INTERACTIVE EFFECT OF NITROGEN, PHOSPHOROUS, AND POTASSIUM FERTILIZATION ON CORN (ZEA MAYS L.)

By

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Abstract: Corn (Zea mays L.) is a high-yielding C4 plant that is cultivated all over the world as a food, feed, and biofuel source. Corn plantations in the southern Great Plains have risen during the last two decades, particularly in rain-fed areas, due to growing corn demand and price. Oklahoma planted 129,000 hectares of grain corn in 2020, up from 121,000 ha in 2017, with just 54.2 percent irrigated. The goal of this study is to explore how NPK fertilization affects grain yield, biomass yield, agronomic and nitrogen efficiency indices, and seasonal mineral nutrient concentration and uptake in corn grown under rain-fed conditions. According to the findings of this study, co-application of P and K had no influence on these parameters. Nitrogen rates were discovered to be the single driving factor impacting all metrics including yield, biomass, NUE, and all macro and micronutrient uptake. It was also discovered that nutrient uptake and removal values varied significantly from previous published research, indicating that these values are affected by soil type, environmental conditions, and crop management approaches. Additional sites years would provide useful nutrient uptake and removal data that will be valuable for making sound nutrient management plans.

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CHAPTER I

LITERATURE REVIEW

Nitrogen in agriculture

Nitrogen (N) is the most important and yield-limiting nutrient in plants, as well as the most widely used nutrient by crops in the world (Dhillon et al., 2020; Girma et al., 2010). According to USDA-NASS, 19 US corn-growing states applied 5.5 billion kg of N fertilizer in 2018, at an anticipated cost of $3.74 billion. Corn accounted for 47% of total annual N applied in the United States, averaging 167 kg ha^{-1} (USDA-NASS, 2018).

Marschner (2011) summarized that plant dry matter contains 1-5% total N, and N is an important part of proteins, nucleic acids, chlorophyll, co-enzymes, phytohormones and secondary metabolites. When the plant has enough N, photosynthesis occurs at a faster rate. As a result, N regulates plant development and the quality of plant products.

Soil acquires N through air fixation by symbiotic and non-symbiotic bacteria, lighting and rainfall, plant and animal waste, and fertilization, whereas leaching, ammonia volatilization, plant loss, and denitrification all cause N losses (Raun et al., 1997).

Nitrogen particularly nitrate is a mobile nutrient in the soil and transported through mass flow. It is also highly mobile in the plant and it is taken up by the plants as ammonium (NH_4^+) and/or nitrate (NO_3^-). Hence, N deficiency symptoms first appears in older leaves. Usually, pale yellow color and stunted growth are the common symptoms of N stress. Nitrogen deficiencies are generally a consequence of N loss or insufficient supply. For example, NO_3^- can be transported from its initial place of application when water is provided in the form of precipitation or excess irrigation. Both ammonium- and nitrate-based fertilizers are prone to runoff and leaching, polluting both surface and groundwater. As a result, good management practices are required for N, as both under and excess application can have negative consequences.

Nitrogen is a very important nutrient in corn because it helps in protein synthesis in plants. Corn requires N in larger amounts than any other nutrient and yield highly depends upon N fertilizer source, timing, amount, method of application (Wang and Below, 1992; Jokela & Randall, 1997).

Nitrogen use efficiency (NUE)

According to Raun & Johnson (1999), NUE in the world is about 33% which is considered very low. Almost, two-thirds of applied N fertilizer is lost through denitrification, volatilization, and leaching. A 1% increase in cereal NUE will save $234 million annually worldwide (Raun & Johnson, 1999) and $1 billion in 2005 (Raun, 2005). Some management techniques like rotation, using improved hybrids, conservation tillage, using NH_4 as N source, in-season and foliar application, irrigation, and precision N management will enhance the NUE in cereal crops (Raun & Johnson, 1999).

Omara et al. (2019) used the same method to calculate NUE in order to see if research efforts and advancements have contributed to an increase in NUE. This group computed that the world NUE improved by 2% and the United States' NUE climbed from 31% to 41%. Due to the small increases in NUE, further management practices should be implemented in the current agricultural period to minimize N losses.

Phosphorous (P)

After nitrogen, P is the second most significant nutrient for yield production, accounting for 0.2 percent of the plant's dry weight (Barber et al., 1963; Schachtman et al., 1998). Corn consumes 15.2% of the world's total P use among all crops (FAO, 2019).

Phosphorous is involved in almost all physiological processes that occur during corn plant growth and development including respiration, cell division, carbohydrate synthesis, and degradation. (Glass et al., 1980; Usuda and Shimogawara, 1993). Biochemically, it forms a link between ribonucleosides in macromolecules such as RNA and DNA (Schachtman et al., 1998; Ozanne, 1980; Marschner, 2011).

Total P is abundant in soils, but most of it is chemically bound with calcium (Ca) in neutral to high pH soils, whereas it is chemically bound with iron (Fe) and aluminum (Al) in lower pH soils. As a result, the amount of P available to plants is limited as a result of the development of these insoluble compounds (Barber, 1995). Therefore, the concentration of plant available P becomes very low (Clarkson et al., 1991). As a result, P management is a critical issue. According to Syers et al. (2008), soil pH and base saturation should be raised to reduce the amount of P bound by Al and Fe in low pH soils.

Because P is relatively immobile in the soil, it is absorbed by the roots through diffusion and mass flow in the form of $H_2PO_4^-$ and HPO_4^{2-} (Barber et al., 1963). Sometimes, organic form of P also contributes to plant nutrition, but it must mineralize into inorganic P by microorganisms (Moro et al., 2021). It is mostly absorbed by roots as $H_2PO_4^-$ through root hairs or the outermost cell layer (Ozanne, 1980). Diffusion accounts for the majority of P intake, while mass flow absorption accounts for 1-2 percent of the total P required by the crop to achieve an acceptable yield (Tinker et al., 2000). Plant roots may extend to meet the plant's Ca and magnesium (Mg) needs, but not for the P need (Barber, 1963). As a result, P is considered the least available nutrient in the soil (Raghothama, 2005).

Plants consume more P than what is lost from individual fields due to leaching and erosion, even though significant P loss occurs around the world (Fardeau, 1995). Phosphorus runoff can cause eutrophication in nearby bodies of water. Attention has been drawn to eutrophication because of outbreaks of dangerous algal blooms (e.g., Cyanobacteria and P. fiesteria). Algae and aquatic weeds thrive because of eutrophication, resulting in a shortage of oxygen (Sharpley et al., 2001; Sims & Kleinman, 2005; Withers et al., 2002). There is also subsurface P leaching (Sims et al., 1998). Phosphorus leaching occurs only when excessive amounts of P are applied in neutral to high pH soils, polluting groundwater. It is one of the main reasons why efficient use of P is becoming increasingly critical.

Plants need P from the start of their life (Grant et al., 2001). Phosphorous is required throughout a corn plant's life cycle, and a shortage of it is evident in the early stages of development (purple color on lower corn leaves) and decreases final yield (Alley et al., 2009). Phosphorus deficiency in corn can be seen in young plants with leaves turning purple (McCauley et al., 2009). Hence, phosphate fertilizers must be used efficiently and in precise quantities to limit detrimental effects on the environment and assure P availability in soil solutions for plant uptake (Chen & Barber, 1990; Girma et al., 2007).

Potassium

Potassium (K) is the third most yield limiting nutrient, taken up by corn in larger amounts than any other nutrient except N. In crop plants, K is important for photosynthesis, osmoregulation, enzyme activation, protein synthesis, ion homoeostasis, monovalent and divalent cation stability, plant turgor, stress tolerance, and enzyme stimulation (Marschner, 2011). In a previous study on corn (Liu, 2011), treatments with K application increased maximum grain filling rate compared to treatments not treated with K, in addition K played a significant role in remobilizing assimilates.

Soil solution K, exchangeable K, non-exchangeable K, and structural K are the four pools of K in soils (Sparks & Huang, 1985). Exchangeable K is held on exchange sites by clay minerals and soil organic matter, while K^+ in non-exchangeable pool is held by tetrahedral layers of 2:1 phyllosilicate. K^+ in structural pool binds to micas and feldspars. Hence, plant available pool is soil solution K solely which has K^+ ions dissolved in soil solution. As plant uptake K^+ from the soil solution pool, it is replenished by exchangeable K, non-exchangeable K, and structural K (Sparks & Huang, 1985). As a result, most of the K in the soil is unavailable to plants. Following uptake of K from the soil solution, exchangeable K released from soil particles moves to the soil solution rapidly; however, releasing K from the other two forms takes longer and is not as immediately available for the plants.

Unlike N and P, K has no special environmental issues related to water quality, but its loss from soils with high soil K levels increases economic losses and accelerates depletion of geologic mineral K reserves (Gilliam et al., 1985).

Plant roots take K from soil solution in the form of the free ion K^+. High-yielding corn varieties rapidly remove K from soil solution, necessitating replenishment at least four times daily. As a result, K fertilizer should be supplied to maintain enough K^+ concentration in soil solution, because the soil K replenishing rate can be too slow to match the plant's K^+ needs at the proper time (Mengel and Kirby, 2012; Barber, 1968).

In 2018, the United States consumed 4.59 million tons of K fertilizer (FAO, 2018) and 22% of the total fertilizers used in US are K-based (USDA, ERS, 2015) of which 56% is used by corn alone (USDA, NASS, 2018). As a result, adequate management of K is required to enhance corn yield. Potassium chloride (KCl) is commonly utilized as a source of K fertilizer which is a natural mineral obtained from deep deposits. Other commercially accessible sources include potassium sulfate and potassium nitrate.

Potassium, like P, is relatively immobile in soils but mobile in plants, and it moves from older leaves to upper plant parts, causing symptoms to develop initially on older leaves. Yellowing begins from the tip along the outside edges of the leaf blade and progresses to the mid-vein, changing the color from yellow to light tan, then brown. The inside of the leaf blade is still green. Once the tan color appears on the leaves, recovery will not occur, even with the addition of K fertilizer (Welch & Flannery, 1985). Deficiency symptoms sometimes also appear as hidden hunger, which appears as growth retardation without symptoms. (Mengel & Kirkby, 2012). This is extremely dangerous because it cannot be visualized easily. Subedi et al. (2009) concluded that corn yield was reduced by 10% without K (Subedi & Ma, 2009).

Corn crop deficient in K have weaker stalks which can lead to lodging, breaking and stalk rot disease. Many studies have shown that increasing the amount of K in the soil strengthens the stalk (Flannery, 1982; Foley & Wernham, 1957). Corn lodging is a major production limiting issue since it decreases the amount of grain that can be harvested (Arnold et al., 1974). Liebhardt and Munson (1976) also identified that K was responsible for reducing stalking lodging in corn. Because of translocation of photosynthate from the stalk to grain starting from R1 stage, its concentration in stalk is decreased but if plant is sufficient in K, it may produce photosynthate at a faster rate and for a longer time. This additional photosynthate would undoubtedly aid in preventing the soluble solid concentration in stalks from becoming too low and thus keep the stalk strong. Potassium also give resistance to the plant to fight diseases and pests (Asante-Badu et al., 2020)

Potassium fertilizer improves agricultural productivity as well as N and P use efficiency (Niu et al., 2013). If sufficient K is present in the plant, NO_3 in the plant will get converted into protein which caused decreased the concentration of NO_3 in plant and hence there will be more uptake of NO_3. Those reasons justify the evaluation for K to be applied as starter application even if the soil is tested sufficient in K under no till conditions (Roth et al. 2003).

Potassium and climate change

Climate change is of huge concern for the globe. Carbon dioxide (CO_2) levels reached 416 ppm and they are rising at an alarming rate with a 6% increase in the last decade (NASA 2021). Leaves that are high in K have larger stomatal openings than those that are low in K, allowing for increased CO_2 diffusion (Welch & Flannery, 1985).

Another concern is water scarcity, as water availability for crops is decreasing because of climate change. According to a study published by the IPCC in 2014, global temperatures are rising, increasing summer dryness. It is hypothesized that K improves agricultural plant drought resistance. According to Hsiao & Lauchli (1986), K plays a vital role in making plants more resistant to water deficiency. They conducted an experiment in corn and found that addition of K fertilizer increased water use efficiency (WUE) and grain yield under water stress conditions due to lower leaf evapotranspiration. Martineau et al. (2017) conducted an experiment to determine the influence of K on WUE, and they also observed that K fertilizer enhanced WUE and grain yield under water stress conditions by enhancing stomatal sensitivity to drought. Meanwhile, a lack of K exacerbated the problem of water stress (Martineau et al., 2017). Therefore, proper K management not only is beneficial to crop yields but also to the environment.

NPK interaction

Nutrient interactions occur when the presence of one nutrient has a favorable or negative impact on the availability, absorption, or function of another nutrient. It is critical to understand the beneficial connection to improve NUE and, as a result, crop returns (Aulakh & Malhi, 2005)**.**

An experiment was conducted by Liu et al. (2011) in a corn-wheat production system to explore multiple NPK treatments and they observed that plots receiving P or K in addition to N resulted in a 7 to 10% increase in grain yield when compared to plots only receiving N. There was a 23% increase in grain yield when NPK fertilizers were applied in combination compared to the unfertilized control treatment. Incomplete fertilizer treatment raised the number of grains each year by 11-17%, while NPK treatment raised grains per year by 18%. Bandel & Griffith (1994) and Duan et al. (2014) reported similar results.

Aulakh and Malhi (2005) derived the data from the study of Chandrakar et al. (1978) in which treatments were single and combined applications of NPK in rice. They computed that addition of K with N and P made the synergistic interaction of nutrients stronger and increased rice yield. An experiment conducted at the University of Florida revealed that corn had a synergetic impact from proper NPK interaction (Usherwood, 2001). It was concluded that adding N and K to corn increased yield by 23 bu ac^{-1}, while adding N and P enhanced yield by 26 bu ac^{-1}; however, when N, P, and K fertilizers were supplied, the yield gain was 15 bu ac^{-1} greater than the total individual yield responses. High levels of P and K, along with N can also accelerate the development of silking (Peaslee et al., 1971).

Aside from increasing grain production, studies suggest that treating with N+P+K enhances root development and biomass compared to treating with N, P, or K alone. The interactive effect of these nutrients changes the length, thickness, and biomass of roots (Duncan et al., 2018). This better root growth can help the plant to tolerate dry spells because roots can absorb nutrients and water from deeper layers. Furthermore, more roots indicate that there will be more organic matter left in the soil when the crop is harvested, which will help to maintain its soil health. Nitrogen and P also have a synergistic interaction, which means that the combined effect of N and P is larger than the sum of their individual effects. When soil requires more P than N, supplying only N can cause reduction in yield (Aulakh and Malhi, 2005).

Adnan (2020) reviewed the role of K in corn and found the effect of K on its own was non-significant, but when combined with phosphorus, it produced higher yields. On the other hand, P showed significant effect when applied alone.

Stromberger et al. (1994) conducted a greenhouse experiment to study how K interacted with N levels and sources, and discovered that high K promoted growth in the presence of high N. In another study, Xu et al. (1992) reported that when maize seedlings treated with NH_4^+ were supplemented with K, they grew more rapidly. High K level promoted root growth, dry matter content and N accumulation in shoots. Arnold et al. (1974) stated that yield increased when K was applied along with N. This interaction reduced stalk lodging and increased crushing strength and rind thickness (Arnold et al., 1974). In contrast, addition of N in the form of Urea had positive effect on K availability because it enhanced soil extractable K to the plants either by reducing K fixation due to competition for binding sites with NH_4^+ or by acidification (MacKenzie et al., 1988).

Surface-applied urea in no-till or minimum-tillage soils can easily lose gaseous N to the atmosphere. Rappaport et al. (1984) conducted laboratory and field experiments and found that adding K in the form of KCl improved the use efficiency of surface applied urea due to reduced volatilization. According to a study done by Johnson in 1997, increasing soil K level increased N utilization; and N rate may be reduced when paired with higher amount of K to maximize profit in corn.

NPK removal

According to the 2010 IPNI report, macro and micronutrients have been reduced in Canadian and US soils during the prior 5 years. The combination of high yielding cultivars and declining soil fertility shows that farmers did not match nutrient uptake and removal with fertilizer applications (Fixen et al., 2010). The United States entered into biofuel crop production for renewable energy in recent years, which necessitates a large amount of crop biomass. When biomass is removed from the field a lot of stored nutrients is exported from soils. Furthermore, high amount of nutrients accumulated in biomass cause problems on the ethanol conversion process (Propheter et al., 2010). Corn harvested solely for grain differs significantly from corn planted for ethanol production, bioenergy, and silage. Corn stover, which was once considered a waste product, is now utilized to make ethanol in some areas. Stover includes all plant components other than grains, such as stalks, leaves, tassels, cobs, husks, and shanks. These are removed from the field, resulting in a significantly greater loss and depletion of soil nutrients than harvesting grains alone (Sindelar et al., 2013). If stover is left in the field in the case of grain only production, nutrients in it will be recycled to the soil. Stover is removed for ethanol and silage production, which removes extra nutrients, such as 23.31kg N, 4.4kg P, and 26.11 kg K per ton of dry matter (Bender et al., 2013).

A recent research by Nunes et al. (2021) found that Stover removal has no major influence on P, N and S nutrients because the amount of their removal with grain is more than with the residue. If the stover is removed, however, K must be closely monitored.

Residues have higher K content than grains. Stover harvest lowers the amount

of exchangeable K and Ca in top soils. If the stover is removed or harvested for any reason, the K supply requires extra attention. Hence, the knowledge of nutrient uptake and removal will aid in understanding nutrient cycle and can be used to change nutrient requirements if corn is planted for ethanol production. From a large data set, Setiyono et al. (2010) compiled that average nutrient concentration in grain were 13.3 g N kg^{-1} 2.6 g P kg^{-1}, and 3.6 g K kg^{-1}, and in stover 8.1 g N kg^{-1}, 0.5 g P kg^{-1}, and 21.8 g K kg^{-1}, and they varied greatly due to environmental and management conditions in grain (4.9-19.6 g N kg^{-1}, 0.6-5.2 g P kg^{-1}, and 1.0-9.7 g K kg^{-1}) and stover (2.2–19.9 g N kg^{-1}, 0.1–4.2 g P kg^{-1}, and 1.5–41.7 g K kg^{-1}). Nutrient removal information also aids in determining how much fertilizer should be applied to the subsequent crop. If a producer applies 100 kg P ha^{-1} and the crop uses 90% of it, assuming no other losses occur only 10% will be left for the following crop, which will require additional fertilizer.

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CHAPTER II

EFFECT OF NITROGEN, PHOSPHOROUS, AND POTASSIUM FERTILIZER ON GRAIN YIELD, BIOMASS, NITROGEN USE EFFICIENCY, AND NUTRIENT REMOVAL

ABSTRACT

Over the past 20 years, corn (Zea Mays L.) plantations in the southern Great Plains have increased, especially in rain-fed environments, as a result of rising demand and price for corn. Although nitrogen (N) is the most important nutrient, but we cannot ignore the importance of phosphorous (P) and potassium (K) in corn production. Field experiment was conducted in 2021 and 2022 to evaluate the effect of co-application of P and K with N in increasing grain yield, biomass yield, nitrogen use efficiency (NUE), and nutrient uptake and removal in rain-fed corn. The results demonstrated that application of P and K does not have any significant effect on corn if soil is above the critical levels for those nutrients. It was noted that the grain and biomass yields significantly relied on the amount of N applied. Nitrogen rate significantly increased plant nutrient uptake and removal. The maximum yield in our study among two site-years was 11 Mg ha^{-1} in the plot receiving 133 kg N ha^{-1} with 20 kg P ha^{-1}. The maximum uptake at maturity in this treatment was 173.9 kg N ha^{-1}, 32.28 kg P ha^{-1}, and 128.05 kg K ha^{-1}. Nitrogen use efficiency, agronomic efficiency (AE), and internal efficiency (IE) decreased with increasing N rate. NUE increased by 5.9 % when K was present, 15.9% when P was present, and 9.7% when both were present with 133 kg N ha^{-1} at EFAW21. Although, applying P or K or both resulted in increasing NUE, AE, NRE, and IE at one site, but this increase was not significantly different from the treatment receiving 133 kg N ha^{-1} only. However, N rate did not affect N recovery efficiency, which is the ratio of change in plant N uptake to N rate.

INTRODUCTION

Corn (Zea mays L.) is a high-yielding C4 plant that is grown as a food, feed and biofuel sources all over the world. With 1.14 billion tons produced in 2019 worldwide, it leads all cereals in output, increasing 2.07% from the previous year (FAOSTAT, 2019). It was planted on 37 million hectares (ha) in the United States in 2021(USDA-NASS, 2021) with an average maize yield about 10.8 Mg ha^{-1} (USDA-ERS, 2020). The US produced 794.2 million metric tons of corn and exported 62.5 million metric tons of it in 2020, making it the world's greatest corn exporter (USDA-ERS, 2020).

