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Interactive Effects of Nitrogen Fertilization and Plant Spacing on Micro-nutrient Uptake in Hybrid Napier Grass (*Pennisetum purpureum*)

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Abstract

*Optimizing agronomic management practices is critical for improving the nutritional quality of forage crops for sustainable livestock production. This study evaluated the interactive effects of nitrogen fertilization rate and plant spacing on micro-nutrient uptake in hybrid Napier grass (*Pennisetum purpureum*). The experiment was conducted at the Teaching and Research Farm of Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, Nigeria, using a 2 × 2 factorial arrangement in a randomized complete block design (RCBD) with three replicates. Treatments consisted of two nitrogen application rates (200 and 400 kg N ha⁻¹, supplied as urea) and two plant spacing (75 and 100 cm). Forage samples were harvested ten weeks after planting and analyzed for zinc (Zn), manganese (Mn), iron (Fe), and copper (Cu) concentrations. Results showed that nitrogen fertilization rate and plant spacing significantly (P < 0.05) influenced micro-nutrient uptake in hybrid Napier grass. Higher nitrogen application (400 kg N ha⁻¹) consistently enhanced Zn, Mn, Fe, and Cu concentrations compared with 200 kg N ha⁻¹. Narrower plant spacing (75 cm) resulted in significantly greater Zn, Fe, and Cu contents, while Mn concentration was not significantly affected by spacing alone. A significant interaction between nitrogen rate and plant spacing was observed, with the combination of 75 cm spacing and 400 kg N ha⁻¹ producing the highest concentrations of all evaluated micro-nutrients. These findings demonstrate that nitrogen fertilization and plant spacing interactively regulate micro-nutrient accumulation in hybrid Napier grass. Optimizing nitrogen input and plant density can therefore improve the micro-nutrient quality of forage, with positive implications for ruminant nutrition and sustainable pasture management.*

***Keywords**: Hybrid Napier grass, nitrogen fertilization, plant spacing, micronutrient uptake, forage quality*

Introduction

Elephant grass (Pennisetum purpureum) is one of the most widely cultivated perennial forage grasses in tropical and subtropical regions due to its high biomass yield, rapid regrowth, and adaptability to diverse agro-ecological conditions. It plays a critical role in ruminant feeding systems, particularly in smallholder and semi-intensive livestock production, where it is commonly used as fresh forage or conserved as silage. When grown under appropriate agronomic management, P. purpureum provides substantial dry matter yield and contributes significantly to livestock productivity (Leta et al., 2013; Asmare, 2016; FAO, 2015).

Recent breeding efforts have led to the development of hybrid Napier grass, derived from crosses between elephant grass and pearl millet, which exhibits improved forage yield, persistence, and nutritive characteristics compared with conventional cultivars. Studies have shown that hybrid Napier grass can produce higher herbage yields within short establishment periods, making it a promising option for addressing seasonal feed shortages in ruminant production systems (Nyambati et al., 2010). However, beyond biomass

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yield, the mineral composition of forage remains a critical determinant of its nutritional value, directly influencing animal health, growth, reproduction, and metabolic efficiency.

Micro-nutrients such as zinc (Zn), manganese (Mn), iron (Fe), and copper (Cu) are essential components of enzymatic systems, immune responses, oxygen transport, and reproductive functions in ruminant animals. Deficiencies or imbalances in these elements can limit animal performance even when forage biomass and crude protein levels are adequate. Consequently, understanding how agronomic practices influence micro-nutrient uptake and accumulation in forage crops is fundamental to improving livestock nutrition and sustainable pasture management (Asmare et al., 2016; FAO, 2015).

Nitrogen fertilization is widely recognized as a major factor controlling forage productivity and nutritional quality. Nitrogen enhances vegetative growth, photosynthetic capacity, and nutrient uptake efficiency, and has been shown to influence both macro- and micro-nutrient concentrations in forage grasses (Ibeawuchi et al., 2008; Ishii et al., 2005). However, excessive or poorly managed nitrogen inputs may alter nutrient balance, underscoring the need for optimized application rates that maximize nutritional benefits while minimizing inefficiencies.

Plant spacing, often expressed as plant density, is another key agronomic variable affecting light interception, root development, soil nutrient exploitation, and intra-specific competition. Appropriate spacing can enhance resource use efficiency and influence nutrient accumulation patterns in forage crops (Ansah et al., 2010; Mihret et al., 2018). Despite its importance, plant spacing is frequently optimized for biomass yield alone, with limited consideration of its effects on forage mineral composition.

