.C., and M.C. Drew. 1980. The development of waterlogging in wheat seedlings. I. Shoot and root growth in relation to changes in the concentration of dissolved gases and solutes in the soil solution. Plant Soil 54:77–94. doi:10.1007/BF02182001

Van der Samn, A.J.M., L.A.C.J. Voesenek, C.W.P.M. Blom., F.J.M Harren, and J. Reuss. 1991. The role of ethylene in shoot elongation with respect to survival and seed output of flooded *Rumes maritimus* L. plants. Functional Ecology 5: 304–313. doi:10.2307/2389269

Voesenek, L.A.C.J., T.D. Colmer, R. Pierik, F.F. Millenaar, and A.J. M. Peeters. 2006. How plants cope with complete submergence. New Phytologist 170:213–226. doi:10.1111/j.1469-8137.2006.01692.x