Corn production in the Southern Great Plains of the US have increased over last two decades (Bushong et al., 2014). This rise is due to increased demand and price of corn attributed to its use as a raw material for ethanol starch, sweeteners, corn oil, beverage and industrial alcohol, and fuel ethanol, in addition to being used for food and fodder (Wallander et al., 2011). But this increase occurs mostly in rain-fed fields while the irrigated hectares remained constant (Bushong et al., 2014). Oklahoma planted 129,000 ha corn for grains in 2020 which increased from 121,000 ha in 2017 (USDA-NASS, 2017; USDA-NASS, 2020), with only 54.2 percent of being irrigated (USDA-NASS Census, 2017). The average grain yield in Oklahoma is only 9.07

Mg ha^{-1} (USDA-NASS, 2020). According to Lobell (2009), average corn yield in rain-fed systems is only around 65% or less of yield potential, giving an opportunity to improve it.

Nutrients are being removed from the soil by crop harvesting, wind or water erosion, leaching to deeper soil layers, volatilization, denitrification etc. That is why we need fertilizers to provide the soil with replenished nutrients. In a survey conducted by USDA-NASS in 18 corn growing states that accounted for 93% of corn grown in US (Oklahoma was not included), it was found that farmers applied nitrogen (N) to 98%, phosphorous (P) to 79%, and potassium (K) to 63% of corn planted acres. The average rate is 167 kg ha^{-1} for N, 77 kg ha^{-1} for P, and 97 kg ha^{-1} for K (USDA-NASS, 2018). Since P and K are also applied in large quantities by US growers, we cannot ignore its importance in corn production and farming profitability.

No doubt, N has the largest impact on any agricultural crop yields (Raun et al., 2005). Moreover, N is considered as producers' best input to increase profitability (Teal et al., 2006), but relying only on N for crop productivity to meet future food needs is not particularly sustainable. Phosphorus and K along with N should be used in a balanced manner, since an imbalance might cause issues in the long run. Some USA farmers are taking advantage of previously applied residual soil P and K supplies (Uri, 1998), but many agricultural fields in the US started losing P & K reserves, and they may be depleted if not replenished (IPNI, 2010; IPNI, 2015).

Phosphorus is important because it has low recovery rate and has finite resources (Smil, 2000). In Oklahoma, phosphorous is one of the most deficient nutrients (Zhang & Raun, 2005). According to Zhang (2001), most of the Oklahoma soils were deficient in P. Effective management of P is necessary because excess accumulation of P in the soil can cause eutrophication in waterbodies and resulting in water quality degradation.

Potassium (K) is known to provide drought tolerance and mitigate effect of water scarcity (Hsiao & Lauchli, 1986). Potassium increases drought tolerance capacity of the crop by regulating the stomatal opening and provide important functions in energy status, charge balance and homeostatis. Potassium helps in conserving water within plant itself (Hussain et al., 2015). When water is becomes unavailable it reduces yield potential especially in rain-fed cropping systems. Long periods of drought, asymmetrical rainfall, and fluctuating temperatures are common in the southern Great Plains (Baath et al., 2018). But sufficient levels of K in plants allow roots to absorb/extract water from soils even when moisture levels are low (Hussain et al., 2015). It is well citied that soil moisture plays important role in nutrient use efficiency (Nagy, 1997). Therefore, application of K is very beneficial because P and K availability depends upon soil moisture availability. Cao et al. (1991) and Ali et al. (1999) found that movement of K from the soil to root-surface increased with application of K fertilizer in wheat.

In no-till soils, corn grain yield increased with P & K fertilization even if soils were sufficient in P and K (Mallarino et al., 1999). Similar results were seen when starter fertilizer containing N, P, and K increased corn yields even in the soil with adequate P and K under no till system (Buah et al., 1999). Welch (1974) also proved that high K soils sometimes need more K for better yield.

Nitrogen use efficiency (NUE) is a major concern because plants use only 33 percent of the applied fertilizer N, and the rest is lost to the environment (Raun & Johnson, 1999). There are many ways to increase NUE such as conservation tillage, in-season N fertilization, irrigation etc. (Raun & Johnson, 1999). Duncan et al. (2018) showed that it can also be increased by addition of P and K along with N. In their experiment on wheat, it was found that balanced P and K along with N in the soil had potential to increase NUE and grain yield. According to Barnes et al.

(1976) when there is sufficient K, K ions significantly promote the absorption and utilization of N and P by crops in the form of compensation charges. Balanced NPK fertilization also showed positive response in both summer corn (Liu et al., 2011) and winter wheat (Bertic et al., 2007). Both experiments showed that addition of P and K with N increased final grain yields. Similar results were obtained by Yousaf et al. (2017) in rice and rapeseed crops. In their experiment, NPK fertilization increased crop yield by 19-41% in rice and 61-76% in rapeseed and the lowest yield was obtained in plots with P and K only.

The objective of this study is to evaluate the effect of NPK fertilization on grain yield, biomass yield, agronomic and nitrogen efficiency indices, and N, P and K uptake in corn under rain-fed conditions.

MATERIAL AND METHODS

Field trials and experimental design

This field study was conducted at two locations, EFAW (36°08'14.1"N 97°06'22.4"W) and Lake Carl Blackwell (LCB) (36°09'04.8"N 97°17'21.5"W) in 2021. While in 2022, the trail was conducted at LCB (36°09'1.64"N 97°17'23.30"W) and Perkins (35°59'37.32"N 97°2'31.41"W). We were able to record data for two site years only out of four because of the raccoon damage at our no-till irrigated LCB site in 2021 and hog damage at Perkins in 2022. Therefore, we will only discuss two locations in this chapter, which are EFAW site in 2021 (EFAW21) and LCB site in 2022 (LCB22).

Both locations were rain-fed and left fallow prior to corn planting. Soil classification at the EFAW site is an Ashport silty clay loam (fine-silty, mixed, superactive, thermic, Fluventic Haplustolls) and LCB is classified as a Pulaski fine-sandy loam (coarse/loamy, mixed non-acid, thermic, Typic, Ustifluvent) (Soil Survey, 2021). Soil chemical properties are presented in Table 1.

A randomized complete block design was used for both locations, which included 12 treatments and three replications. Each treatment plot is measured 3x6 m and consisted of four rows of corn plants, with an alley of 3 m between each replication. The 12 treatments evaluated different combinations of nitrogen (N), phosphorous (P), and potassium (K) fertilizer rates along with control treatment (Table 2). Nitrogen rates evaluated included 0, 67, and 133 kg N ha^{-1}. Phosphorus and K rates were 0 and 20 kg P ha^{-1} and 0 and 60 kg K ha^{-1}, respectively. All fertilizers were applied as pre-plant using a barber metered feed fertilizer spreader using appropriate settings to achieve the desired fertilizer application rates. This machine was calibrated to ensure the appropriate amount of fertilizer was delivered. After applying a particular nutrient, hopper of the spread was cleaned with pressurized air. Fertilizer sources for N, P, and K included urea (N-P_2O_5-K_2O, 46-0-0), triple super phosphate (0-46-0), and Muriate of potash (0-0-60).

Composite pre-plant soil samples were taken from both locations at 0-15 cm depth from each replication. Soil samples were dried at 65° C for 12 hours and passed through 2 mm sieve in preparation for chemical analysis. Chemical analyses conducted included pH, ammonium- (NH_4-N), nitrate-nitrogen (NO_3-N), and plant available P and K. Analysis of soil nitrate and ammonium nitrogen was performed using 5 g of soil sample extracted with 25 ml of 1 M KCl solution. These samples were filtered after shaking for 30 minutes and analyzed using a Lachat flow injection autoanalyzer. Mehlich 3 (M3) extractant (Mehlich, 1984) was used for plant available P and K determination. To do this, 2 g of the soil samples were weighed and mixed with 20 ml of M3 extractant. Samples were filtered after shaking for 5 minutes and analyzed by an Inductively Coupled Plasma-Atomic Emission Spectroscopy. Results of the pre-plant routine soil tests are shown in Table 1. Approximately four days after pre-plant fertilizer applications were made, when the soil moisture level and soil temperature were both adequate, corn hybrid ‘DKC66-29’ (DeKalb Genetics Corporation, IL) was planted at both sites using John Deere Max Emergence 2 7300 four row planter. Row spacing was 76 cm with a population of 49,400 seeds ha^{-1} at EFAW21 and 56,810 seeds ha^{-1} at LCB22. Integrated Pest Management was performed according to Oklahoma State University recommendations. Summary of field activities can be found in Table 3.

Data collection

Data collection began 20 days after planting (DAP). Stand-count data was gathered to determine the germination rate of each plot by counting the emerged corn plants in the center two rows. Growth stages used were determined according to Abendroth et al. (2011).

Plant tissue samples were collected from the side two rows of each plot at growth stage R6 (maturity) for nutrient analysis (Abendroth et al., 2011). A one-meter length of row was randomly selected, then plants were cut at 5 cm above ground level to avoid soil contamination. Fresh weight was taken after collecting plant samples and then weighed again after oven-drying to determine subsample aliquot dry weight and dry biomass accumulation. Dry plant samples were then ground and passed through 1-mm sieve. Dried and ground samples were labelled and brought to the lab for analysis.

Upon crop maturity, the middle two rows of each plot were harvested using a Kincaid 8XP plot combine equipped with a Harvest Master Yield monitor in which the final grain yield reported was adjusted to 15.5% moisture.

For grain N and crude protein (CP) analysis, a LECO CN 828 instrument was used (LECO corp. St. Joseph, MI). One hundred and fifty mg of dried and ground grain samples from each plot were encapsulated in a tin foil and loaded in the machine. These samples were combusted at 950 °C in a reticulated ceramic crucible inside the machine's furnace in a pure oxygen environment. The moisture was then separated by thermoelectric cooler until the combusted gases were delivered into a ballast volume, where they equilibrate and mix before being exposed to a flowing stream of inert gas for analysis. The aliquot gas was carried to thermal conductivity cells for detection of nitrogen as N_2.

Total P and K in plant samples were determined by nitric acid digestion and inductively coupled plasma spectrometry quantification. Nutrient concentrations (Nc) were provided in percentage and the values of total aboveground macronutrient uptake were calculated by Equation 1.

$$Plant\ Macronutrient\ Uptake\ (kg\ ha^{-1}) = Dry\ Biomass\ (kg\ ha^{-1})\ \times Nc \qquad (1)$$

Nitrogen Use Efficiency Indices calculations

Nitrogen use efficiency (NUE) indices are used to quantify the effectiveness of plants to convert available N into grain and biomass (Ciampitti and Vyn, 2011). Calculations of Agronomic efficiency (AE), Nitrogen recovery efficiency (NRE), and Internal Efficiency (IE) were based on Woli et al. (2016), and Ciampitti and Vyn (2011). While NUE was calculated as described by Arnall et al. (2009) as uptake efficiency.

$$NUE(\%) = \frac{\text{Grain N uptake in fertilized plot} - \text{Grain N uptake in control plot}}{\text{N applied}} \times 100 \qquad (2)$$

Agronomic efficiency shows the effect of applied N on increasing grain yield as compared to the control with no N.

$$AE\ (\Delta\ \mathrm{kg\ kg^{-1}}) = \frac{Grain\ yield\ in\ fertilizied\ plot - Grain\ yield\ in\ control}{N\ applied} \quad (3)$$

Nitrogen recovery efficiency indicates the ability of aboveground plant to recover N from applied N at physiological maturity as compared to the control. It defines the proportion of applied N recovered in plants at maturity.

$$NRE\ (\Delta\ \mathrm{kg\ kg^{-1}}) = \frac{Plant\ N\ uptake\ in\ fertilizied\ plot - Plant\ N\ uptake\ in\ control}{N\ applied} \quad (4)$$

where plant N uptake is the total nitrogen uptake in aboveground plant parts calculated on dry basis (0 % moisture).

Internal efficiency is the ability of plant to convert accumulated N to grain yield (Equation 5).

$$IE\ (\mathrm{kg\ kg^{-1}}) = \frac{Grain\ yield}{Plant\ N\ uptake} \quad (5)$$

Statistical Analysis

Data were analyzed using SAS version 9.4 (SAS Institute, Cary, NC, USA). ANOVA was applied using PROC-GLIMMIX procedure and the mean separation procedure was done by Tukey. Each site and year were analyzed separately. Treatments were used as fixed effect and replications as random. All nutrient uptake values were calculated on dry weight basis.

RESULTS

Grain Yields

Analysis of Variance (ANOVA) for interaction effect and main effects of factors showed that there was no NPK interaction for yield at both locations (Table 4). Among all interactions, only NP interaction was significant (*p*=0.037) at EFAW21 site (Fig. 3). Apart from this NP interaction, there was no other interaction effect on grain yield at either location. However, only N main effect was significant (*p*=0.0001) at both locations and years. Grain yield varied from 5.5 Mg ha^{-1} to 10.9 Mg ha^{-1} at EFAW21, whereas it varied from 0.42 Mg ha^{-1} to 4.41 Mg ha^{-1} at LCB22 (Figure 2) (Table 4). At EFAW21, the highest yield was recorded where P was applied with 133 kg ha^{-1} N which was 14.2 % more than that of 133 kg ha^{-1} N only. At LCB22, the highest yield was noticed when K was applied with 133 kg ha^{-1} N, which was 7% more than that of 133 kg ha^{-1} N only. Although, co-application of P or K with N enhanced grain yield slightly, the ANOVA showed no significant difference of co-application of P or K with N. (Table 4). Corn yield might respond only 50% to 70% of time to annual P application when STP is equal to or lesser than 20 mg kg^{-1} (Dodd and Mallarino, 2005). Rehm et al. (2010) also found no response of corn grain yield in their starter fertilizer experiment. This might explain why P fertilizer was unable to increase grain yield significantly, especially at LCB22 site. It was noted that the grain yield significantly relies on the amount of N applied, since it increases with increasing N (Figure 4). The N x P interaction at EFAW demonstrated that P showed positive response only when its co-application was carried out with 133 N ha^{-1} rather than 67 kg ha^{-1} N (Figure 3). This again suggests that N plays major role in increasing grain yield.

Biomass Yields

There was no interaction between N x P x K for aboveground dry biomass production at either location. However, there was an interaction effect of N x P (*p*= 0.04) and main effect of N at EFAW21 (Fig. 3). This interaction effect was similar to that on grain yield, which implies that the effect of applied P can be seen only when applied with higher amount of N. At EFAW21, there was main effect of K (*p*= 0.02) also, which resulted in 6.5% more biomass when K was present in NPK treatments regardless of N rate in the combination. At LCB22, ANOVA resulted in main effect of N only. The highest biomass was achieved when 20 kg P ha^{-1} was applied with 133 kg N ha^{-1} at EFAW21 and at LCB22 when 60 kg K ha^{-1} was applied with 133 kg N ha^{-1}. However, these were not statistically different when compared to other treatments receiving only 133 kg N ha^{-1}. Similar to grain yield, N was also the key factor in biomass production, since treatments with no N were significantly different from those receiving either 67 kg ha^{-1} N or 133 kg ha^{-1} N at both locations. Total aboveground dry biomass at full maturity ranged from 9762 kg ha^{-1} to 17257 kg ha^{-1} at EFAW21, and 2663.1 kg ha^{-1} to 7936.8 kg ha^{-1} at LCB22 (Table 4).

Nutrient uptake and removal

Significant effects ($p<0.05$) were observed for plant nitrogen uptake (PNU) for the N rate and for the interaction N x P x K at EFAW21, according to ANOVA presented in Table 4. At EFAW21, significant effects were observed for grain nitrogen uptake (GNU) for the N rate and the N x P interaction. Maximum PNU as well as GNU occurred when 20 kg P ha^{-1} was applied with 133 kg N ha^{-1} at EFAW21. But these were not statistically different when compared to other treatments receiving only 133 kg N ha^{-1}. At LCB22, significant effects were observed for PNU for the N rate and P rate, whereas only N rate was significant for GNU. At this site, P had negative effect on PNU, causing 17 % decrease in PNU when P was present in NPK combination. The highest PNU and GNU was observed when 133 kg N ha^{-1} was applied. Nitrogen rate was significant at all locations as there was increase in PNU and GNU with increasing rate of N (Fig 4). It was expected and consistent with the results from Halvorson and Bartolo (2014).

There were interaction effects of N x P x K, N x P, and main effect of N for plant P uptake (PPU) at maturity at EFAW21 (Table 6). Grain P uptake (GPU) at this site was affected significantly by interaction of N x P, main effect of N, and main effect of P. However, only main effect of N was observed for both PPU and GPU at LCB22. At maturity, plant K uptake (PKU) was affected significantly by the main effect of N and main effect of K, while grain K uptake (GKU) was affected by interaction of N x P and main effect of N at EFAW21. However, at LCB22, both PKU and GKU was solely affected by N rate. Our study showed that N, P, and K uptake by maturity increased linearly with N rates which is consistent with the findings of Ciampitti et al. (2013), Feil et al., (1993) and Hanway (1962). According to the study conducted by Setiyono et al. (2010), maximum total N uptake, P uptake, and K uptake was 232.2 kg ha^{-1}, 35.2 kg ha^{-1}, and 269.1 kg ha^{-1} respectively for the crop with grain yield of 12.01 Mg ha^{-1}. However, the maximum yield in our study among two site-years was 11 Mg ha^{-1} in plot receiving sufficient N with P at EFAW21. The maximum uptake in this treatment was 173.9 kg N ha^{-1}, 32.28 kg P ha^{-1}, and 128.05 kg K ha^{-1}.

Agronomic and Nitrogen Use Efficiency indices

The statistical data presented in Table 5 revealed that there was interactive effect of N x P x K, interactive effect of N x P, main effect of N, and main effect of K for Agronomic Efficiency (AE) at EFAW21, whereas only main effect of N was observed at LCB22. Significant effect of N x P interaction and main effect of N was observed for NUE at EFAW 21, and only main effect of N was observed at LCB22 for NUE. However, there were no significant difference observed at EFAW21 for N recovery efficiency (NRE), but only main effect of P was noted at LCB22 for NRE. In contrast to other measures, main effect of N was not observed for NRE at either location. Internal Efficiency (IE) was significantly affected by N x P x K interaction and main effect of N at EFAW21; however, at LCB22 only main effect of N was seen. NUE increased by 5.9% when K was present, 15.9% when P was present, and 9.7% when both were present with

133 kg N ha^{-1} at EFAW21. Although, applying P or K or both resulted in increased NUE, AE, NRE, and IE at EFAW21, but this increase was not significantly different from the treatment receiving 133 kg N ha^{-1} only. Similar to grain yield, biomass, PNU, and GNU only N rate was influencing these NUE indices significantly except for NRE.

DISCUSSION

The variation in mean grain yield between two site-years were due to the difference in soil physical and chemical characteristics at these locations (Table 1). EFAW21 site had higher residual N in the soil that could have also increased overall grain yield in 2021. Additionally, this discrepancy might be explained by weather variations between the two years (Fig. 1). Mean grain yield was reduced in 2022 at LCB because of uneven rainfall and higher temperature (Fig. 1). However, the 2021 season had good distribution of rainfall during growth stages of the crop which might also contributed to higher mean grain yield.

This study clearly reveals the importance of N rate for improving grain yield, biomass, and nutrient uptake. Treatments receiving 133 kg N ha^{-1} had the highest yield, biomass, and nutrient uptake at both sites as compared to lower rates of N. Other treatments receiving 0 kg N ha^{-1} or 67 kg N ha^{-1} did not have enough N needed for its potential growth. The lack of N could hinder the overall growth of crop. Moreover, addition of P and K were not statistically different within each rate of N indicating N deficiency is the most limiting factor for overall rainfed corn production. These results were consistent as reported by Ma et al. (2016), Halvorson and Bartolo (2014), and (Liang and MacKenzie, 1994). There was an interaction effect of N x P at EFAW21 site for grain yield, biomass, GNU, NUE, and AE. This interaction states that P responds well only when applied with higher amount of N. Cole et al. (1963) found that P uptake rates were highly correlated with N level in plant roots and there was a connection between P uptake and N metabolism. This positive N x P interaction was also reported by Fageria (2001), and Schlegel and Havlin (2017). Aulakh and Malhi (2005) also reported that N and P interaction is the most critical for increasing yield. Biomass at EFAW was affected significantly by K application ($p=0.02$), but there was not significant effect on grain yield. This means that K application increased only stover biomass. Grain yield, nutrient uptake, and NUE indices did not respond to P and K fertilizer statistically at either location. Although these parameters slightly increased with addition of P with higher rate of N at EFAW21 but there was no statistical difference ($p<0.05$) of applied P and K along with N. This slight response could be explained by higher P uptake by the plants. Phosphorous uptake increased by 21% when P was applied with 133 kg N ha^{-1}. P is involved in almost all physiological processes that occur during corn plant growth and development including respiration, cell division, carbohydrate synthesis, and degradation. Another possible explanation could be soil temperature. Cooler temperatures slow diffusion, the process by which plants absorb P, which could lead to a P deficiency. By utilizing the well-known "pop-up" effect, which is the temporary solubilization of P fertilizer in cool soils, even small amounts of P fertilizer, which may be present at or near the seed, can ensure that plants have an adequate supply of P at the start of their growth.