Although several studies have examined the effects of nitrogen fertilization or plant spacing on forage yield and chemical composition, information on their interactive effects on micro-nutrient uptake in hybrid Napier grass remains limited, particularly under tropical field conditions. Given the increasing demand for nutritionally balanced forage to support sustainable livestock production, there is a need to quantify how nitrogen application rates and plant spacing jointly influence micro-nutrient accumulation in this important forage species.

Therefore, this study investigated the interactive effects of nitrogen fertilization rate and plant spacing on the uptake of zinc, manganese, iron, and copper in hybrid Napier grass (Pennisetum purpureum). The findings aim to provide agronomic insights that support improved forage nutritional quality and informed pasture management decisions for ruminant production systems.

Materials and Methods

Study Area

The experiment was conducted at the Teaching and Research Farm of Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, Nigeria, located within the derived savanna agro-ecological zone. The area is characterized by a tropical climate with distinct wet and dry seasons, which is suitable for forage crop production. Prior to plot establishment, baseline soil characteristics were determined to assess soil fertility status.

Experimental Design and Treatments

The study employed a 2 × 2 factorial arrangement in a randomized complete block design (RCBD) with three replications. The factors consisted of two nitrogen fertilization rates and two plant spacing. Nitrogen was applied at rates of 200 and 400 kg N ha^{-1}, supplied in the form of urea. Plant spacing treatments were 75 cm and 100 cm between plants.

The treatment combinations were as follows:

200 kg N ha^{-1} with 75 cm plant spacing

200 kg N ha^{-1} with 100 cm plant spacing

400 kg N ha^{-1} with 75 cm plant spacing

400 kg N ha^{-1} with 100 cm plant spacing

Each treatment was replicated three times, resulting in a total of twelve experimental plots.

Land Preparation and Crop Establishment

The experimental field measured approximately 37 m × 15 m and was cleared, ploughed, and leveled prior to plot layout. Twelve plots measuring 4 m × 4 m were established, with 1 m alleys between plots to minimize edge effects. Stem cuttings of hybrid Napier grass (Pennisetum purpureum) were sourced from a certified pasture research farm in Adamawa State, Nigeria. Stem cuttings were initially established in a nursery and irrigated daily to ensure uniform sprouting. Nine days after planting, viable seedlings were transplanted to the prepared plots according to the designated plant spacing treatments. Nitrogen fertilizer was applied two weeks after transplanting as a basal application. Weed control was carried out manually throughout the experimental period to minimize competition for nutrients, water, and light.

Soil Sampling and Analysis

Soil samples were collected prior to fertilizer application using a soil auger at a depth of 0–15 cm. Within each replicate, sub samples were taken from five randomly selected locations and composited to obtain a representative sample. Soil samples were air-dried, sieved, and analyzed following standard analytical procedures as described by the Association of Official Analytical Chemists and the Agricultural Experimental Station protocols (AES, 1998).

Forage Sampling and Dry Matter Determination

Forage samples were harvested ten weeks after planting. A 1 m × 1 m quadrant was randomly placed within each plot, and all above-ground biomass within the quadrant was harvested. Samples were weighed fresh and subsequently oven-dried at 60 °C for 72 h to determine dry matter content.

Micro-nutrient Analysis

Dried forage samples were milled to pass through a 1 mm sieve prior to chemical analysis. Micronutrient concentrations, including zinc (Zn), manganese (Mn), iron (Fe), and copper (Cu), were determined following wet digestion procedures as described by Sahrawi *et al.* (2002). Elemental concentrations

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were quantified using appropriate spectrometric techniques, and results were expressed on a dry matter basis (mg kg^{-1}).

Statistical Analysis

Data obtained were subjected to analysis of variance (ANOVA) using the General Linear Model procedure of SPSS software (version 20.0). The effects of nitrogen fertilization rate, plant spacing, and their interaction were tested. When significant differences were detected ($P < 0.05$), treatment means were separated using Duncan's Multiple Rang

Results

Plant spacing significantly influenced the micro-nutrient composition of hybrid Napier grass (*Pennisetum purpureum*) (Table 1). Forage grown at 75 cm spacing recorded significantly higher ($P < 0.05$) concentrations of zinc (Zn), iron (Fe), and copper (Cu) compared with plants grown at 100 cm spacing. Specifically, Zn concentration increased from 49.87 mg kg^{-1} at 100 cm spacing to 52.03 mg kg^{-1} at 75 cm spacing, while Fe concentration increased from 148.98 to 156.27 mg kg^{-1}. Copper concentration followed a similar trend, with higher values observed at the narrower spacing.

In contrast, manganese (Mn) concentration was not significantly affected ($P > 0.05$) by plant spacing, although slightly higher numerical values were recorded at 75 cm spacing. These results indicate that closer plant spacing enhances the accumulation of certain micro-nutrients, particularly Zn, Fe, and Cu, in hybrid Napier grass. Nitrogen fertilization rate had a significant effect ($P < 0.05$) on all evaluated micro-nutrients (Table 1). Application of 400 kg N ha^{-1} resulted in significantly higher concentrations of Zn, Mn, Fe, and Cu compared with the 200 kg N ha^{-1} treatment. Zinc concentration increased from 47.28 mg kg^{-1} at 200 kg N ha^{-1} to 54.63 mg kg^{-1} at 400 kg N ha^{-1}.

While Fe concentration increased from 147.74 to 157.51 mg kg^{-1}. Similarly, Mn concentration increased from 25.88 to 30.32 mg kg^{-1}, and Cu concentration increased from 12.96 to 13.31 mg kg^{-1} with higher nitrogen application. These findings demonstrate a positive response of micro-nutrient uptake to increased nitrogen availability in hybrid Napier grass. A significant interaction ($P < 0.05$) between nitrogen fertilization rate and plant spacing was observed for Zn, Mn, Fe, and Cu concentrations (Table 1). The combination of 75 cm plant spacing with 400 kg N ha^{-1} consistently produced the highest micro-nutrient concentrations across all measured elements. Under this treatment, Zn, Mn, Fe, and Cu concentrations reached 56.17, 31.31, 162.77, and 13.49 mg kg^{-1}, respectively. Conversely, the lowest micro-nutrient concentrations were generally recorded at 100 cm spacing combined with 200 kg N ha^{-1}. These results indicate that nitrogen fertilization and plant spacing interact synergistically to influence micro-nutrient accumulation in hybrid Napier grass.

Summary of Micro-nutrient Response

Overall, micro-nutrient accumulation in hybrid Napier grass increased with higher nitrogen fertilization and was enhanced under narrower plant spacing. While nitrogen application rate exerted a strong main effect on all micro-nutrients, plant spacing primarily influenced Zn, Fe, and Cu concentrations. The significant interaction between these two factors highlights the importance of integrated nutrient and spacing management for improving forage micro-nutrient quality.

Discussion

The present study demonstrates that nitrogen fertilization rate and plant spacing exert significant and interactive effects on micro-nutrient uptake in hybrid Napier grass (*Pennisetum purpureum*). The observed increases in zinc (Zn), manganese (Mn), iron (Fe), and copper (Cu) concentrations under higher nitrogen application and narrower plant spacing highlight the sensitivity of forage micro-nutrient composition to agronomic management practices. The enhanced micro-nutrient concentrations recorded at the higher nitrogen application rate (400 kg N ha^{-1}) are consistent with the established role of nitrogen in stimulating vegetative growth, root development, and nutrient absorption capacity.

Nitrogen availability has been shown to increase root surface area and metabolic activity, thereby improving the plant's ability to acquire micro-nutrients from the soil solution (Ishii *et al*., 2005). The increased uptake of Zn, Mn, Fe, and Cu observed in this study suggests that adequate nitrogen supply may enhance micro-nutrient transport and assimilation pathways in hybrid Napier grass, supporting previous findings that nitrogen fertilization influences not only biomass yield but also forage mineral quality (Ibeawuchi *et al*., 2008).

Plant spacing also played a significant role in micro-nutrient accumulation, with narrower spacing (75 cm) resulting in higher Zn, Fe, and Cu concentrations compared with wider spacing (Akinde, 2016). This response may be attributed to improved canopy structure, enhanced light interception, and more efficient utilization of soil nutrients at higher plant densities. Increased root overlap under closer spacing may promote more intensive exploitation of available soil micro-nutrients, while also influencing rhizosphere interactions that facilitate nutrient mobilization (Ansah *et al.,* 2010; Mihret et al., 2018).

The lack of a significant spacing effect on Mn concentration suggests that manganese uptake may be less sensitive to plant density or more strongly governed by soil chemical factors (Akinde, 2025). The significant interaction between nitrogen fertilization rate and plant spacing observed in this study underscores the importance of integrated agronomic management. The combination of 75 cm spacing and 400 kg N ha^{-1} consistently produced the highest concentrations of all evaluated micro-nutrients, indicating a synergistic effect between nitrogen availability and plant density.