EFAW21 site did not respond statistically to P and K because this site was above the critical levels for soil test P and K (Table 1). In Oklahoma, soils are considered P and K sufficient for corn production if soil test P (STP) and soil test K (STK) is above 32.5 and 125 mg/kg, respectively (Zhang and Arnall, 2013). However, EFAW21 site was over 95% sufficient for P and 100% for K. Therefore, only P responded hardly and K did not have any effect. These results were similar to that of Roth et al. (2003), and Subedi and Ma, (2009). A study by Liu et al. (2011) showed that K addition increased corn grain yield but it was not observed in our study.

Buah et al. (1999) and Mallarino et al. (1999) found that grain yield can be increased even if P and K were sufficient in the soil. However, our study showed no response of P and K statistically. Current study shows that N plays more important role in increasing grain yield in rainfed corn (Fig 4). This suggests no advantage of applying P and K fertilizers when the soil is sufficient or near sufficient for these nutrients. These results agree with the findings of Mallarino et al. (1991). Phosphorus application was expected to show response at LCB22 because this site was deficient in STP. This might be due to environment factor which had uneven distribution of rainfall and drought conditions especially at the time of tasseling (Fig 1). The drought undoubtedly hindered the crop to reach its yield potential. Other possible explanation could be that sometimes soil testing methodologies do not account for the slow release of sorbed P and the mineralization of soil organic P (Steffens, 1994).

Increasing N rate decreased NUE, AE, IE (Table 5). These findings were consistent with the observations of Halvorson and Bartolo (2014), Qian et al. (2012), Bundy and Carter (2013), and Guillard et al. (1995). While NRE, which is the ratio of change in PNU to N rate, was not affected by N rate. This was consistent with the findings of Halvorson and Bartolo (2014) who also reported that NRE did not change with increasing N rate for corn. Duncan et al. (2018) showed that NUE can be increased by addition of P and K along with N in wheat. Although addition of P and K improved NUE but it was not statistically different from N only. Johnson and Reetz (1995) reported that K increased NUE in corn. Welch (1976) also proved that high K soils sometimes needed more K for better yields, but our results were not significant when K was applied with higher rate of N.

The current study shows that the grain yield, biomass, NPK uptake, NUE had significant effect with different rate of N. Increased grain yields with increasing N rate were also found by

Bruns and Ebelhar (2006). While a study by Halvorson and Bartolo (2014) reported that nutrient uptake increased with increased rate of N. Our research supported previous findings from Ciampitti et al. (2013), Feil et al. (1993), and Hanway (1962) by demonstrating that N, P, and K uptake at maturity increases linearly with N rates.

CONCLUSION

Fertilization is one of the most crucial factors that influence the growth of rain-fed corn. An appropriate fertilizer combination of N, P and K fertilizers can enhance grain yield and quality, reduce costs and minimize the impact of farming on the environment. However, using excessive or inappropriate amount of fertilizers cannot guarantee to increase grain yield, but may result in economic loss, low nutrient use efficiency, and various environmental problems. The effect of co-application of P and K along with N on grain yield, biomass, nutrient uptake, and NUE indices are inconsistent among sites and years in this study. Potassium application increased stover biomass significantly at EFAW21, while P application increased PNU at LCB22. But these effects were not consistent for both locations. Only N application affected all the parameters significantly at both locations. Grain yield, aboveground dry biomass, PNU, GNU, PPU, GPU, PKU, and GKU enhanced with the increasing rate of N. On the other hand, NUE, AE, IE decreased with increasing N rate. However, N rate did not affect NRE, which is the ratio of change in plant N uptake to N rate. Corn only responded to P fertilizer if applied with sufficient amount of N in P deficient soils. Although application of P and K with N resulted in improved NUE, AE, and IE, but it is not significantly different from N only. There is no benefit of applying K to increase grain yield or NUE when the soil test K is above the critical level (100% sufficient). The results from this study confirmed that N is the most important nutrient when P and K are sufficient or near sufficient in the soils. Production of rainfed corn largely depends on the environment in addition to plant nutrients. Therefore, more information may be gained from additional studies that can include more rates of P and N in various environmental settings.

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FIGURES AND TABLES

Table 2.1: Basic soil properties at Efaw in 2021, and Lake Carl Blackwell (LCB) Oklahoma in 2022.

Year	Location	pH	Organic Carbon %	NH_4-N	NO_3-N	P ǂ	K ǂ
				mg kg^{-1}			
2021	Efaw	5.6	0.83	42.5	23.3	24	201
2022	LCB	6.1	0.63	3.3	<0.1	11.5	69.5

ǂ P& K are plant available phosphorous (P) and potassium (K) using Mehlich 3, respectively.

Table 2.2. Nitrogen (N), phosphorous (P), and potassium (K) application rates employed at both Efaw and LCB locations.

Treatment code	Nutrient rates (kg ha^{-1})		
	N	P	K
N0P0K0	0	0	0
N0P0K1	0	0	60
N0P1K0	0	20	0
N0P1K1	0	20	60
N1P0K0	67	0	0
N1P0K1	67	0	60
N1P1K0	67	20	0
N1P1K1	67	20	60
N2P0K0	133	0	0
N2P0K1	133	0	60
N2P1K0	133	20	0
N2P1K1	133	20	60

Table 2.3: Dates for major field activities performed during the crop season at both sites

Location	Soil Samples taken	Pre-plant fertilizer Application	Planting date	Stand Count Date	Harvesting
Efaw	04/01/2021	04/02/2021	04/06/2021	04/26/2021 05/10/2021	08/26/2021
LCB	04/06/2022	04/07/2022	04/06/2022	04/26/2022	08/15/2022

Table 2.4. Analysis of Variance for interaction effects and main effects of factors for grain yield (GY), dry biomass, plant nitrogen uptake (PNU), and grain nitrogen uptake (GNU) by site year.

		Site Year							
		EFAW21				LCB22			
Source of Variation	*df*	Yield (Mg ha^{-1})	Biomass (kg ha^{-1})	PNU (kg ha^{-1})	GNU (kg ha^{-1})	Yield (Mg ha^{-1})	Biomass (kg ha^{-1})	PNU (kg ha^{-1})	GNU (kg ha^{-1})
		Mean Squares							Mean Squares
N	2	***	***	***	***	***	***	***	***
P	1	ns	ns	ns	ns	ns	ns	*	ns
N x P	2	*	*	ns	*	ns	ns	ns	ns
K	1	ns	*	ns	ns	ns	ns	ns	ns
N x K	2	ns	ns	ns	ns	ns	ns	ns	ns
P x K	1	ns	ns	ns	ns	ns	ns	ns	ns
N x P x K	2	ns	ns	*	ns	ns	ns	ns	ns
S.E.		0.45	581.5	6.37	5.14	0.37	757.6	5.04	4.74
Treatment		Means				Means			
N0P0K0		5.55 d	9762.4 e	77.0 f	56.4 e	0.42 d	2760.9 c	21.7 de	4.2 d
N0P0K1		5.59 d	10958 e	68.7 f	53.4 e	0.95 dc	3608.5 bc	24.2 de	8.4 d
N0P1K0		5.61 d	10175 e	69.8 f	52.3 e	1.62 bdc	4146.9 bac	30.4 dec	16.1 dc
N0P1K1		7.01 dc	11832 edc	84.5 ef	65.4 e	0.87 dc	2663.1 c	18.1 e	8.8 d
N1P0K0		9.34 ba	14048 bc	112.5 ed	104.3 dc	3.75 a	7240.4 ba	48.1 bdac	45.1 ba
N1P0K1		9.50 ba	16000 ba	136.2 bdc	108.4 bdc	3.31 ba	6096.9 bac	53.8 bac	40.9 bac
N1P1K0		8.55 bc	13895 bdc	116.1 d	95.6 d	2.71 bac	5010.2 bac	38.0 bdec	33.3 bc
N1P1K1		9.45 ba	14411 bac	122.3 dc	100.7 dc	3.35 ba	5476.5 bac	39.9 bdec	41.3 bac
N2P0K0		9.62 ba	15055 ba	152.9 bac	124.1 bac	4.10 a	7352.8 a	72.2 a	56.1 ba
N2P0K1		10.13 ba	15839 ba	158.2 ba	132.0 ba	4.41 a	7936.8 a	72.3 a	57.9 a
N2P1K0		10.99 a	17257 a	173.9 a	145.4 a	3.66 a	6868.4 ba	62.7 ba	50.2 ba
N2P1K1		10.76 a	16166 ba	164.4 ba	137.1 a	3.66 a	7313.9 a	60.5 ba	52.7 ba

*, **, and *** are significant at the 0.05, 0.01, and 0.001 levels respectively. ns: not significant at the 0.05 level. Treatment means values represent the mean of three replications. Different letter within each column denotes the significant difference at *P<0.05 by Tukey test*

Table 2.5. Analysis of Variance for interaction effects and main effects of factors for nitrogen use efficiency (NUE), agronomic efficiency (AE), nitrogen recovery efficiency (NRE), and internal efficiency (IE) by site year.

Site year	Source of Variation	*df*	NUE %	AE (kg kg^{-1})	NRE (kg kg^{-1})	IE (kg kg^{-1})
EFAW21	N	2	**	***	ns	***
	P	1	ns	ns	ns	ns
	N x P	2	**	***	ns	ns
	K	1	ns	*	ns	ns
	N x K	2	ns	ns	ns	ns
	P x K	1	ns	ns	ns	ns
	N x P x K	2	ns	*	ns	*
	S.E.		4.46	3.48	0.06	3.05
	Treatments					
	N0P0K0		-	-	-	71.8 bac
	N0P0K1		-	-	-	81.8 a
	N0P1K0		-	-	-	80.8 ba
	N0P1K1		-	-	-	82.6 a
	N1P0K0		70.7 ba	50.8 a	0.56	83.8 a
	N1P0K1		76.9 a	53.0 a	0.91	70.0 bac
	N1P1K0		57.9 bc	38.9 b	0.61	73.6 bac
	N1P1K1		65.4 bac	52.3 a	0.71	77.3 bac
	N2P0K0		50.6 c	27.7 b	0.59	63.1 c
	N2P0K1		56.5 bc	31.4 b	0.63	64.0 c
	N2P1K0		66.5 bac	38.0 b	0.74	63.2 c
	N2P1K1		60.3 bac	36.2 b	0.67	65.5 bc
LCB22	N	2	**	***	ns	***
	P	1	ns	ns	*	ns
	N x P	2	ns	ns	ns	ns
	K	1	ns	ns	ns	ns
	N x K	2	ns	ns	ns	ns
	P x K	1	ns	ns	ns	ns
	N x P x K	2	ns	ns	ns	ns
	S.E.		5.86	4.15	0.06	13.64
	Treatments					
	N0P0K0		-	-	-	32.2
	N0P0K1		-	-	-	38.5
	N0P1K0		-	-	-	52.6
	N0P1K1		-	-	-	53.8
	N1P0K0		53.9	42.2 a	0.37	73.1
	N1P0K1		47.7	35.6 ba	0.46	58.9
	N1P1K0		36.4	26.6 ba	0.22	69.8
	N1P1K1		48.2	36.2 ba	0.25	79.8
	N2P0K0		35.5	23.9 ba	0.37	63.2
	N2P0K1		36.8	26.2 ba	0.37	67.1
	N2P1K0		31.0	20.6 b	0.30	53.1
	N2P1K1		32.9	20. b	0.28	64.7

*, **, and *** are significant at the 0.05, 0.01, and 0.001 levels respectively. ns: not significant at the 0.05 level. Treatment means values represent the mean of three replications. Different letter within each column denotes the significant difference at *P<0.05 by Tukey test*

Table 2.6. Least square means of total nutrient uptake by aboveground whole plant at R6 growth stage and nutrient removal by grain.

		N		**P**		**K**	
Site-year	Treatment	--------------------kg ha^{-1}---------------		-------------------kg ha^{-1}---------------		--------------------kg ha^{-1}---------------	
		Whole Plant	Grain	Whole Plant	Grain	Whole Plant	Grain
EFAW 21	N0P0K0	77.0 f	56.4 e	19.18 dc	16.41 dc	79.14 c	24.13 dc
	N0P0K1	68.7 f	53.4 e	18.95 dc	14.73 d	102.79 bc	23.61 d
	N0P1K0	69.8 f	52.3 e	15.62 d	14.72 d	81.74 c	22.74 d
	N0P1K1	84.5 ef	65.4 e	19.36 dc	19.67 dc	99.11 bc	29.83 bdc
	N1P0K0	112.5 ed	104.3 dc	22.71 bdc	23.69 bac	101.50 bc	37.20 ba
	N1P0K1	136.2 bdc	108.4 bdc	29.12 ba	24.88 bac	145.52 a	38.30 ba
	N1P1K0	116.1 d	95.6 d	24.32 bdac	20.69 dc	109.98 bac	33.17 bdac
	N1P1K1	122.3 dc	100.7 dc	22.38 bdc	22.63 bdc	111.07 bac	37.49 ba
	N2P0K0	152.9 bac	124.1 bac	26.54 bac	22.46 bdc	123.08 ba	36.24 bac
	N2P0K1	158.2 ba	132.0 ba	23.66 bdac	22.42 bdc	122.07 ba	38.11 ba
	N2P1K0	173.9 a	145.4 a	32.28 a	29.79 ba	128.05 ba	45.46 a
	N2P1K1	164.4 ba	137.1 a	30.54 ba	31.54 a	128.07 ba	45.50 a
	S.E.	5.96	4.77	1.72	1.73	7.55	2.44
LCB22	N0P0K0	21.70 de	4.22 d	5.82 b	1.16 e	24.92 c	2.11 e
	N0P0K1	24.24 de	8.43 d	8.08 ba	2.37 e	31.44 bac	4.27 e
	N0P1K0	30.35 dec	16.10 dc	10.55 ba	5.22 dec	33.82 bac	9.16 edc
	N0P1K1	18.15 e	8.82 d	6.35 b	3.26 e	24.30 bc	5.83 ed
	N1P0K0	48.14 bdac	45.07 ba	15.69 a	12.56 bac	60.33 ba	19.18 bac
	N1P0K1	53.79 bac	40.90 bac	14.67 ba	12.37 bac	63.85 a	19.66 bac
	N1P1K0	37.96 bdec	33.33 bc	12.06 ba	8.76 bdec	45.09 bac	14.16 bedc
	N1P1K1	39.91 bdec	41.27 bac	14.92 ba	10.37 bdac	56.26 bac	15.87 bdc
	N2P0K0	72.24 a	56.12 ba	13.29 ba	15.58 ba	54.19 bac	24.47 ba
	N2P0K1	72.31 a	57.89 a	16.31 a	17.68 a	59.99 ba	27.67 a
	N2P1K0	62.70 ba	50.16 ba	13.93 ba	11.59 bdac	48.82 bac	18.74 bac
	N2P1K1	60.46 ba	52.70 ba	14.10 ba	14.54 ba	54.33 bac	22.14 ba
	S.E.	5.04	4.74	1.96	1.42	7.81	2.50

Treatment means values represent the mean of three replications. Different letter within each column denotes the significant difference at *P<0.05 by Tukey test.*

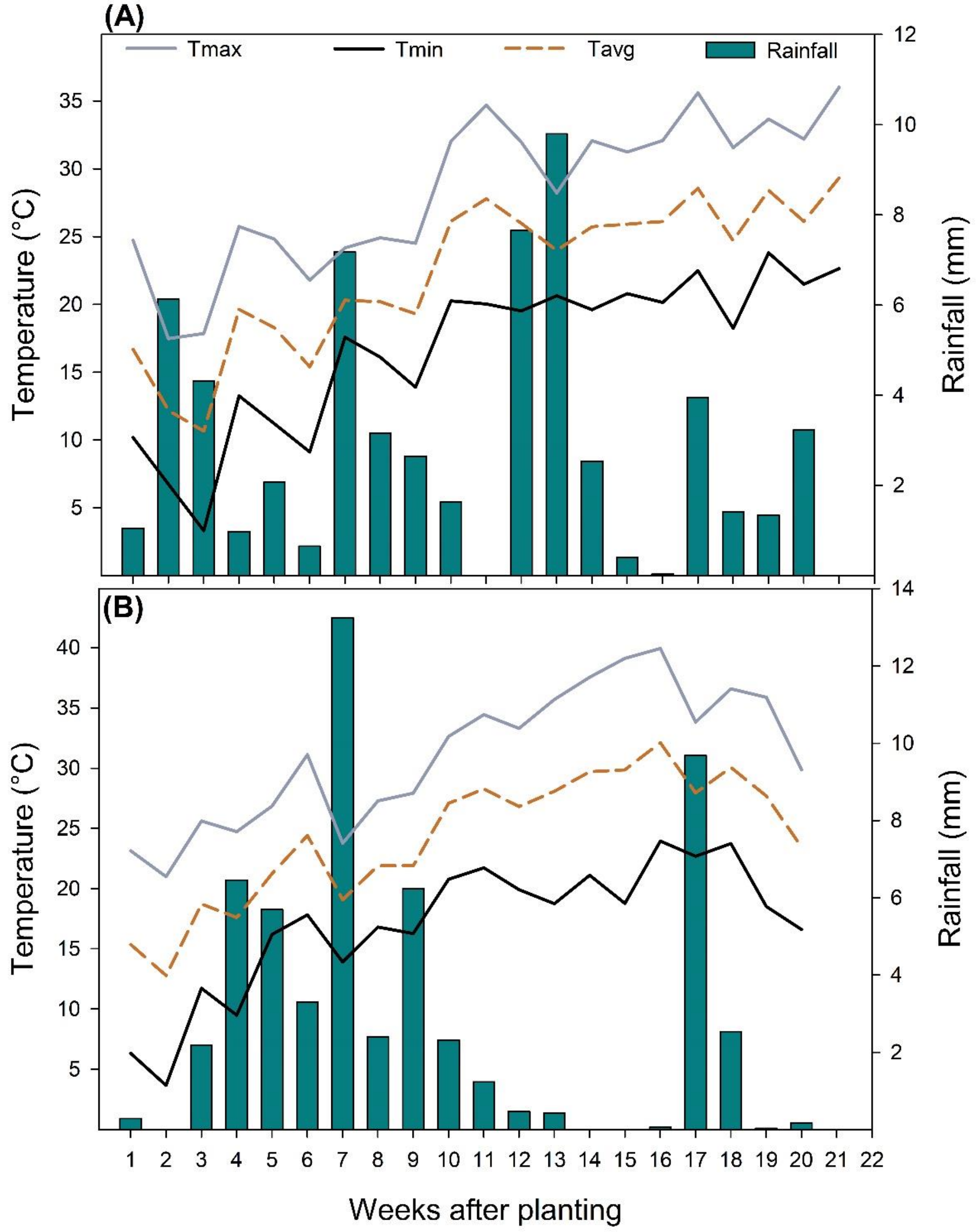

Fig 2.1: Weekly total rainfall (mm), and maximum temperature (Tmax), minimum temperature (Tmin), and average temperature (Tavg) for EFAW 2021 (A), and LCB 2022 (B).

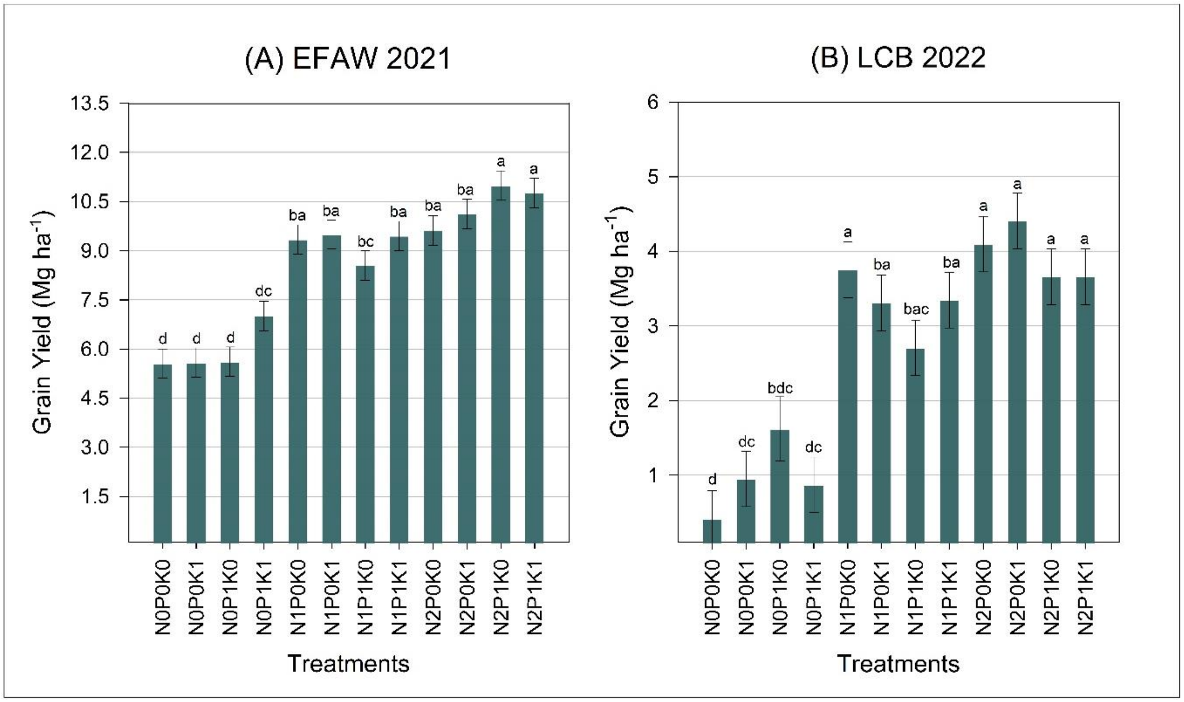

Fig 2.2: Grain yield as affected by treatments with bars representing mean grain yield and the lines representing standard error at EFAW 2021 (A); and LCB 2022(B)

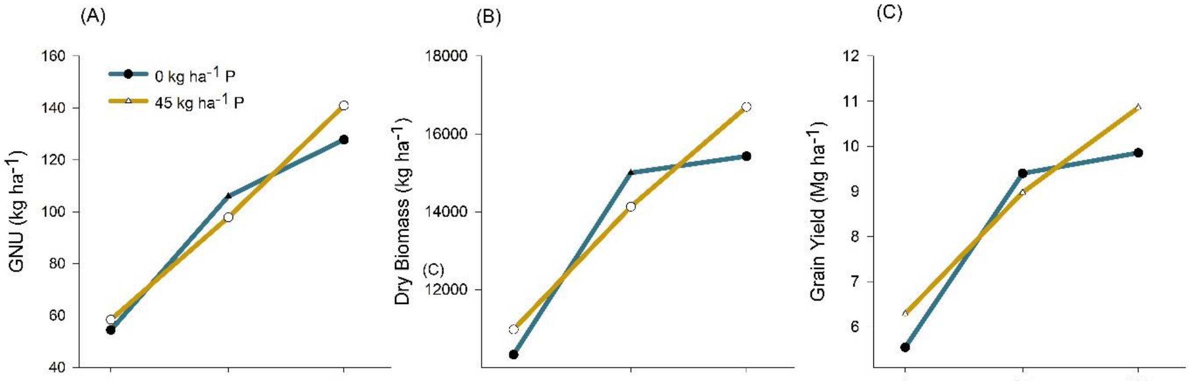

Fig 2.3: Grain nitrogen uptake (GNU, A), Dry biomass (B), and Grain yield (C) as affected by the interaction of nitrogen and phosphorous at EFAW 2021.

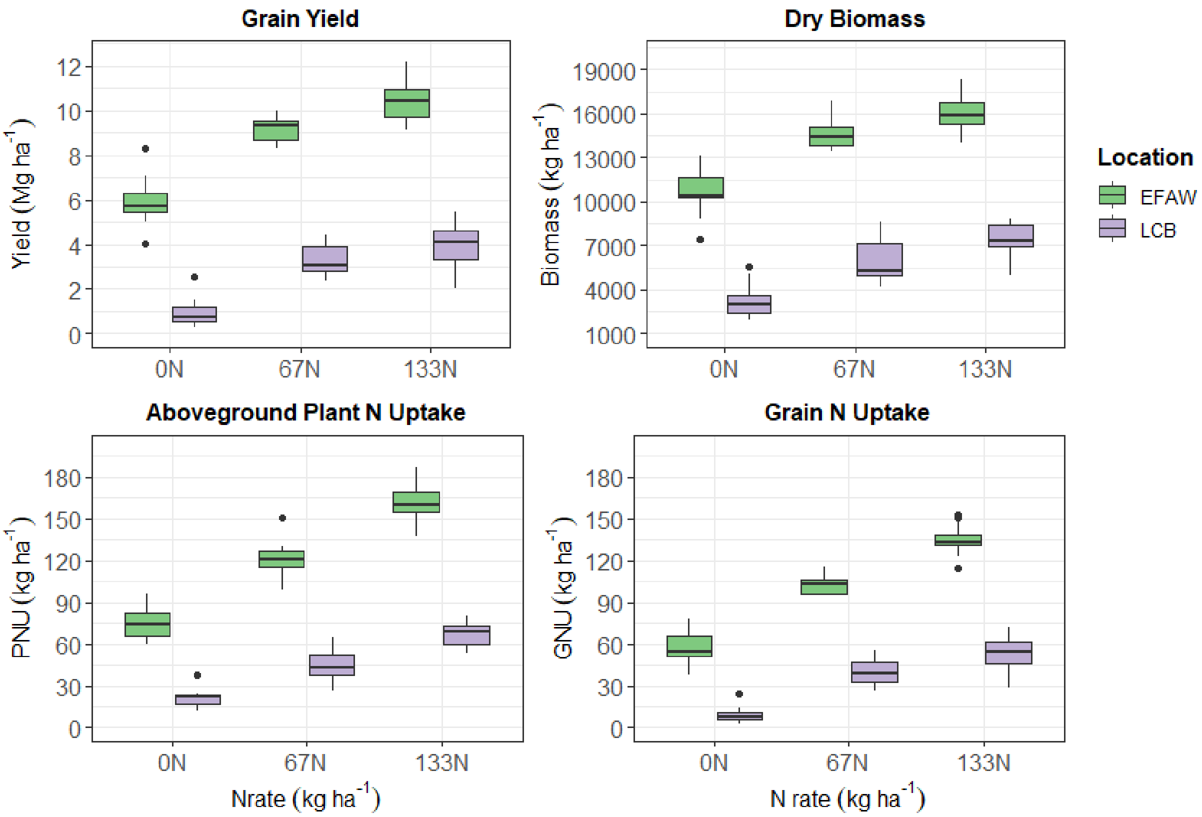

Fig 2.4: Effect of nitrogen rate on grain yield, dry biomass, plant nitrogen uptake (PNU), and grain nitrogen uptake (GNU) at EFAW 2021 and LCB 2022 site years.

CHAPTER III

NITROGEN, PHOSPHOROUS, AND POTASSIUM UPTAKE IN RAIN-FED CORN AS AFFECTED BY NPK FERTILIZATION

ABSTRACT

Effective nutrient management necessitates the knowledge of nutrient uptake at various growth stages and removal by the harvested portion. Information on nutrient accumulation was provided by some older literature as well as few researchers focused on this issue in this modern period with modern hybrids and improved corn cultivation practices. While almost all the studies were conducted in northern states of the US, information for the Southern Great Plains is still limited. Therefore, a 2-year field study was conducted to evaluate the effect of nitrogen (N), phosphorous (P) and potassium (K) fertilization on N, P, and K contents in aboveground plant at different growth stages in a rain-fed corn production system. Here we show that nutrient uptake values, pattern, and dynamics depend on environmental conditions, soil type, and management practices. We found that N concentration in plant was linearly affected by N application rate. Furthermore, plant P and K concentrations were not affected by any NPK fertilization rates. Our results demonstrated that total N, P, and K uptake was primarily driven by N

application rate. Total nutrient uptake increased linearly with increased N rate. Co-application of P and K along with N had no significant effect on nutrient concentration and uptake.

INTRODUCTION

Effective nutrient management requires the knowledge of nutrient uptake at different growth stages and removal by the harvested portion. When it comes to fertilization decisions, knowing the accumulation timing and quantity of nutrients removed is helpful (Ciampitti *et al.*, 2013). These values may differ from an average crop nutrient value because of different environmental conditions and agronomic techniques (Heckman *et al.*, 2003). There should be a re-evaluation of important nutrients uptake by corn for specific locations, which can then be utilized to make better fertilizer recommendations that will help current hybrids to achieve their maximum yield potential (Bender *et al.*, 2013).

Information on nutrient accumulation was provided by some older literature (Hanway, 1962; Peck et al., 1969; Walker & Peck, 1972, 1974; Mackay *et al.*, 1987), as well as few researchers focused on this issue in this modern period with modern hybrids and improved corn cultivation practices (Mallarino and Higashi, 2009; Ciampitti and Vyn, 2011, 2014; Bender *et al.*, 2013; Stammer and Mallarino, 2018; Woli *et al.*, 2018). While almost all those studies were conducted in the northern states, and information for the southern great plains is still limited. Some research has been done on nitrogen (N) uptake in Oklahoma but still lacking on how N, phosphorous (P), and potassium (K) fertilization practices affect these nutrients uptake and their concentrations in corn plants (Freeman *et al.*, 2007; Girma *et al.*, 2011). It was reported in

Oklahoma that corn N uptake was 68.8 to 114 kg N ha^{-1} (Freeman *et al.*, 2007). According to Girma *et al.*, (2011) the maximum N accumulation was 42 kg N ha^{-1} in check plot to 131 kg N ha^{-1} in a plot received 224 kg N ha^{-1} for corn grown in Oklahoma. Nitrogen uptake and removal increases with increases in N application rate (Halvorson and Bartolo, 2014; Halvorson & Johnson, 2009; Sindelar et al., 2013). According to Zone et al. (2020), P and K fertilization marginally but consistently increased leaf P and K concentrations of corn grown in Ohio with 1.05% increase in P and 3.17% increase in K. Grain P concentration was also shown directionally positive increase in 51% (21 out of 41) of their corn trials, while grain K increased in 72% (21 out of 29) of trials. Modern hybrids uptake more N and P as compared to earlier varieties (Woli et al., 2018). They found that 178 and 213 kg N ha^{-1} is taken up by the corn plants at R6 growth stage in 1960 and 2000 era hybrids, respectively. According to Ciampitti et al. (2013) 195 kg N ha^{-1} was accumulated by R6 for 2000 era hybrids. Bender et al. (2013) conducted a research in Illinois with modern corn hybrids planted at higher densities. They found that plants removed 286 kg N ha^{-1}, 114 kg P ha^{-1}, 202 kg K ha^{-1}, 26 kg S ha^{-1} and the grains removed 166 kg N ha^{-1}, 90 kg P ha^{-1}, and 66 kg K ha^{-1}. In a study conducted by Stammer and Mallarino (2018), P concentration was 4.8 to 5.3 g kg^{-1} and K concentration was 18.8 to 25.3 g kg^{-1} in the whole plant at V5-V6 stage. Discrepancy in these studies suggest that nutrient concentration as well as nutrient uptake varies greatly with plant growth stage, cultivation practices, variety, soil fertility, and environmental conditions. Therefore, nutrient uptake knowledge is only useful when it is specified to local growing conditions. According to the 2010 IPNI report, macro and micronutrients have been reduced in Canadian and US soils during the prior 5 years (Fixen et al., 2010). The combination of high yielding cultivars and declining soil fertility shows that farmers did not match nutrient uptake and removal with fertilizer applications

(Fixen et al., 2010). Therefore, the primary goal of this study is to evaluate the effect of N, P, and K fertilization on N, P, and K contents in aboveground plant at different growth stages in a rain-fed corn production system. A second objective is to evaluate the seasonal nutrient uptake pattern and accumulation as a function of time as affected by NPK fertilization.

MATERIAL AND METHODS

Field trials and experimental design

This field study was planted at four locations, EFAW (EFAW21, 36°08'14.1"N 97°06'22.4"W) and Lake Carl Blackwell (LCB21, no reportable results due to raccoon damage, 36°09'04.8"N 97°17'21.5"W) in 2021. While in 2022, the trail was conducted at LCB (LCB22, 36°09'1.64"N 97°17'23.30"W) and Perkins (PRK22, no reportable results due to damages by wild hogs after R1 stage, 35°59'37.32"N 97°2'31.41"W). Soil classification at the EFAW site is an Ashport silty clay loam (fine-silty, mixed, superactive, thermic, Fluventic Haplustolls) while, LCB is classified as a Pulaski fine-sandy loam (coarse/loamy, mixed non-acid, thermic, Typic, Ustifluvent), and Perkin's soil is Teller sandy loam (fine-loamy, mixed, active, thermic, Udic Argiustoll) (Soil Survey, 2021).

A randomized complete block design was used for all locations, which included 12 treatments and three replications. Each treatment plot was measured 3x6 m and consisted of four rows of corn plants, with an alley of 3 m between each replication. The 12 treatments evaluated different combinations nitrogen (N), phosphorous (P), and potassium (K) fertilizer rates for maize applied preplant (Table 1). Nitrogen rates evaluated included 0, 67, and 133 kg N ha^{-1}. The rates were 0 and 20 kg P ha^{-1} and 0 and 60 kg K ha^{-1}. All fertilizers were applied as pre-plant using a barber metered feed fertilizer spreader using appropriate settings to achieve the desired fertilizer application rates. This machine was calibrated to ensure the appropriate amount of fertilizer was delivered. After applying a particular nutrient, hopper of the spreader was cleaned with pressurized air. Fertilizer sources for N, P, and K included urea ($N-P_2O_5-K_2O$, 46-0-0), triple super phosphate ($N-P_2O_5-K_2O$, 0-46-0), and Muriate of potash ($N-P_2O_5-K_2O$, 0-0-60).

Composite pre-plant soil samples were taken from both locations at 0-15 cm depth from each replication. Soil samples were dried at 65° C for 12 hours and passed through 2 mm sieve in preparation for chemical analysis. Chemical analyses conducted included pH, ammonium (NH_4-N), nitrate (NO_3-N), plant available P and K. Analysis of soil nitrate and ammonium nitrogen was performed using 5 g of soil sample extracted with 25 ml of 1 M KCl solution. These samples were filtered after shaking for 30 minutes and analyzed using a Lachat flow injection autoanalyzer (Gavlak *et al.*, 2003). Mehlich 3 (M3) extractant (Mehlich, 1984) was used for P and K determination. To do this, 2 g of the soil samples were weighed and mixed with 20 ml of M3 extractant. Samples were filtered after shaking for 5 minutes and analyzed by an Inductively Coupled Plasma-Atomic Emission Spectroscopy. Results of the pre-plant routine soil tests are shown in Table 2.

Approximately four days after pre-plant fertilizer applications were made, when the soil moisture level and soil temperature were both adequate, corn hybrid ‘DKC66-29’ (DeKalb Genetics Corporation, IL) was planted at both sites using John Deere Max Emergence 2 7300 four row planter. Row spacing was 76 cm with a population of 49,400 seeds ha^{-1} at EFAW21 and 69,160 seeds ha^{-1} at LCB22. Integrated Pest Management was performed according to Oklahoma State University recommendations. Summary of field activities can be found in Table 3.

Data collection

Data collection began 20 days after planting (DAP). Stand-count data was gathered to determine the emergence percentage of each plot by counting the emerged corn plants in the center two rows. Growth stages used were determined according to Abendroth et al. (2011).

Plant samples were collected from the side two rows of each plot at V6/V7, VT, R2, and R6 growth stage for nutrient analysis (Abendroth et al., 2011). A one-meter length of row was randomly selected, then plants were cut at 5 cm above ground level. Fresh weight was taken after collecting plant samples and then weighed again after oven-drying to determine subsample aliquot dry weight and dry biomass accumulation. Dry plant samples were then ground and passed through 1-mm sieve.

Because rain-fed crop often loses biomass per unit area over time, data for nutrient uptake curves and seasonal nutrient uptake patterns were determined per plant basis. We counted the number of plants in the 1-meter rows each time we took biomass sample. To determine the amount of biomass per plant, the total biomass from these meter rows was determined and then divided by the number of plants.

Upon crop maturity, the middle two rows of each plot were harvested using a Kincaid 8XP plot combine equipped with a Harvest Master Yield monitor in which the final grain yield reported was adjusted to 15.5% moisture.

For grain N and crude protein (CP) analysis, a LECO CN 828 instrument was used

(LECO corp. St. Joseph, MI). One hundred and fifty mg of dried and ground grain samples from each plot were encapsulated in a tin foil and loaded in the machine. These samples were combusted at 950 °C in a reticulated ceramic crucible inside the machine's furnace in a pure oxygen environment. The moisture was then separated by thermoelectric cooler until the combusted gases were delivered into a ballast tank, where they equilibrate and mix before being exposed to a flowing stream of inert gas for analysis. The aliquot gas was carried to a thermal conductivity cell for detection of nitrogen as N_2.

Total P and K in plant samples were determined by nitric acid digestion and inductively coupled plasma spectrometry quantification. Nutrient concentrations (Nc) were provided in percentage and the values of total aboveground macronutrient uptake were calculated by Equation 1. While Equation 2 and 3 were used for the estimation of nutrient removal by grains and whole plants, respectively.

$$Plant\ Macronutrient\ Uptake\ (kg\ ha^{-1}) = Dry\ Biomass\ (kg\ ha^{-1})\ \times Nc \qquad (1)$$

$$Grain\ Macronutrient\ Uptake\ (kg\ ha^{-1}) = Yield\ (kg\ ha^{-1})\ \times Nc \qquad (2)$$

$$Plant\ Macronutrient\ Uptake\ (g\ pl^{-1}) = Dry\ Biomass\ (g\ pl^{-1})\ \times Nc \qquad (3)$$

Nutrient harvest index is the partitioning efficiency of nutrient to grain (Bender *et al.*, 2013). Following grain nutrient harvest indices are calculated on the basis of grain nutrient uptake and total aboveground plant nutrient uptake:

Grain Nitrogen Harvest Index (GNHI) was calculated to estimate the amount of nitrogen portioned to the grain by plant

$$GNHI = \frac{\text{Nitrogen uptake in grain}}{\text{Nitrogen uptake by whole plant}} \times 100 \qquad (4)$$

Grain Phosphorous Harvest Index (GPHI) was calculated to determine how much K was portioned to the grain by plant.

$$GPHI = \frac{\text{Phosphorous uptake in grain}}{\text{Phosphorous uptake by whole plant}} \times 100 \qquad (5)$$

Grain Potassium Harvest Index (GKHI) was estimated to quantify the amount of K portioned to the grain by plant.

$$GKHI = \frac{\text{Potassium uptake in grain}}{\text{Potassium uptake by whole plant}} \times 100 \quad (6)$$

Statistical Analysis

Data were analyzed using SAS version 9.4 (SAS Institute, Cary, NC, USA). ANOVA was applied using PROC-GLIMMIX procedure and the mean separation procedure was done by Tukey. Each site and year were analyzed separately. Treatments was used as fixed effect and replications as random. All nutrient uptake values were calculated on dry weight basis.

RESULTS AND DISCUSSIONS

We were able to record the yield data for two site years only out of four. Corn ears were damaged by raccoons at LCB21 site at maturity, while almost all the crop was destroyed at PRK22 by wild hogs after R1 stage. Therefore, we will only report data from EFAW21 and LCB22.

Nutrient concentration in whole plant at early growth stage (V6/V7) and plant components at physiological maturity (R6)

Nitrogen concentration

There was a main effect of N rate on N concentration at V6/V7 growth stage at EFAW21, and LCB22 (Table 4). Nitrogen concentrations in plants increased with the increase in N rates (Fig. 2). However, addition of P or K or both did not have any significant effect of decreasing or increasing N concentration. Terman et al. (1977) also documented that P had no effect of increasing N concentration in corn plants.

At Maturity, there was a main effect of N rate on N concentration in stover, ear, and grain at both locations. Nitrogen concentrations in these plant components were significantly greater when higher rate of N was applied (Fig. 2). These results were similar to Bruns and Ebelhar (2006) who also found that N concentrations in stover and grain increased as N fertility level increased. Kurtz and Smith (1966) also found that increasing N fertility increased protein content in corn grain which is the major form of N found in corn grain. This positive effect of increased N concentration by N rates has been often reported. The maximum grain N concentration in our study was 14.0 g kg^{-1} which is consistent with the findings of Heckman et al. (2003), Setiyono et al. (2010), and Bender et al. (2013).

Phosphorous and potassium concentration

There was no significant effect observed at V6 growth stage for P and K concentrations at EFAW21 (Table 4). However, the main effect of N (p=0.001) and main effect of K (p=0.013) was observed at LCB22 for P concentration. Similar effect of N (p=0.005) and K (p=0.003) was observed for K concentration at this site. Phosphorus concentration in whole plant ranged from 2.49 g kg^{-1} to 2.82 g kg^{-1} at EFAW21 and 3.89 g kg^{-1} to 4.97 g kg^{-1} at LCB22. While K concentration ranged from 39.9 g kg^{-1} to 52.4 g kg^{-1} at EFAW21 and 33.6 g kg^{-1} to 50.5 g kg^{-1} at LCB22. Difference in P concentrations between the two sites was due to the dilution effect caused by more biomass at EFAW21 site. However, this effect was not observed for K concentration, which might support luxury uptake of K.

At maturity, stover P concentration was significantly affected by N rate at LCB22 site only. Stover P concentration decreased linearly with N rate at this site. Stover P concentration was reduced by half where 133 kg N ha^{-1} was applied. Phosphorus concentration in stover tend to decrease as N rate increased but was not significantly different. Stover P concentration ranged from 0.83 g kg^{-1} to 2.5 g kg^{-1} at LCB22, and 0.37 g kg^{-1} to 0.65 g kg^{-1} at EFAW21. Stover P concentration at EFAW21 falls within the range reported by Setiyono et al. (2010). While ear P concentration were similar among all treatments at both locations which varied from 1.9 g kg^{-1} to 2.5 g kg^{-1} at EFAW21 and 2.6 g kg^{-1} to 3.3 g kg^{-1} at LCB22.