This interaction suggests that optimal micro-nutrient uptake in hybrid Napier grass cannot be achieved through nitrogen management or spacing adjustments in isolation, but rather through coordinated optimization of both factors. From a livestock nutrition perspective, the observed micronutrient concentrations under optimal treatment combinations are

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particularly relevant. Zinc, iron, manganese, and copper are essential for enzymatic activity, immune function, metabolic regulation, and reproductive performance in ruminants.

Improved micro-nutrient content in forage can reduce the risk of mineral deficiencies, enhance feed efficiency, and potentially lower the need for mineral supplementation in grazing systems (FAO, 2015; Asmare *et al.,* 2016). Thus, agronomic practices that enhance forage mineral quality contribute directly to animal health and productivity (Akinde *et al.,* 2024). The findings of this study align with broader efforts to improve forage quality through sustainable management practices (Akinde *et al.,* 2025).

While higher nitrogen inputs increased micro-nutrient uptake, careful consideration of fertilizer rates remains necessary to balance nutritional benefits with environmental and economic concerns. Future studies could explore lower nitrogen thresholds, soil–plant nutrient dynamics, and seasonal variations to further refine fertilizer recommendations for hybrid Napier grass production. Overall, this study provides evidence that nitrogen fertilization rate and plant spacing interactively regulate micro-nutrient accumulation in hybrid Napier grass.

By identifying management combinations that enhance forage micro-nutrient quality, the results offer practical guidance for improving ruminant nutrition and promoting sustainable pasture-based production systems.

Conclusion

This study demonstrated that nitrogen fertilization rate and plant spacing significantly influence micro-nutrient uptake in hybrid Napier grass (*Pennisetum purpureum*). Higher nitrogen application (400 kg N ha^{-1}) consistently enhanced zinc, manganese, iron, and copper concentrations, indicating the important role of nitrogen availability in regulating forage micro-nutrient accumulation. Narrower plant spacing (75 cm) further improved the uptake of zinc, iron, and copper, while manganese concentration was less responsive to spacing alone.

The significant interaction between nitrogen fertilization rate and plant spacing highlights the need for integrated agronomic management. The combination of higher nitrogen input with narrower spacing was most effective for maximizing zinc, manganese, and iron concentrations, whereas copper accumulation was enhanced under closer spacing across nitrogen rates, with several treatment combinations showing comparable responses.

From a forage nutrition perspective, improving micro-nutrient concentrations through appropriate fertilizer and spacing management can contribute to better-quality forage for ruminant livestock, potentially reducing the risk of mineral deficiencies in pasture-based systems. However, nitrogen application strategies should be optimized to balance nutritional benefits with environmental and economic considerations.

Overall, the findings indicate that coordinated management of nitrogen fertilization and plant spacing offers a practical approach to improving the micro-nutrient quality of hybrid Napier grass. This information provides a useful basis for refining pasture management practices aimed at enhancing sustainable livestock production in tropical agro-ecosystems.

Table 1

Effects of nitrogen fertilization rate and plant spacing on micro-nutrient concentrations of hybrid Napier grass (*Pennisetum purpureum*)

Treatment	**Zn (mg kg^{-1})**	**Mn (mg kg^{-1})**	**Fe (mg kg^{-1})**	**Cu (mg kg^{-1})**
Plant spacing				
75 cm	52.03^a	28.83	156.27^a	14.16^a
100 cm	49.87^b	27.37	148.98^b	12.11^b
SEM	1.10	0.77	2.01	0.24
Nitrogen fertilization rate				
200 kg N ha^{-1}	47.28^b	25.88^b	147.74^b	12.96^b
400 kg N ha^{-1}	54.63^a	30.32^a	157.51^a	13.31^a
SEM	1.10	0.77	2.01	0.24
Interaction (Spacing × Nitrogen)				
75 cm × 200 kg N ha^{-1}	47.90^c	26.35^c	149.77^c	14.83^a
75 cm × 400 kg N ha^{-1}	56.17^a	31.31^a	162.77^a	13.49^a
100 cm × 200 kg N ha^{-1}	46.66^c	25.40^c	145.71^d	11.09^b
100 cm × 400 kg N ha^{-1}	53.09^b	29.33^b	152.25^b	13.12^a
SEM	1.56	1.09	2.88	0.34

Notes: Values are means of three replications. Means within the same column followed by different superscript letters differ significantly at $P < 0.05$. SEM = standard error of the mean; Zn = zinc; Mn = manganese; Fe = iron; Cu = copper.

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