At maturity, only main effect of K was observed in stover for increasing stover K concentration at EFAW21. The K concentration in stover ranged from 14 to 17.5 g kg^{-1}, while K concentration in ear ranged from 4.4 to 5.4 g kg^{-1} at this site. Presence of K in NPK combination increased K concentration in stover by 12.5%. The positive effect of K may be due to the fact that K enhances tissue turgor pressure, which regulates the opening and closing of stomata (Marschner, 1995). However, this effect of K application was not seen at LCB22 site. Stover K concentration and ear K concentration varied from 8.6 to 12.5 g kg^{-1} and 5.5 to 8.5 g kg^{-1} respectively at this site. Stover K concentration in our study is close to that reported by Ciampitti et al. (2013), which was 15 g kg^{-1}. However, stover K concentration was lower than 21.8 g kg^{-1} reported by Setiyono et al. (2010). Phosphorus and K concentrations of stover and ear were similar among different treatments even if they were accumulated significantly differently among biomass samples.

The treatment effect was non-significant for P and K concentrations in grains. This may be due to increased grain yield by sufficient fertilization, which increased P and K dilution. This dilution effect could cause similar P and K concentration even when compared with controls.

Bélanger and Richards (1999) also observed this effect. Phosphorus concentration decreased with increasing N rates due to dilution, which was consistent with the results of Schlegel and Havlin (2017). Mallarino and Higashi (2009) also supported that K concentration in corn grain did not get affected by K application. Phosphorus concentrations in grain ranged from 2.20 g kg^{-1} to 2.93 g kg^{-1} at EFAW21 and 2.86 g kg^{-1} to 3.89 g kg^{-1} at LCB22. While K concentration in grain ranged from 3.77 g kg^{-1} to 4.31 g kg^{-1} at EFAW21 and 5.21 g kg^{-1} to 6.39 g kg^{-1} at LCB22. Difference between these two sites could be due to difference of test weight of grains. Mean test weight for LCB22 site was higher as compared to EFAW21, which could have caused dilution of nutrients in grain. This suggests that nutrients dilution occurs in corn grain in high yielding environment. This was also observed by Lollato et al. (2019) in wheat. Grain P concentration in our study was consistent with that reported by Setiyono et al. (2010), which was 2.6 g kg^{-1}. Other studies reported higher grain P concentration. Ferreira et al. (2012) conducted experiment with different cultivars in Brazil and reported P concentration ranged from 2.9 g kg^{-1} to 5.1 g kg^{-1} and K concentration ranged from 3.7 g kg^{-1} to 10.3 g kg^{-1}. Bender et al. (2013) reported 3.3 g kg^{-1} P, and 4.4 g kg^{-1} K in grain. According to Heckman et al. (2003), the average grain P concentration was 3.34 g kg^{-1} and the average grain K concentration was 4.8 g kg^{-1}. These values were calculated from corn cultivars grown in north-eastern US states. Ciampitti et al. (2013) reported grain P concentration from 3.4 to 4.0 g kg^{-1} and grain K concentration between 4.9 and 5.1 g kg^{-1}. While Mallarino and Higashi (2009) reported that grain K concentration of 3.5 g kg^{-1} in their study. Phosphorus and K concentration from LCB22 site falls within these ranges reported by different researchers. However, even the highest P concentration at the EFAW21 site was below these values, while the K concentration was within the published ranges. The reason for this could be discrepancy between yield level and study environment. Almost all those comparable studies were conducted in high yielding environment especially in the Midwest of US. Favorable environment can provide better conditions for diffusion of these nutrients from soil to roots (Heckman et al., 2003).

Total plant biomass accumulation and NPK uptake at early growth stage and maturity

Plant biomass accumulation

Analysis of Variance shows that there was no interaction effect of N x P x K on biomass at V6 growth stage at both locations ($p \leq 0.05$) (Table 5). However, application of K had significant effect at EFAW21 ($p = 0.0435$), as the biomass was reduced by 15.48 % with the application of K fertilizer. Only EFAW21 site did not respond to N rate for biomass accumulation at V6 ($p = 0.08$). Biomass at EFAW21 ranged from 1138 kg ha^{-1} to 1665 kg ha^{-1}. At LCB22, biomass ranged from 307.6 kg ha^{-1} to 1014 kg ha^{-1}. Application of K with N increased biomass seldomly at LCB22. This may be due to positive response of K to provide drought resistance to plants which is beneficial to rain-fed crops. A study by Martineau et al. (2017) reported that K fertilizer enhanced water use efficiency under water stress conditions by enhancing stomatal sensitivity to drought. Hsiao and Lauchli (1986) found K played a vital role in making plants more resistant to water deficiency.

At crop maturity, interaction effect of N x P and main effect of N ($p < 0.0001$) and main effect of K ($p = 0.02$) were observed for biomass accumulation for EFAW21, while only main effect of N was observed at LCB22 site (Table 6). Potassium application increased dry biomass by 6.25 % at EFAW21. However, the highest aboveground biomass at EFAW21 was observed where 20 kg ha^{-1} P was applied with 133 kg ha^{-1} N, which is similar to the findings of Karlen et al. (1988). The maximum biomass at maturity was found at LCB22 when K was applied with 133 kg ha^{-1} N. Nitrogen rate increased biomass by 36% and 50% at EFAW21, and 80% and

123% at LCB22 when N was applied at 67 kg N ha^{-1} and 133 kg N ha^{-1} respectively as compared to the control. The role of K in increasing dry biomass can be also explained by the fact that K provides strength to stalk (Flannery, 1982; Foley & Wernham, 1957) and enhances resistance to plant diseases and pests (Asante-Badu et al., 2020). These could be the main reasons why the corn plant produced higher biomass when K was applied. However, K did not increase grain yield as discussed in previous chapter, which suggests that K was able to enhance stover biomass only. This can also be confirmed through low GKHI (Table 7), which proved that luxury uptake of K occurred.

Total nitrogen uptake

There was a significant difference for N uptakes during early growth stage at both locations attributed to N rates. Applied P or K, however, did not have any significant effect at this stage at either location (Table 5). Nitrogen uptake ranged from 22.2 kg ha^{-1} to 43.3 kg ha^{-1} at EFAW21. It almost doubled when 133 kg N ha^{-1} was applied as compared to the control. At LCB22, N uptake increased more than three folds when higher rate of N was applied and it ranged from 8.1 kg ha^{-1} to 31.4 kg ha^{-1}. When P or K or both were applied with N in 2021, N uptake decreased. In contrast, N uptake increased in 2022, when P and K were present with N. However, both the reduction and increment were insignificant and inconsistent.

At maturity stage (R6), there was interaction effect of N x P x K and main effect of N rate at EFAW21 (Table 6). Addition of P or K or both with 133 kg ha^{-1} N increased the nitrogen uptake by 3 to 13% as compared to 133 kg ha^{-1} N only. Application of 67 kg N ha^{-1} resulted in 34.5% less N uptake as compared to 133 kg ha^{-1} N. The main effect of N and main effect of P was observed at LCB22. As compared to 0-N, addition of 67 kg N ha^{-1} increased N uptake by two folds and 133 kg N ha^{-1} increased N uptake by three folds at LCB22. Application of P decreased N uptake by 7 kg ha^{-1}, which was not expected. But, it is clear from this study that total N uptake at maturity depends greatly on N input. Nitrogen uptake at maturity in the fertilized plots was 60% higher at EFAW21 and 43% lower at LCB22 than the findings of Freeman et al. (2007). They computed that total N uptake for irrigated corn in Oklahoma was 108.2, 108.5, and 114.4 kg N ha^{-1}, when N was applied at the rate of 118, 236, 354 kg ha^{-1}. However, our current mean value was 162 kg N ha^{-1} at EFAW21 and 68.15 kg N ha^{-1} when 133 kg ha^{-1} N was applied alone or in NPK combination (Fig. 5). The highest N accumulated across two sites when P was applied with 133 kg ha^{-1} N, but this value was far less than the findings of Woli et al. (2018), Bender et al. (2013), and Karlen et al. (1988). This uptake value was even less than that reported by Hanway in 1962 (mean= 201 kg N ha^{-1}).

Total phosphorous uptake

The statistical data presented in Table 5 revealed that at early growth stage, P uptake was not significantly affected by any interaction effect and main effect at EFAW21. There was main effect of N ($p< 0.0001$) and main effect of K ($p = 0.03$) on total P uptake at LCB22. Phosphorus uptake increased with the increase of N rate. Application of 133 kg N ha^{-1} resulted in P uptake increase by almost two folds when compared to treatments with 0-N at LCB22. Co-application of K also increased P uptake at early growth stage at LCB22.

At maturity, there was interaction effect of N x P x K, N x P, and main effect of N for P uptake in above ground whole plant at EFAW21 (Table 6). Grain P uptake at this site was affected significantly ($p\leq0.05$) by interaction of N x P, main effect of N, and main effect of P. However, only main effect of N was observed for whole plant P uptake as well as grain P uptake at LCB22 (Table 6). Grain P uptake increased by 11.7% with P application at EFAW21, but this effect was not observed at LCB22. The maximum whole plant P uptake was 32.28 kg ha^{-1} and the maximum grain P uptake was 31.54 kg ha^{-1} at EFAW21 (Fig. 5). While, at LCB22, the highest whole plant P uptake was 16.31 kg ha^{-1} and grain P uptake was 17.68 kg ha^{-1}. Increases in total aboveground biomass resulted in more P uptake by whole plant as well as grain. Positive interaction of N x P for P absorption was also noted by Fageria (2001). Phosphorus uptake in stover and grain increased with the increase in N supply, which was also supported by Setiyono et al. (2010), Ma et al. (2016), and Ciampitti et al. (2013). According to Wilkinson et al. (1999), P uptake was increased by N rate because of increase in the root length and the ability of roots to explore and absorb more P. Moreover, NH_4^+ ions from N fertilizer compete with other cations, which then increase soil P solubility by releasing P fixed on oxide surfaces of clay minerals. Grain N, P, and K uptake increased with N rates linearly, which were similar to the observations of Ciampitti et al. (2013), Feil et al., (1993) and Hanway (1962).

Total potassium uptake

During early growth stage, neither interaction effect nor main effect was observed for K uptake at EFAW21. While, at LCB22, there was main effect of N and main effect of K for K uptake. Nitrogen application increased K uptake at early growth stage at LCB22. Application of K also increased K uptake by 20.7 % at LCB22 (Table 5).

At maturity, the whole plant K uptake was affected significantly by the main effect of N and main effect of K, while grain K uptake was affected by interaction of N x P and main effect of N at EFAW21. However, at LCB22, both whole plant K uptake and grain K uptake was solely affected by N rate (Table 6). Total K uptake in whole plant and grain was significantly ($p \leq 0.05$) greater at 133 kg N ha^{-1} rate than lower levels of N (Table 6). However, K concentration in the whole plant and grain was unaffected by varying N application rates. (Table 4). This suggests that the uptake was affected due to increase in dry biomass. In our study, we found that N, P, and

K contents were controlled by N rates. This finding is consistent with previous studies (Kamprath, 1987; Wang et al., 2007; and Setiyono et al., 2010).

Nutrient Harvest Index

Nitrogen Harvest Index (NHI), Phosphorous Harvest Index (PHI), and Potassium Harvest Index (KHI) were computed and presented in Table 7. Results showed that NHI increased linearly with N rates, while PHI and KHI were similar among treatments at EFAW21 site. However, at LCB22, all these nutrient harvest indices increased with increased N application rates. In the study of Bender et al. (2012), NHI ranged from 0.51 to 0.62, PHI ranged from 0.70-0.82, while KHI ranged from 0.27 to 0.37. Ciampitti et al. (2013) documented mean NHI value of 0.55 to 0.70, mean PHI value of 0.70 to 0.85 and KHI 0.28. While, Setiyono et al. (2010) reported NHI 0.64, PHI 0.84 and KHI 0.17. Similar values were reported by Bender et al. (2012). However, NHI, PHI, and KHI in our study were higher than what have been reported by published studies at both locations when 133 kg N ha^{-1} was applied.

Seasonal nutrient uptake pattern

Nitrogen uptake (mg per plant) was significantly affected by N rate across all growth stages at EFAW21 (Table 8a) and LCB22 (Table 8b) except for R6 growth stage at EFAW21, where interaction effect of N x P x K was also observed along with main effect of N. Applied P or K did not have significant effect on seasonal nutrient uptake at any growth stages. Nitrogen rate, however, significantly increased N uptake at all growth stages at both locations.

Phosphorus uptake was not affected by N rate until maturity at EFAW21. At R6 growth stage, N rate increased P uptake. Application of 67 kg N ha^{-1} increased P uptake by 14.7%, while 133 kg N ha^{-1} increased P uptake by 54.6% as compared to the control at this site. While at

LCB22, P uptake was affected by N rate at V6 and interaction of N x K at VT growth stage. Bennett et al. (1962) reported that when N uptake of plant increased, it became physiologically active which further caused higher uptake of P. Large amount of N compounds are formed in plant due to high uptake of N and some of these compounds contains P. While some of other plant compounds require P even for their formation. These physiological changes in plant cause plant to uptake higher P if available. This explanation was also supported by Cole et al. (1963).

Potassium uptake was not significantly affected ($p \leq 0.05$) at V6 and R2 growth stage at EFAW21. At VT growth stage, only main effect of K was observed, while at R6, main effect of N (p=0.000) as well as main effect of K (p=0.003) was observed at this site. Potassium application increased K uptake by plants. Potassium concentration in grain was not significantly affected by K rate but K concentration in stover was affected by K rate. Application of K increased stover K concentration, which further caused higher K accumulation. At LCB22, K uptake was affected at V6 growth stage only, where K uptake was increased by N rate.

The maximum uptake values of N, P, and K per plant at early growth stage were higher at both locations than found by Rosa et al. (2019) and Bermudez & Mallarino (2004). To our knowledge all studies have reported uptake pattern on the basis of total nutrient uptake per hectare (Karlen et al., 1988; Hanaway et al., 1962, Woli et al., 2016; and Bender et al., 2013). However, there was only N rate which was influencing N, P, and K uptake at almost all growth stages at both sites; therefore, the only effect of N rate was shown in seasonal nutrients uptake curves (Fig 3a-c).

Nutrient accumulation timing

The timing of nutrient accumulation is presented on nutrient contribution during particular growth period (Fig 4 a-c). There was no significant difference of applied P or K with timing of nutrients uptake at any growth stage. However, there was difference of N rate at some growth stages at EFAW21. This difference was due to difference in total uptake of that particular nutrient. When 133 kg N ha^{-1} was supplied, 39% of total N uptake was done by VT stage whereas 43.2% and 47.4% of total N uptake was observed when N input decreased to 67 kg N ha^{-1} and 0 kg N ha^{-1} respectively at EFAW21. About half of N was taken in well fertilized plots between VT and R2 stage, which was R1 growth stage. About 14% to 25% of P uptake was done till VT growth stage and 60 to 80% of total K uptake was done at VT stage at EFAW21. Nitrogen and P uptake follows different pattern, while K follows same pattern as reported by Karlen et al. (1988), Woli et al. (2017), Ciampitti et al. (2013), and Bender et al. (2013).

Karlen et al. (1988) reported that 65% N, 46% P, and 88% uptake was done by R1 growth stage. According to Hanway et al. (1962), 65% N, 50%, and 90% of K uptake occurred by R1 growth stage. Ciampitti et al. (2013) reported that 70% N, 49% P, and 122% K uptake was done by R1. According to Bender et al. (2013), 67% N, 46% P, and 66% K uptake was completed by R1 growth stage. The variability among treatments for uptake percentage depends on total nutrient uptake at maturity and amount of available nutrients. Nutrient uptake pattern differences from other studies could be explained by environmental difference which includes differences in weather, soil-type, hybrids, irrigation status, agronomic management and other factors. Further field research is necessary in light of these varied results.

CONCLUSION

It is important to adapt sound nutrient management practices, which balance inputs and outputs of nutrients. To achieve this goal, nutrients removed by the crop should be replaced.

Local nutrient removed values are an important part of making effective nutrient management plan and sustainable agriculture. In this study we observed that nutrient uptake and concentration in plants depends greatly on environment and management. Nitrogen concentration in whole plant at early growth stage was linearly affected by N application. Similar trend in N concentration was observed for all plant components at maturity. Phosphorous and K concentration was not affected by NPK rate. Total N, P, and K uptake was primarily driven by N rate at all growth stages. Almost all K uptake occurred in the vegetative stage of corn plant. Uptake pattern in our rain-fed corn study was very different from published studies because of the difference in environment and management practices. Almost all of those studies were conducted in high yielding environment with more rain or better irrigation facilities. Removal values in our study questions about the usefulness of average values from those empirical studies in rainfed environment. More studies are required on variety of soils under different environments to compute the nutrient uptake and removal values for rain-fed corn. Additional sites years would provide useful nutrient uptake and removal data that will be valuable for making sound nutrient management plans.

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TABLES AND FIGURES

Table 3.1: Basic soil properties at Efaw in 2021, and Lake Carl Blackwell (LCB) Oklahoma in 2022.

Year	Location	pH	Organic Carbon %	NH_4-N	NO_3-N	P ǂ	K ǂ
				mg kg^{-1}			
2021	Efaw	5.6	0.83	42.5	23.3	24	201
2022	LCB	6.1	0.63	3.3	<0.1	11.5	69.5

ǂ P& K are plant available phosphorous (P) and potassium (K) using Mehlich 3, respectively

Table 3.2: Dates for major field activities performed during the crop season at both sites

Location	Soil Samples taken	Pre-plant fertilizer Application	Planting date	Stand Count Date	Harvesting
Efaw	04/01/2021	04/02/2021	04/06/2021	04/26/2021 05/10/2021	08/26/2021
LCB	04/06/2022	04/07/2022	04/06/2022	04/26/2022	08/15/2022

Table 3.3. Nitrogen (N), phosphorous (P), and potassium (K) application rates employed at both Efaw and LCB locations.

Treatment code	Nutrient rates (kg ha^{-1})		
	N	P	K
N0P0K0	0	0	0
N0P0K1	0	0	60
N0P1K0	0	20	0
N0P1K1	0	20	60
N1P0K0	67	0	0
N1P0K1	67	0	60
N1P1K0	67	20	0
N1P1K1	67	20	60
N2P0K0	133	0	0
N2P0K1	133	0	60
N2P1K0	133	20	0
N2P1K1	133	20	60

Table 3.4. The least square means of nutrient concentrations at early growth stage (V6/V7), physiological maturity (R6), and grain. Corn plants were separated into stover (stalk + leaves) and ear (cob + husk) at R6 stage. Grain samples were collected during harvesting.

Site-year	Treatment	**Nitrogen (g kg^{-1})**			
		-----------------------Plant component-----------------------			
		V6	R6		Grain
		(Whole plant)	Stover	Ear	
EFAW21	N0P0K0	19.72 b	4.62 ba	9.10 bdc	10.07 edf
	N0P0K1	19.81 b	3.02 b	8.81 dc	9.53 ef
	N0P1K0	20.52 ba	4.18 ba	8.39 d	9.32 f
	N0P1K1	21.37 ba	4.26 ba	8.48 d	9.32 f
	N1P0K0	23.66 ba	4.71 ba	8.71 dc	11.16 edc
	N1P0K1	23.85 ba	4.66 ba	9.62 bdac	11.41 bdc
	N1P1K0	24.89 ba	4.68 ba	9.16 bdac	11.19 edc
	N1P1K1	26.11 ba	5.25 ba	9.41 bdac	10.66 edf
	N2P0K0	26.55 ba	5.86 a	10.33 bac	12.89 ba
	N2P0K1	27.82 a	5.86 a	10.28 bac	13.09 ba
	N2P1K0	24.91 ba	5.93 a	10.69 ba	13.27 a
	N2P1K1	26.73 ba	5.92 a	10.81 a	12.74 bac
	S.E.	1.8	0.53	0.33	0.32
		Phosphorous (g kg^{-1})			
	N0P0K0	2.54	0.65	2.55	2.91
	N0P0K1	2.69	0.56	2.42	2.62
	N0P1K0	2.55	0.41	2.14	2.62
	N0P1K1	2.82	0.43	2.19	2.80
	N1P0K0	2.62	0.45	1.98	2.53
	N1P0K1	2.75	0.49	2.46	2.62
	N1P1K0	2.64	0.45	2.27	2.42
	N1P1K1	2.66	0.44	1.94	2.39
	N2P0K0	2.73	0.44	2.23	2.34
	N2P0K1	2.61	0.38	1.89	2.22
	N2P1K0	2.49	0.37	2.40	2.70
	N2P1K1	2.88	0.50	2.31	2.93
	S.E.	0.16	0.07	0.20	0.16
		Potassium (g kg^{-1})			
	N0P0K0	43.47	14.40	5.45	4.31
	N0P0K1	46.79	16.90	4.94	4.22
	N0P1K0	39.90	13.97	4.70	4.05
	N0P1K1	49.18	16.27	4.70	4.24
	N1P0K0	46.46	15.72	4.54	3.99
	N1P0K1	49.24	17.39	5.02	4.03
	N1P1K0	42.66	14.89	5.07	3.88
	N1P1K1	46.36	17.16	4.45	3.97
	N2P0K0	45.33	15.81	4.74	3.78
	N2P0K1	47.72	16.50	4.59	3.78
	N2P1K0	42.36	15.54	4.51	4.13
	N2P1K1	52.46	17.49	4.47	4.23
	S.E.	3.49	0.98	0.24	0.16

Table 3.4. Continued

Site-year	**Treatment**	**Nitrogen ($g\ kg^{-1}$)**			
		----------Plant components----------			
		V6 (Whole plant)	R6 Stover	Ear	Grain
LCB22	N0P0K0	26.32 bdc	5.54 ba	8.79	9.90 bc
	N0P0K1	24.66 d	5.71 ba	9.73	9.43 c
	N0P1K0	24.51 d	5.21 b	9.82	10.20 bc
	N0P1K1	23.74 d	5.68 ba	11.43	9.78 bc
	N1P0K0	27.07 bdac	4.54 b	9.09	11.73 bac
	N1P0K1	27.59 bdac	7.88 ba	11.30	12.73 ba
	N1P1K0	25.15 dc	5.58 ba	10.07	12.04 bac
	N1P1K1	28.79 bdac	5.32 ba	9.76	11.76 bac
	N2P0K0	29.88 bac	8.99 a	10.86	13.47 a
	N2P0K1	30.94 ba	7.47 ba	10.81	12.87 a
	N2P1K0	31.95 a	7.39 ba	11.14	13.23 a
	N2P1K1	31.12 ba	6.01 ba	10.40	14.00 a
	S.E.	1.0	0.76	0.92	0.51
		Phosphorous ($g\ kg^{-1}$)			
	N0P0K0	4.30 ba	2.25 ba	2.69	3.39
	N0P0K1	4.97 a	2.48 ba	2.92	2.87
	N0P1K0	4.81 ba	2.59 a	2.91	2.95
	N0P1K1	4.73 ba	2.21 ba	2.95	3.37
	N1P0K0	4.22 ba	1.74 bac	2.66	3.47
	N1P0K1	4.50 ba	1.87 bac	3.32	3.32
	N1P1K0	3.98 b	1.82 bac	3.10	3.57
	N1P1K1	4.20 ba	1.98 bac	2.75	3.60
	N2P0K0	4.24 ba	0.83 c	2.78	3.60
	N2P0K1	4.36 ba	1.26 bc	2.85	3.70
	N2P1K0	3.89 b	1.24 bc	2.93	3.89
	N2P1K1	4.42 ba	0.83 c	2.98	3.83
	S.E.	0.21	0.26	0.24	0.24
		Potassium ($g\ kg^{-1}$)			
	N0P0K0	44.28 ba	10.90	8.22	6.40
	N0P0K1	50.52 a	10.59	8.29	5.37
	N0P1K0	45.61 ba	10.55	6.59	5.33
	N0P1K1	45.01 ba	9.78	8.51	6.17
	N1P0K0	39.76 ba	9.66	6.84	5.22
	N1P0K1	46.45 ba	12.51	7.20	5.27
	N1P1K0	38.97 ba	10.29	7.32	5.82
	N1P1K1	45.06 ba	10.57	6.71	5.56
	N2P0K0	41.43 ba	8.63	5.61	5.70
	N2P0K1	41.08 ba	9.47	5.79	5.90
	N2P1K0	33.60 b	9.27	5.55	6.30
	N2P1K1	43.50 ba	9.04	6.28	6.00
	S.E.	2.6	1.2	0.76	0.37

Values represent the mean of three replications. Different letter within each column denotes the significant difference at *$P<0.05$ by Tukey test.* Columns with no letter indicates that there was no significant difference observed among treatments.

Table 3.5. Analysis of Variance for interaction effects and main effects of N, P, and K for total aboveground biomass accumulation, N, P, and K uptake at V6/V7 growth stage.

Site-year	**Source of Variation**	***df***	**Biomass (kg ha^{-1})**	**N uptake (kg ha^{-1})**	**P uptake (kg ha^{-1})**	**K uptake (kg ha^{-1})**
				Mean Squares		
EFAW 21	N	2	ns	***	ns	ns
	P	1	ns	ns	ns	ns
	N x P	2	ns	ns	ns	ns
	K	1	*	ns	ns	ns
	N x K	2	ns	ns	ns	ns
	P x K	1	ns	ns	ns	ns
	N x P x K	2	ns	ns	ns	ns
	S.E.		158.18	3.86	0.43	8.13
	Treatment			Means		
	N0P0K0		1269.9	24.8ab	3.2	55.5
	N0P0K1		1150.1	22.2b	3.0	52.8
	N0P1K0		1293.8	26.5ab	3.3	51.9
	N0P1K1		1138.1	24.1ab	3.3	57.6
	N1P0K0		1629.3	38.1ab	4.3	75.2
	N1P0K1		1509.5	33.9ab	4.0	72.4
	N1P1K0		1269.9	31.3ab	3.4	54.7
	N1P1K1		1245.9	31.4ab	3.3	56.5
	N2P0K0		1629.3	43.3a	4.4	72.6
	N2P0K1		1305.8	36.5ab	3.4	62.2
	N2P1K0		1665.2	41.6ab	4.2	70.7
	N2P1K1		1233.9	32.3ab	3.6	65.2
LCB 22	N	2	***	***	***	***
	P	1	ns	ns	ns	ns
	N x P	2	ns	ns	ns	ns
	K	1	ns	ns	**	**
	N x K	2	ns	ns	ns	ns
	P x K	1	ns	ns	ns	ns
	N x P x K	2	ns	ns	ns	ns
	S.E.		77.09	2.28	0.31	3.06
	Treatments					
	N0P0K0		307.6 c	8.1 c	1.33 c	13.62 d
	N0P0K1		439.4 bc	10.8 bc	2.19 bc	22.19 bdc
	N0P1K0		463.3 bc	11.3 bc	2.17 bc	20.66 bdc
	N0P1K1		439.4 bc	10.4 bc	2.08 bc	19.69 dc
	N1P0K0		738.9 ba	20.0 ba	3.11 ba	29.43 bac
	N1P0K1		774.8 ba	21.4 ba	3.48 ba	35.91 ba
	N1P1K0		798.8 ba	20.0 ba	3.17 ba	30.90 bac
	N1P1K1		846.7 a	24.6 a	3.56 ba	37.98 a
	N2P0K0		810.7 ba	24.0 a	3.40 ba	33.10 bac
	N2P0K1		1014.4 a	31.4 a	4.43 a	41.80 a
	N2P1K0		918.6 a	29.4 a	3.58 ba	30.97 bac
	N2P1K1		786.8 ba	24.6 a	3.46 ba	33.98 bac

*, **, and *** are significant at the 0.05, 0.01, and 0.001 levels respectively. ns: not significant at the 0.05 level.

Treatment means values represent the mean of three replications. Different letter within each column denotes the significant difference at *P<0.05 by Tukey test*

Table 3.6. ANOVA results for total biomass, N-Uptake, P-uptake, and K-uptake by whole aboveground plant at R6 growth stages (Physiological maturity).

			Significance of *F* Ratio					
			Whole Plant			**Grain**		
Site-year	Source of Variation	*df*	**N**	**P**	**K**	**N**	**P**	**K**
EFAW21	N	2	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
	P	1	0.28	0.47	0.54	0.28	0.02	0.06
	N × P	2	0.01	0.002	0.23	0.01	0.0008	0.01
	K	1	0.26	0.57	0.003	0.26	0.18	0.11
	N × K	2	0.69	0.14	0.07	0.69	0.94	0.78
	P × K	1	0.96	0.59	0.07	0.96	0.14	0.30
	N × P × K	2	0.08	0.04	0.11	0.08	0.45	0.40
LCB22	N	2	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
	P	1	0.02	0.74	0.20	0.54	0.78	0.88
	N × P	2	0.16	0.40	0.47	0.15	0.22	0.16
	K	1	0.81	0.60	0.36	0.76	0.59	0.58
	N × K	2	0.49	0.55	0.65	0.83	0.86	0.86
	P × K	1	0.24	0.36	0.72	0.93	0.85	0.50
	N × P × K	2	0.65	0.10	0.50	0.24	0.19	0.21

*Significant at 0.05 level, **Significant at 0.01 level, ***Significant at 0.001 level, ns-not significant.

Table 3.7. The least square means of Grain nitrogen harvest index (GNHI), grain phosphorous harvest index (GPHI), and grain potassium harvest index (GKHI) by site year.

Different letters within each column denotes the significant difference at *$P<0.05$ by Tukey test*

Site year	Treatments	GNHI	GPHI	GKHI
EFAW21		Means		
	N0P0K0	0.72 b	0.85	0.30
	N0P0K1	0.78 ba	0.77	0.24
	N0P1K0	0.75 ba	0.95	0.29
	N0P1K1	0.77 ba	1.01	0.30
	N1P0K0	0.94 a	1.06	0.37
	N1P0K1	0.8 ba	0.91	0.27
	N1P1K0	0.82 ba	0.85	0.30
	N1P1K1	0.82 ba	1.02	0.34
	N2P0K0	0.81 ba	0.86	0.30
	N2P0K1	0.83 ba	0.95	0.31
	N2P1K0	0.84 ba	0.92	0.36
	N2P1K1	0.83 ba	1.07	0.36
LCB22		Means		
	N0P0K0	0.22 b	0.21 c	0.09 d
	N0P0K1	0.34 b	0.31 c	0.15 dc
	N0P1K0	0.54 ba	0.46 bc	0.26 bdac
	N0P1K1	0.54 ba	0.51 bc	0.24 bdac
	N1P0K0	0.86 a	0.81 bac	0.32 bdac
	N1P0K1	0.75 ba	0.87 bac	0.30 bdac
	N1P1K0	0.85 a	0.72 bac	0.32 bdac
	N1P1K1	0.93 a	0.81 bac	0.31 bdac
	N2P0K0	0.85 a	1.22 a	0.48 a
	N2P0K1	0.86 a	1.09 ba	0.46 a
	N2P1K0	0.70 ba	0.75 bac	0.40 bac
	N2P1K1	0.89 a	1.02 ba	0.41 ba

Table 3.8a. Least square means for nutrient uptake per plant at different growth stages during the entire crop season at EFAW21

Treatment	**Nitrogen (mg pl^{-1})**			
	---Growth Stage---			
	V6	VT	R2	R6
N0P0K0	576.0 ab	842.8 b	990.1 dc	1785.4 f
N0P0K1	515.2 b	890.6 b	1020.2 bdc	1590.6 f
N0P1K0	615.1 ab	798.3 b	1035.8 bdc	1616.1 f
N0P1K1	559.3 ab	765.6 b	924.0 d	1957.0 ef
N1P0K0	883.2 ab	1074.2 ba	1286.2 bdac	2606.2 ed
N1P0K1	786.3 ab	1318.9 ba	1595.0 bdac	3158.0 bdc
N1P1K0	726.0 ab	1230.8 ba	1526.2 badc	2692.0 d
N1P1K1	729.0 ab	1254.2 ba	1520.6 badc	2833.4 dc
N2P0K0	1003.5 a	1643.0 a	1966.2 a	3545.3 bac
N2P0K1	847.0 ab	1366.2 ba	1678.0 bdac	3668.2 ba
N2P1K0	963.4 ab	1397.2 ba	1799.3 ba	4032.2 a
N2P1K1	748.7 ab	1382.6 ba	1732.1 bac	3811.9 ba

	Phosphorous (mg pl^{-1})			
N0P0K0	75.0 a	93.7 a	136.0 a	444.7 dc
N0P0K1	70.0 a	106.0 a	154.6 a	439.4 dc
N0P1K0	76.7 a	88.3 a	146.1 a	362.2 d
N0P1K1	76.3 a	94.8 a	140.2 a	448.9 dc
N1P0K0	99.1 a	108.1 a	161.9 a	526.6 bdc
N1P0K1	93.7 a	130.8 a	199.5 a	675.2 ba
N1P1K0	78.0 a	118.7 a	181.4 a	563.9 bdac
N1P1K1	75.5 a	111.5 a	164.3 a	518.9 bdc
N2P0K0	102.2 a	137.7 a	206.9 a	615.4 bac
N2P0K1	79.1 a	96.0 a	162.8 a	548.6 bdac
N2P1K0	96.8 a	115.7 a	194.6 a	748.5 a
N2P1K1	82.4 a	106.0 a	177.3 a	708.1 ba

	Potassium (mg pl^{-1})			
N0P0K0	1286.6 a	1817.8 ba	1912.9 a	1835.0 c
N0P0K1	1224.3 a	1904.3 ba	1967.4 a	2383.4 bc
N0P1K0	1202.9 a	1523.8 b	1619.6 a	1895.3 c
N0P1K1	1336.5 a	1797.2 ba	1827.1 a	2298.0 bc
N1P0K0	1743.6 a	2003.3 ba	2173.1 a	2353.5 bc
N1P0K1	1679.7 a	2403.3 a	2614.1 a	3374.1 a
N1P1K0	1267.9 a	1781.2 ba	1954.9 a	2550.1 bac
N1P1K1	1310.7 a	1990.8 ba	2230.8 a	2575.4 bac
N2P0K0	1682.4 a	2001.5 ba	2225.0 a	2853.8 ba
N2P0K1	1442.9 a	1913.6 ba	2051.1 a	2830.4 ba
N2P1K0	1640.0 a	1814.4 ba	1972.0 a	2969.1 ba
N2P1K1	1511.8 a	2181.9 ba	2374.3 a	2969.5 ba

Different letters within each column denotes the significant difference at $P<0.05$ *by Tukey test*

Table 3.8b. Means for Nutrient uptake per plant at different growth stages during the crop season at LCB22.

Treatment	Nitrogen (mg pl^{-1})							
	Growth stage							
	V6		VT		R2		R6	
N0P0K0	194.8	e	777.8	d	888.4	b	1015.4	dc
N0P0K1	262.9	edc	865	dc	1061.2	b	909.1	d
N0P1K0	279.7	ebdc	776.3	d	1153.7	ba	1285.9	bdc
N0P1K1	240.3	ed	846.4	dc	1081.7	b	1115.1	dc
N1P0K0	454.9	ebdac	1431.3	bdac	1510.3	ba	1145.2	dc
N1P0K1	526.1	bac	1096.5	bdc	1663.1	ba	1705.3	bac
N1P1K0	499.4	bdac	1136.8	bdac	1268.2	ba	1409.3	bdc
N1P1K1	571	a	1174.2	bdac	1530.7	ba	1339.7	bdc
N2P0K0	555	a	1420.1	bdac	2050.8	a	2250.3	a
N2P0K1	695.4	a	1917.1	a	1542.1	ba	1799.5	bac
N2P1K0	680.7	a	1541	bac	1657.9	ba	1959.8	ba
N2P1K1	544.4	ba	1686	ba	1685.3	ba	1792.5	bac
	Phosphorous (mg pl^{-1})							
N0P0K0	31.9	d	262.9	ba	377.3	a	321.2	a
N0P0K1	53.2	bdc	296.6	ba	384	a	277.4	a
N0P1K0	53.8	bdc	244.9	ba	385.1	a	413.9	a
N0P1K1	48.2	dc	255	ba	487.9	a	328.9	a
N1P0K0	70.8	bac	302.3	ba	402.2	a	319.8	a
N1P0K1	86.0	bac	178.6	b	437.4	a	436.7	a
N1P1K0	79.5	bac	271.9	ba	407.1	a	415.7	a
N1P1K1	82.5	bac	222.5	ba	509.2	a	358.6	a
N2P0K0	79.0	bac	242.1	ba	429.7	a	331.7	a
N2P0K1	97.8	a	318.6	a	341	a	396.3	a
N2P1K0	83.0	ba	256.2	ba	367.8	a	415.1	a
N2P1K1	76.3	bac	298.6	ba	395.1	a	408.8	a
	Potassium (mg pl^{-1})							
N0P0K0	325.4	d	1027.8	a	1919.1	a	1369.4	a
N0P0K1	537.8	bdc	1914.2	a	2006.2	a	1186.7	a
N0P1K0	514.2	dc	1501.6	a	1912.6	a	1406.7	a
N0P1K1	456.6	dc	1554.1	a	2155.1	a	1344.9	a
N1P0K0	668.1	bdac	2006.3	a	2644.0	a	1398.7	a
N1P0K1	887.9	ba	1429.3	a	2675.2	a	1992.9	a
N1P1K0	774.6	bac	2152.0	a	2453.7	a	1620.5	a
N1P1K1	880.5	ba	1493.0	a	2900.6	a	1502.6	a
N2P0K0	766.4	bac	1700.4	a	2554.0	a	1583.9	a
N2P0K1	918.5	a	2414.5	a	1648.3	a	1544.7	a
N2P1K0	718.2	bac	1490.4	a	2305.8	a	1513.7	a
N2P1K1	748.3	bac	2221.8	a	2440.7	a	1690.2	a

Different letters within each column denotes the significant difference at *$P<0.05$ by Tukey test*

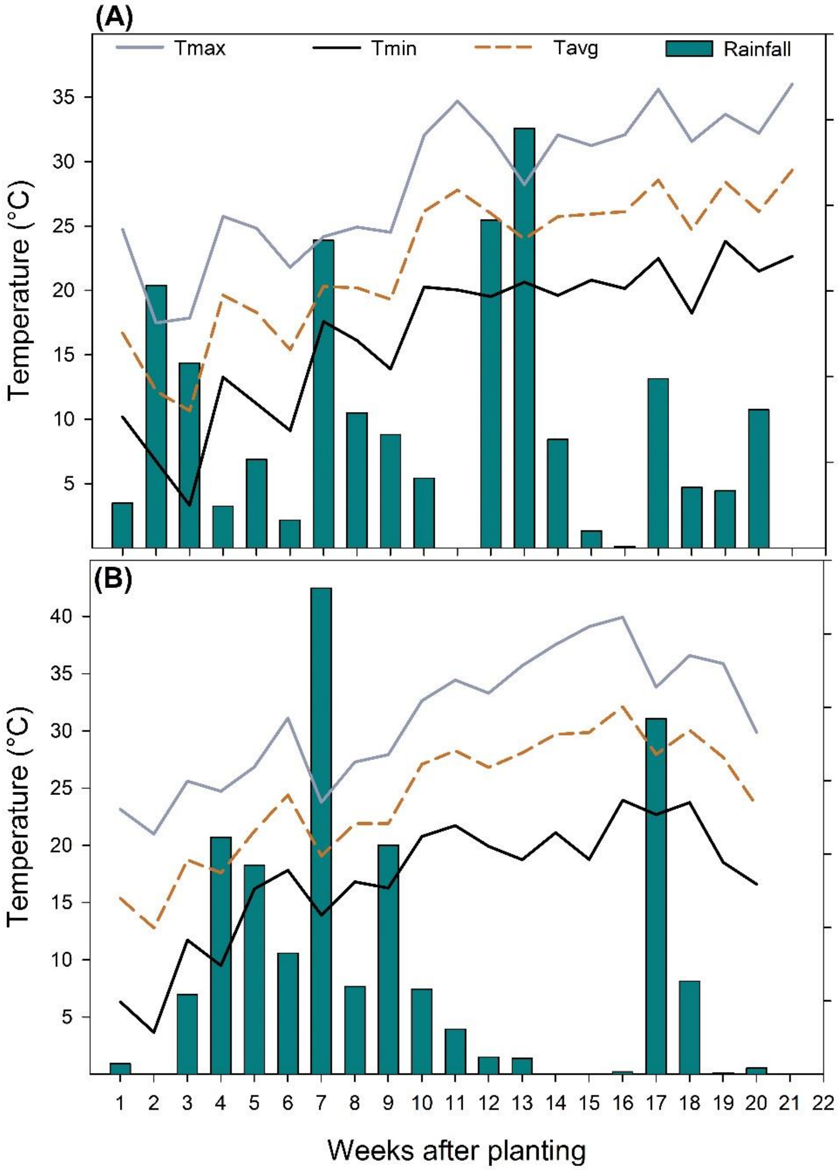

Fig 3.1: Weekly total rainfall (mm), and maximum temperature (Tmax), minimum temperature (Tmin), and average temperature (Tavg) for EFAW 2021 (A), and LCB 2022 (B).

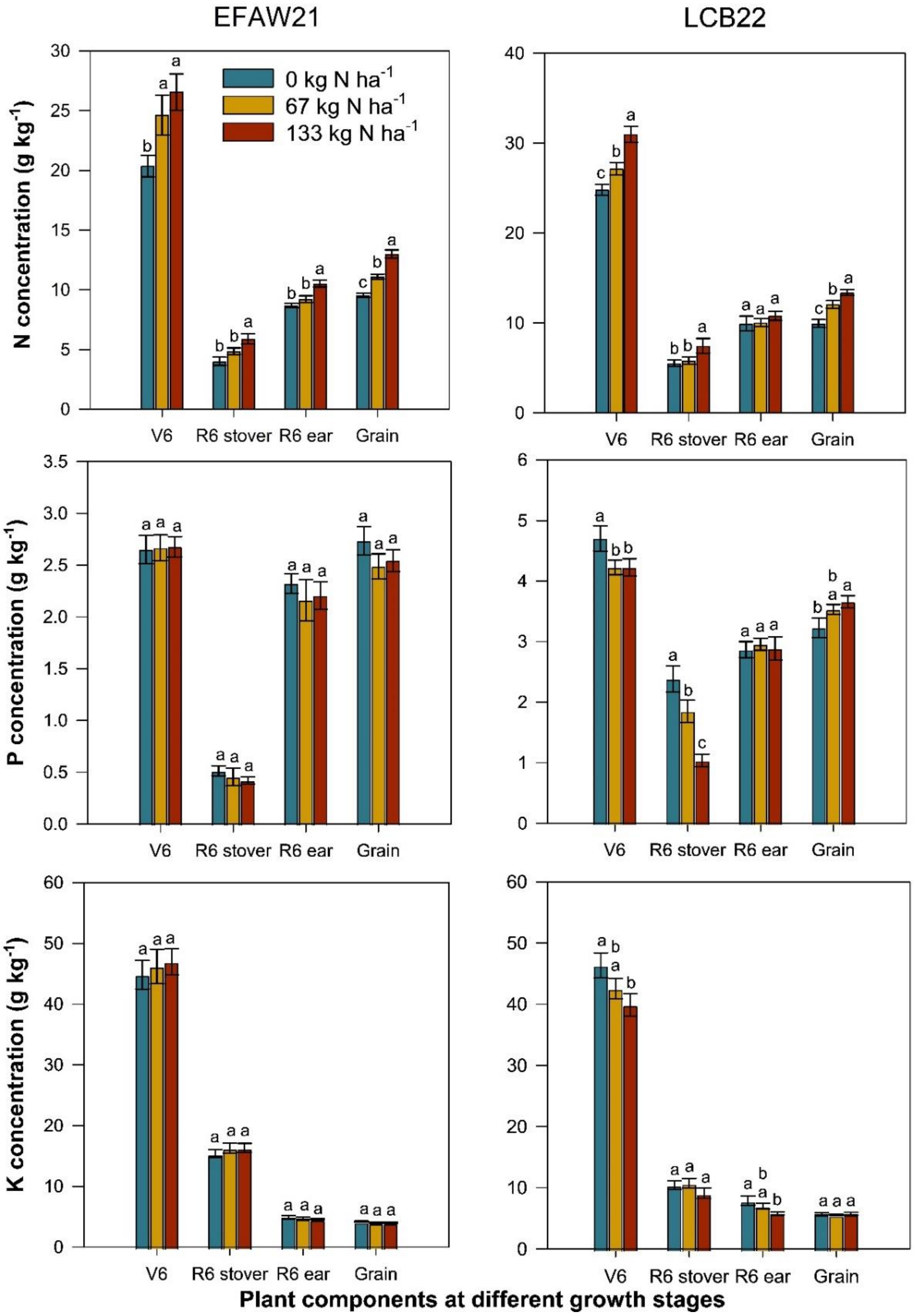

Fig. 3.2. Mineral concentrations in whole plant at growth stage V6, Stover (stalk + leaves) and ear (cob + husk) at R6, and grain as affected by N rates. Bar values are means + standard error. Within each growth stage different letters are significantly different by Tukey (P<0.05).

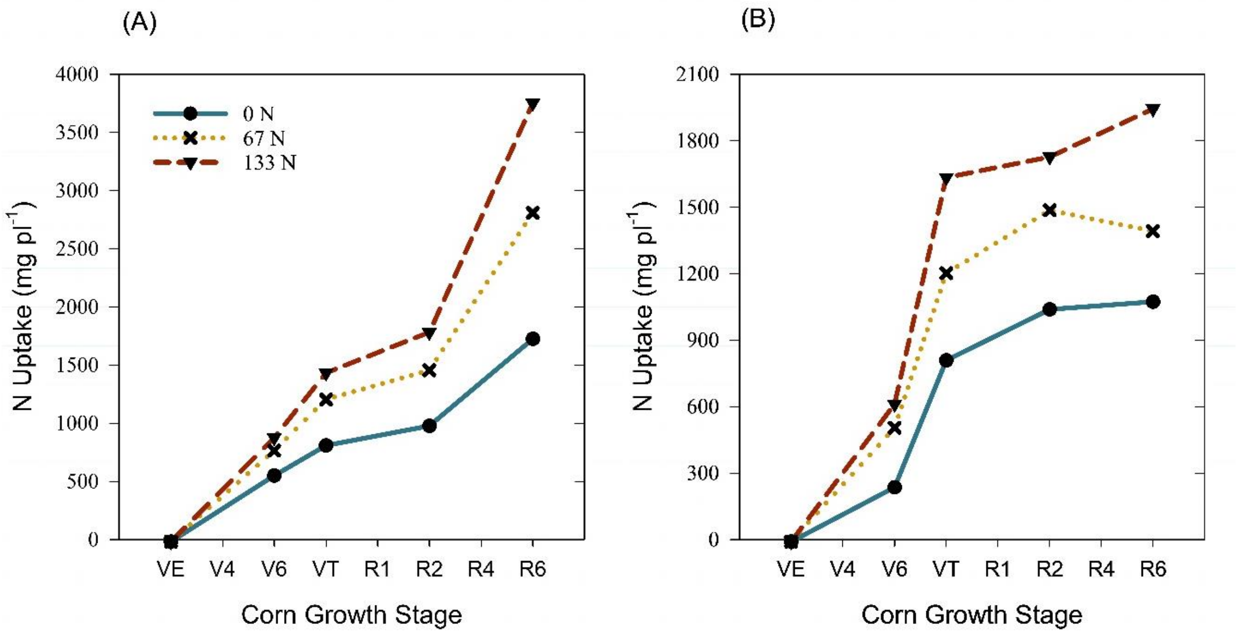

Fig 3.3a: Nitrogen rate effect on N uptake at different growth stages. (A) EFAW21 (B)LCB22

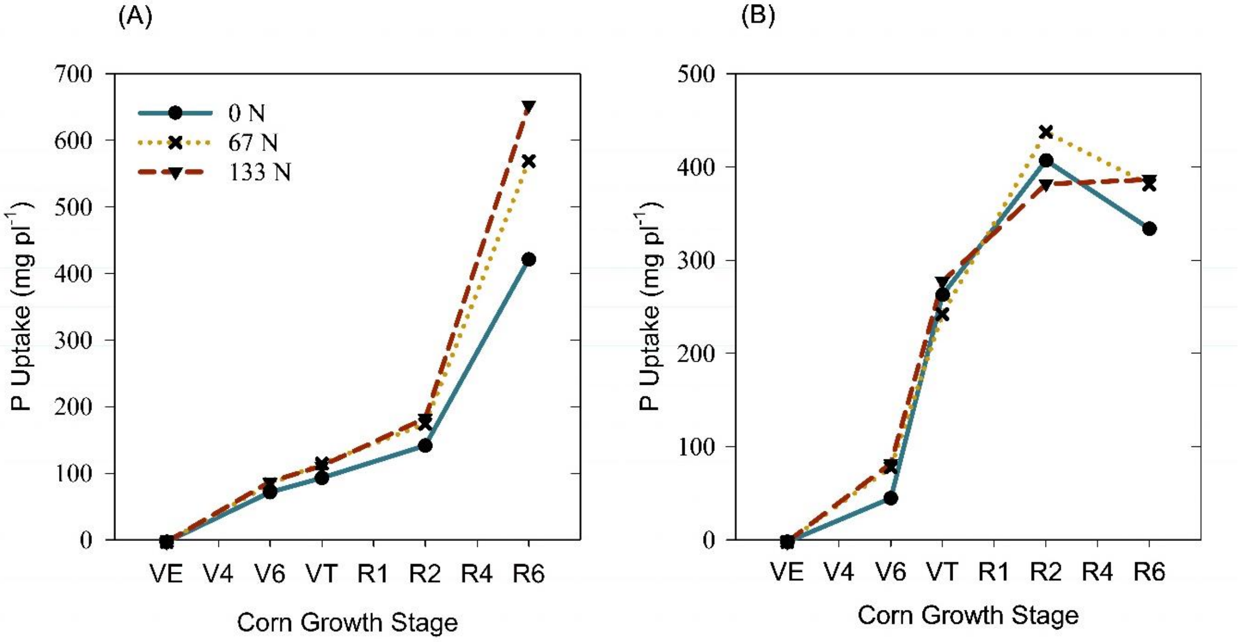

Fig 3.3b Nitrogen rate effect on P uptake at different growth stages. (A) EFAW21 (B)LCB22

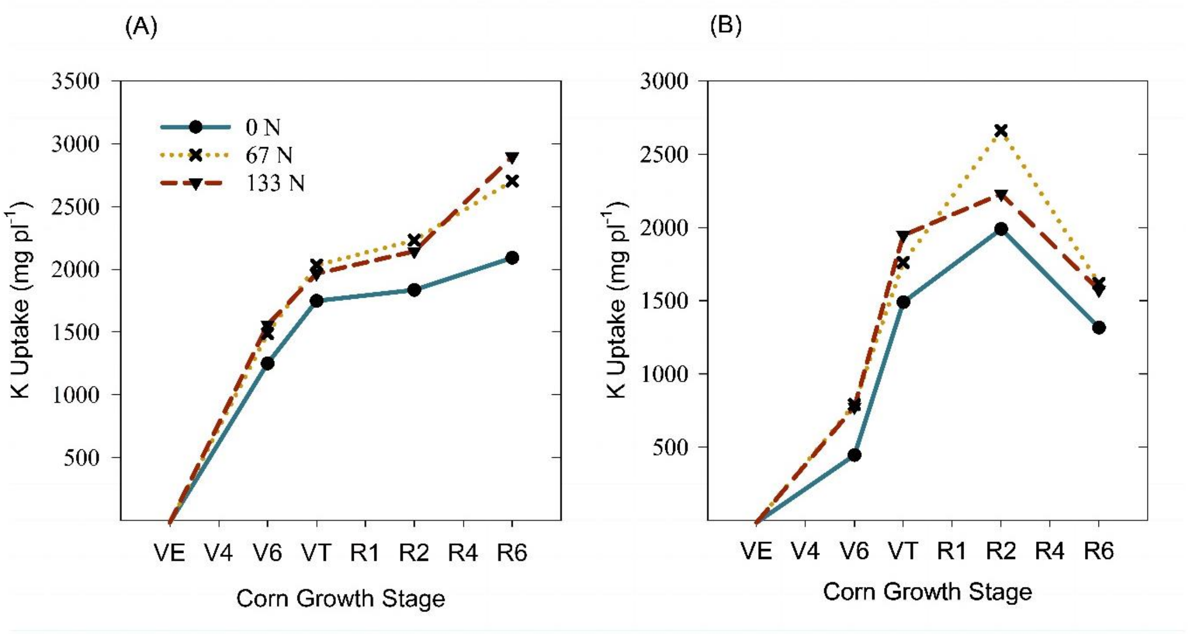

Fig 3.3c Nitrogen rate effect on K uptake at different growth stages. (A) EFAW21 (B)LCB22

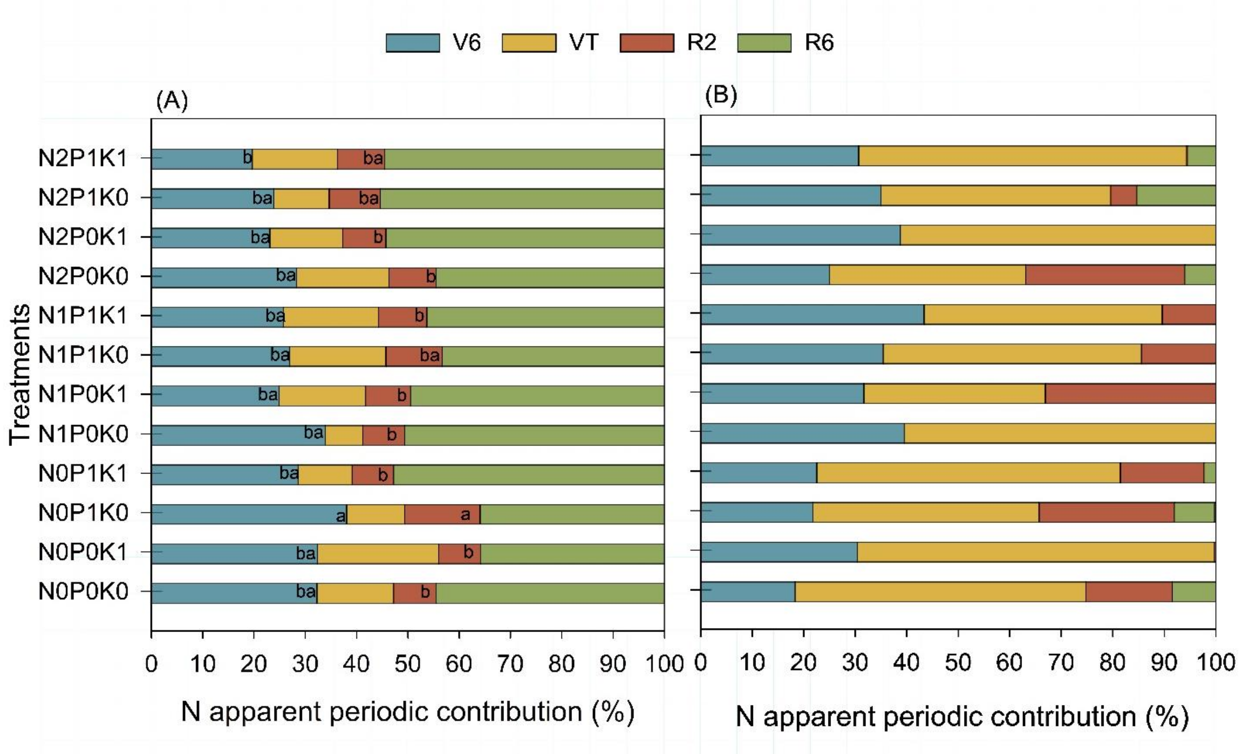

Fig 3.4a: Nitrogen contribution (%) of periodic accumulation from Emergence to V6, V6 to VT, VT to R2, and R2 to R6 (maturity)to final total N uptake (g per plant). Bars with the same letter are not significantly different and bars with no letters are non-significant at P ≤ 0.05. (A) EFAW21 (B)LCB22

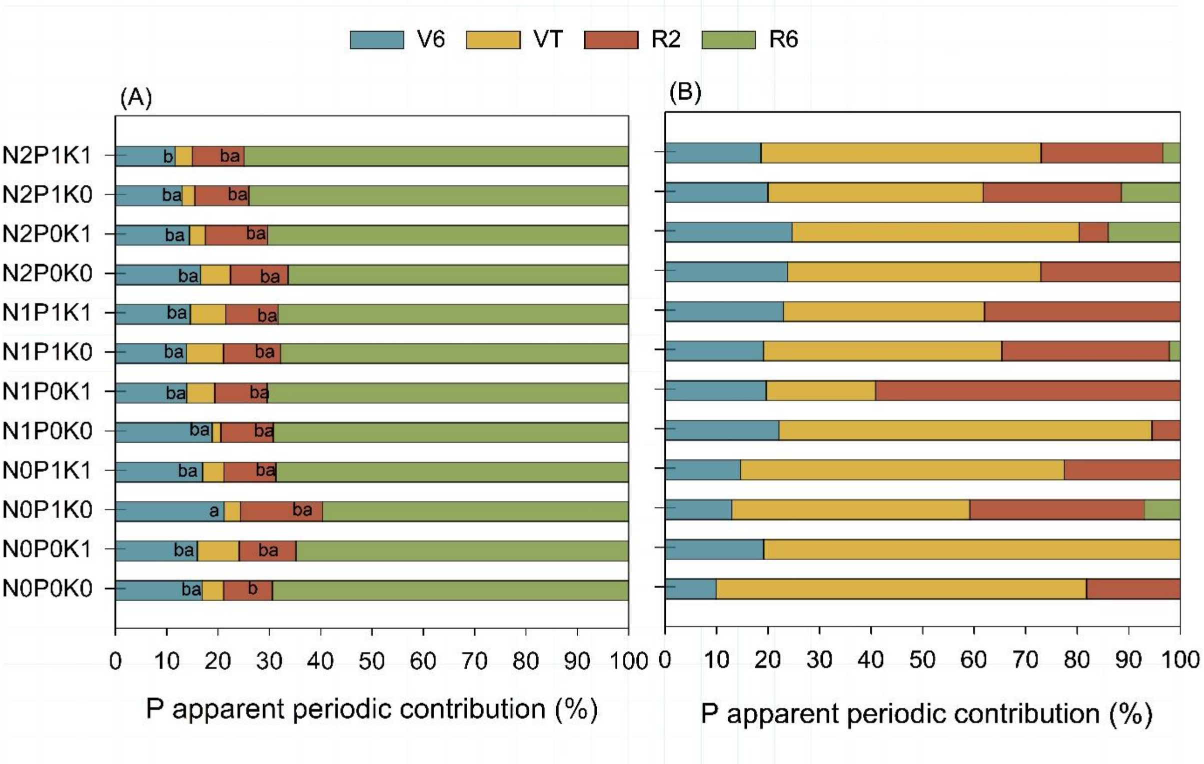

Fig 3.4b: Phosphorous contribution (%) of periodic accumulation from Emergence to V6, V6 to VT, VT to R2, and R2 to R6 (maturity)to final total P uptake (g per plant). Bars with the different letters are significantly different and bars with no letters are non-significant at P ≤ 0.05. (A) EFAW21 (B)LCB22

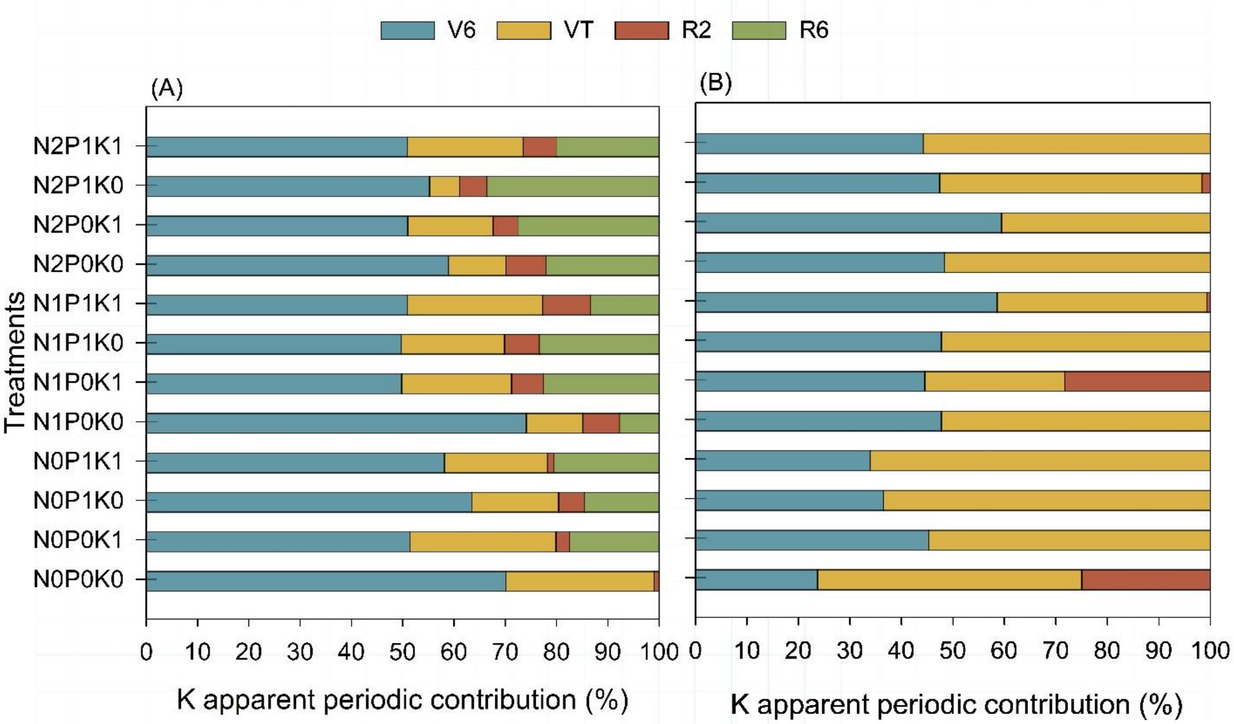

Fig 3.4c: Potassium contribution (%) of periodic accumulation from Emergence to V6, V6 to VT, VT to R2, and R2 to R6 (maturity)to final total K uptake (g per plant). Bars with no letter are not significantly different at P ≤ 0.05. (A) EFAW21 (B)LCB22

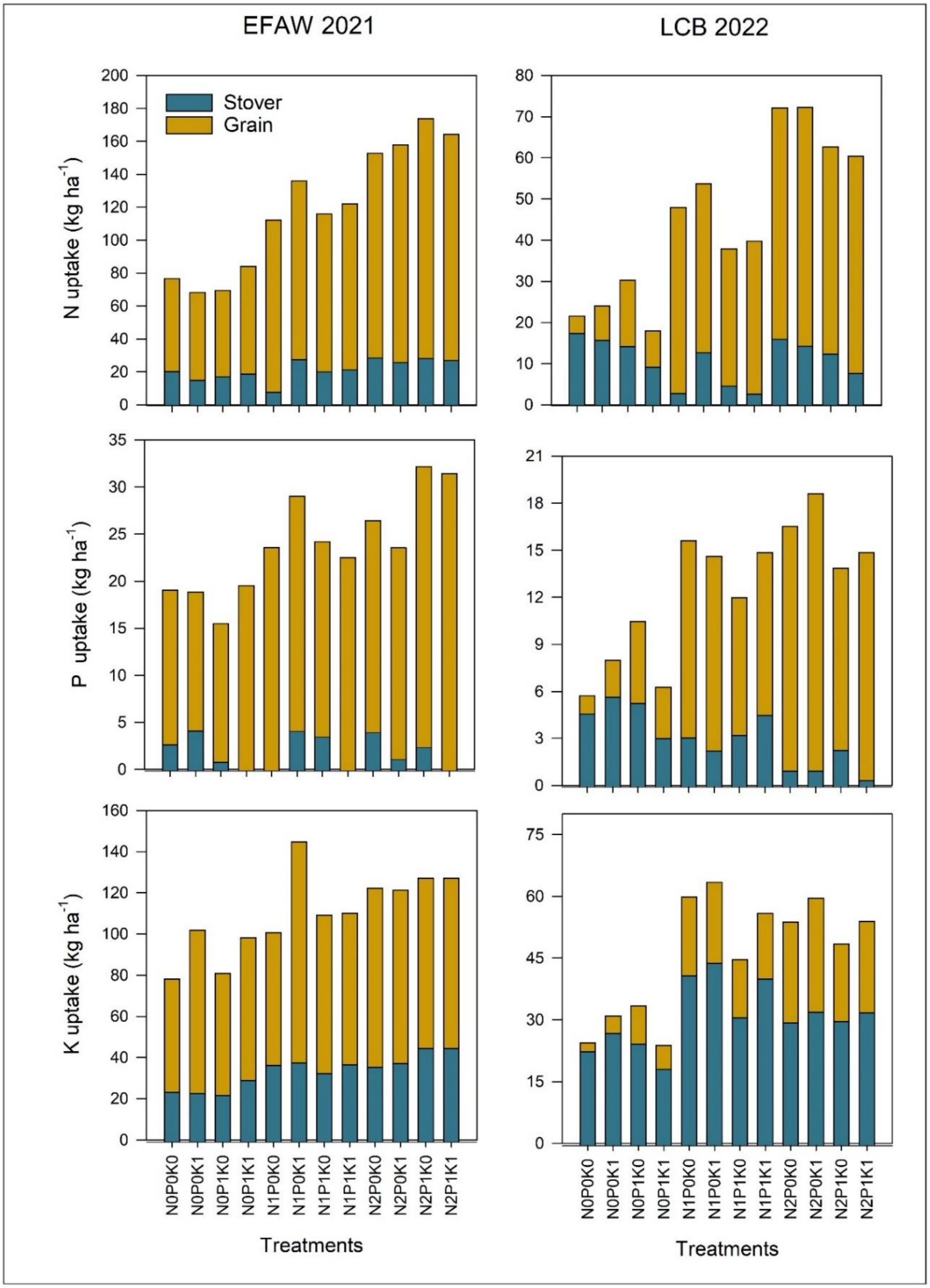

Fig 3.5. Total nutrient uptake at maturity with horizontal bars representing total nutrient uptake by aboveground whole plant and segmentation shows uptake proportion in stover and grain at EFAW 2021 and LCB 202

CHAPTER IV

MICRONUTRIENTS CONCENTRATION AND CONTENT IN RAIN-FED CORN AS AFFECTED BY NITROGEN, PHOSPHOROUS AND POTASSIUM FERTILIZATION

ABSTRACT

Understanding the relationship between macro and micronutrients is required for effective nutrient management in crop production. Growers usually apply nitrogen (N), phosphorus (P), and potassium (K) fertilizers for corn which further interacts with micronutrients in either a synergistic or antagonistic way, affecting their availability in the soil and plant uptake. A 2-year field experiment was conducted to determine the effect of NPK fertilization on micronutrient uptake of rain-fed corn. Randomized complete block design was employed with twelve treatments replicated three times. Different combinations of N, P, and K fertilizer rates for corn applied pre-plant were studied in the treatments. Corn plant accumulated nutrients in the following order: iron (Fe) > manganese (Mn)>zinc (Zn) > copper (Cu). Nitrogen application rate was the primarily driving factor affecting nutrient concentrations in the plant and uptake. We found that micronutrient concentrations (except Cu) in corn grain were increased by the rate of N increase. Furthermore, we found that Cu concentration in the plant as well as in grain was unrelated to nutrient supply, biomass, yield, and other nutrient concentrations. Our results

demonstrated that grain Fe concentrations were positively correlated with grain Mn concentration. With the increase in N application rate, corn plant contained more Mn, Fe, Zn, and Cu at early growth stage as well as at physiological maturity. Co-application of P with N did not have any significant effect on grain micronutrient concentration and uptake. However, application of K increased uptake of grain Mn, Fe, and Cu.

INTRODUCTION

Corn (Zea Mays L.), which originated in Mexico, has now become one of the world's most prominent cereal crops due to its wide range of uses. Its functionality is not only limited to source of food and fodder, but it is also being used for ethanol production which accounts for about 40% US corn production (Ranum et al., 2014). Corn is a staple food for 4.5 billion people of 94 developing nations, and the United States is the world's leading corn exporter (Shiferaw et al., 2011).

Although the three most critical nutrients for crop development are nitrogen (N), phosphorus (P), and potassium (K), there are 14 additional elements necessary for plant growth that are frequently overlooked during management. The fact that plants require a balanced mix of nutrients for their growth was proved many decades ago (Liebig, 1855). Micronutrients, unlike macronutrients, are only required in trace amounts yet are nonetheless essential for plant growth (Marschner, 2012). Hence, micronutrients should not be neglected while raising a healthy crop. Farmers often apply high amounts of N, P, and K to avoid crop deficits, but often remiss when it comes to micronutrients. However, NPK interacts with micronutrients in either a synergistic or antagonistic way, affecting their availability in the soil and plant uptake (Ma & Zheng, 2018; Manásek et al., 2013). Some studies suggested that modern farming practices with high-yielding cultivars have a negative interaction with micronutrients because excess N, P, and K lead to micronutrient deficiency in the crop (Cakmak et al., 2002). Plant growth and yield are affected by micronutrient uptake, as is the quality and standard of the produce. Micronutrient-deficient maize grain can cause nutrient deficiency in individuals who use corn as a staple meal, as well as affecting the quality of livestock feed and fodder (Campen & Glahn, 1999). A vast population relies on corn as a main food source, hence corn grains should provide enough micronutrients for both human and animal diets. As a result, quality grain and fodder production must also be emphasized.

According to a NAAS survey from 2018, most corn producers in the United States only apply N, P, and K to their fields. Fertilization practices and management can change the soil environment, affecting the availability and uptake of micronutrients (Li et al., 2007). The impact of these fertilizers on micronutrient availability and uptake is not well understood and is still unclear (Ma & Zheng, 2018). For example, some scientists believe that increasing N rates can increase micronutrient uptake (Ciampitti & Vyn, 2013; Manásek et al., 2013; Soliman et al., 1992) whereas others disagree and have demonstrated that N rate has negative effect on micronutrient uptake (Rui et al., 2004 ; Ma & Zheng, 2018). Furthermore, Zhang et al. (2004) found that increasing rate of N in NPK combination can reduce zinc (Zn), and iron (Fe) uptake. The effect of Fertilizer P on micronutrients depends upon soil water availability (Gaj et al., 2016), since high availability of P decreased the availability of water-soluble micronutrient under field conditions (Bierman & Rosen, 1994). Phosphorus fertilizer reduced Zn uptake in corn by 37% and increased Mn uptake by 111% as compared to control (Jurkovic et al., 2006). They also illustrated that P fertilizer had no influence on copper (Cu) and Fe uptake. Many other researchers also supported that increased P content in the soil negatively affected Zn, Cu and Fe uptake by corn (Karamanos, 2013; Li et al., 2007; Shukla & Singh, 1979; Zhang et al., 2017) and negatively affected grain Cu and Mn (Gaj et al., 2016). The disparity among different researches is most likely due to different uptake and internal use efficiency among different genotypes, environment, and management practices where trials were conducted (Ma & Zheng, 2018). Therefore, it is vital to explore how co-application of P and K along with different rates of N affects mineral nutrient uptake for corn grown in the Southern Great Plains.

The knowledge of influence of soil fertility and fertilization practices on micronutrient uptake, nutrient harvest index, and the amount of nutrient translocated from plant to grain, is required under the influence of different fertilizer practices in order to produce quality grains (Gaj et al., 2016). Many studies have looked at how varying yield levels, cultivars, plant density, and N rate impact micronutrient accumulation dynamics and patterns (Bender et al., 2013; Ciampitti & Vyn, 2013; Woli et al., 2018). However, little is known about the effects of combining P and K with N on micronutrient uptake and dynamics. Furthermore, those experiments were done in a high-yielding environment with irrigated corn planted at high density, which is substantially different from the Southern Great Plains environment and crop management practices (Singh et al., 2021). However, it is widely known that nutrient uptake is influenced by the environment as well as crop management approaches (Ciampitti & Vyn, 2013). Therefore, there is a need to explore how combination of NPK fertilizer can impact on micronutrient concentration and total uptake for corn grown in Southern Great Plains environment. This knowledge will help in synchronization of crop demand and supply of micronutrients, improving the efficiency of micronutrient management.

Therefore, the objective of this study is to (i) Investigate the micronutrient uptake in response to different NPK fertilizer combinations (ii) Assess the impact of NPK fertilizers on micronutrient concentration at different corn growth stages (iii) Study how the concentrations of Zn, Fe, Cu, Mn in corn grain affected by different fertilizer combinations.

MATERIAL AND METHODS

Experiment Location

This field study was planted at four locations, EFAW (EFAW21, 36°08'14.1"N 97°06'22.4"W) and Lake Carl Blackwell (LCB21, no reportable results after R2 due to raccoon damage) (36°09'04.8"N 97°17'21.5"W) in 2021. While in 2022, the trail was conducted at LCB (LCB22, 36°09'1.64"N 97°17'23.30"W) and Perkins (PRK22, no reportable results after R1 due to damages by wild hogs) (35°59'37.32"N 97°2'31.41"W). These sites were different in terms of soil types as EFAW site is an Ashport silty clay loam (fine-silty, mixed, superactive, thermic, Fluventic Haplustolls) and LCB is classified as a Pulaski fine-sandy loam (coarse/loamy, mixed non-acid, thermic, Typic, Ustifluvent), while Perkin's soil is Teller sandy loam (fine-loamy, mixed, active, thermic, Udic Argiustoll) (Soil Survey, 2021). Composite pre-plant soil samples were taken from all sites at 0-15 cm depth from each replication. Soil samples were dried at 65° C for 12 hours and passed through a 2 mm sieve in preparation for chemical analysis. Soil properties for all experimental sites are presented in Table 1. All soils had slightly acidic or close to neutral pH (5.3 to 6.8).

Experimental design

For all sites, a randomized complete block design with 12 treatments and three replications was used. Each treatment plot was 18 m^2 (3x6 m) in size and comprised of four rows of corn plants. Each replication block was separated from each other by 3m alley. Different combinations of nitrogen (N), phosphorous (P), and potassium (K) fertilizer rates for corn applied pre-plant were studied in the 12 treatments. Nitrogen rates evaluated included 0, 67, and 133 kg N ha^{-1} while P and K rates were 0 and 20 kg P ha^{-1} and 0 and 60 kg K ha^{-1}, respectively. All fertilizers were applied as pre-plant using a barber metered feed fertilizer spreader using appropriate settings to achieve the desired fertilizer application rates. This machine is designed in a way to avoid drift of fertilizers between plots. Fertilizer sources for N, P, and K included urea (46-0-0), triple super phosphate (0-46-0), and Muriate of potash (0-0-60). Corn hybrid 'DKC66-29' (DeKalb Genetics Corporation, IL) was planted in 2021 at both locations and DKC63-99RIB was planted in 2022 at both locations using John Deere Max Emergence 2 7300 four row planter. Row spacing was 76 cm with a population of 49,400 seeds ha^{-1} at EFAW21, PRK22, and 69,160 seeds ha^{-1} at LCB21 and LCB22. Weed and pest management was done according to Oklahoma State University recommendations. No major weed or pest problem was observed during the growing season of the crop.

Plant sampling and nutrient analysis

For calculating in-season nutrient accumulation, plant samples were taken at four growth stages described according to Abendroth et al. (2011): (i) Vegetative leaf six stage (V6), (ii) Tasseling stage (VT), (iii) Reproductive blistering Stage (R2), and (iv) Physiological maturity (R6). One-meter length of row was randomly selected from side rows of each plot, then whole plants were cut at 5 cm above ground level to avoid any contamination of soil. At R2 and R6 stage, corn plants were partitioned into stover and ear. Plant samples were oven-dried, grinded and analyzed for manganese (Mn), zinc (Zn), copper (Cu), and iron (Fe) using inductively coupled plasma atomic emission spectroscopy (ICP-AES) after wet acid digestion. Details of sampling, preparation, and analyzing methods are explained in Chapter 2 and 3. We were only able to get yield data from two of the four site years. Raccoons damaged corn ears at the LCB21 site at maturity (R6). While wild hogs destroyed nearly all of the crop at PRK22 after the R1 stage. As a result, we will present vegetative growth stage (V6 and VT) data from all locations but for reproductive stage and grains, we will only present data from EFAW21 and LCB22.

Calculation of nutrient accumulation

Nutrient concentration (Nc) was provided in parts per million (ppm) and the values of total aboveground nutrient uptake were calculated by Equation 1 and Equation 2.

$$Plant\ Micronutrient\ Uptake\ (g\ ha^{-1}) = Dry\ Biomass\ (kg\ ha^{-1})\ \times Nc/1000 \qquad (1)$$

$$Grain\ Micronutrient\ Uptake\ (g\ ha^{-1}) = Yield\ (kg\ ha^{-1})\ \times Nc/1000 \qquad (2)$$

Statistical Analysis

Nutrient uptake was calculated using equations 1 and 2. This data was then analyzed using PROC GLIMMIX function of SAS 9.4 (SAS Institute, 2009). All fertilizer input (N rate, P rate, and K rate) were used as fixed treatments while replication was used as random factor. Means were separated and compared using adjust=Tukey option in LSMEANS at alpha level of 0.05. All results were presented on dry weight basis except for grains at 15.5% moisture level. Correlation analysis between nutrient concentrations was performed using SAS CORR (SAS Institute, 2009).

RESULTS

Micronutrient concentration in whole aboveground plant at vegetative growth stages

Zinc (Zn)

Main effect of N rate was observed at V6 (p=0.001) and VT (p=0.001) stage for Zn concentration at EFAW21 (Table 3). However, at LCB22, only main effect of P was noted at V6 stage, which increased Zn concentration from 34.0 to 36.8 mg kg^{-1}. However, main effect of P was faded at VT where only main effect of N rate was observed. At LCB21, interaction effect of N x P x K (*p*=0.016) and main effect of N rate (p=0.001) was observed at V6 stage, while only main effect of N rate (*p*=0.0001) was observed at VT stage. While Zn concentration at PRK22 site was not affected at V6 and VT growth stages. At vegetative growth stages, the effect of NPK treatments was inconsistent across site-years. At V6, Zn concentration was not affected by any treatment at both sites in 2022. However, in 2021 there was significant differences between treatments. Nitrogen had completely opposite effect at 2021 sites where Zn concentration at EFAW21 increased significantly with increased N rate while Zn concentration at LCB21 decreased with increased N rates.

Manganese (Mn)

The statistical data presented in Table 3, showed that Mn concentration was affected by main effect of N rate (*p*=0.0007) and main effect of K (*p*=0.005) at V6 and VT stage at EFAW21. Application of N increased Mn concentration in whole aboveground plant at V6 and VT at EFAW21 (Fig 3). Presence of 20 kg K ha^{-1} increased Mn concentration by 9.3%. However, at LCB22, only main effect of N (*p*=0.0001) was observed at V6. Nitrogen rate increased Mn concentration linearly in whole aboveground plant at V6 (Fig 3). At LCB22, Mn concentration increased from 66.65 to 67.51 mg kg^{-1} when 133 kg N ha^{-1} was applied instead of 0-N. At VT growth stage, main effect of N rate faded at this site. At LCB22 and PRK22, Mn concentration was not significantly affected by any treatment at V6 and VT growth stage.

Copper (Cu)

There was a main effect of N rate for Cu concentration at V6 and VT growth stages at all locations (*p*=0.001) except PRK22 (Table 3). However, at LCB22, interactive effect of P x K was also observed along with main effect of N. Whole aboveground plant Cu concentration increased significantly at V6 and VT stage as N application rate increased from 0 to133 kg N ha^{-1}.

Iron (Fe)

There was no interaction as well as main effect of N, P, and K observed for Fe concentration in whole plant at V6 and VT growth stages at all locations except PRK22 (Table 3). Fe concentration was affected significantly by P application which increased Fe concentration from 98.97 to 110.7 g kg^{-1} at V6 stage. At VT stage, Fe concentration at PRK22 was also affected by the interaction of N x K. Fe concentration at three site-years except PRK22 were similar across all treatments at both growth stages.

Micronutrient concentration in stover (leaves + stem) and ear (cob + husk) at reproductive growth stages

Zinc

As stated earlier, we will only report reproductive stage data from EFAW21 and LCB22. Stover Zn concentration at R2 stage was affected by main effect of N rate (*p*= 0.019) and interactive effect of N x P at EFAW21 and main effect of N rate at LCB22 (Table 3). Main effect of K (*p*=0.03) was noted for ear Zn concentration at R2 stage at EFAW21, where application of K increased ear Zn concentration from 42.47 mg kg^{-1} to 46.85 mg kg^{-1}. Stover Zn concentration at R6 stage was affected by the interaction of N x P and N x K at EFAW21, while main effect of N rate was observed at LCB22. When K was present with 133 kg N ha^{-1}, Zn concentration reduced from 29.17 to 20.54 mg kg^{-1} at EFAW21. However, N reduced stover Zn concentration significantly at R6 stage at LCB22. However, no statistical difference was observed in ear Zn concentration at R6. Grain Zn concentration increased linearly with N rate at both locations. Grain Zn concentration ranges from 19.95 to 24.54 mg kg^{-1} and 23.85 to 34.92 mg kg^{-1} at EFAW21 and LCB22 respectively (Fig 1). Nitrogen rate increased Zn concentration at EFAW21 but decreased at LCB22 in stover. Grain Zn concentration increased significantly with increased N application rate at both locations.

Manganese

At EFAW21, Mn concentration in stover was affected by main effect of N (p=0.03) and main effect of K (p=0.007) at R2 growth stage, while ear Mn concentration was affected by main effect of K only (p=0.004). At maturity, main effect of N (p=0.01) and main effect of K (p=0.03) was observed for stover Mn concentration, while no significant difference was noted for ear Mn concentration (Table 3). Application of K increased Mn concentration significantly in stover and ears till R2 stage. After this stage, the effect of K was only observed in stover, where the ears at R6 was not significantly affected by K application. The interaction effect of N x P (p=0.02) and main effect of N (p=0.003) was noted for grain Mn. Mn concentration in grain was affected by N rate (p=0.0001) substantially, where application of 133 kg N ha^{-1} increased Mn concentration from 6.7 to 7.5 mg kg^{-1} as compared to 0-N (Fig 3). However, at LCB22, no significant difference was noted for grain Mn concentration (Table 3). Although, no statistical difference was observed in ears at R2 and R6 for Mn concentration at this site, but N rate decreased Mn concentration.

Copper

Stover Cu concentration at R2 stage was significantly affected by N rate at EFAW21 (*p*=0.0001), while interaction of P x K (*p*=0.02) and main effect of N rate (*p*=0.0001) was observed at LCB22. The interaction of N x P (*p*=0.0001) and main effect of N rate (*p*=0.04) was observed for ear Cu concentration at R2 stage at LCB22. However, at EFAW21, main effect of N (*p*=0.009) and main effect of K (*p*=0.0012) was noted for ear Cu concentration at R2 stage, where application of N and K significantly increased Cu concentration. Ear Cu concentration at R6 stage was non-significant at both locations. Grain Cu concentration was not significantly affected at LCB22, while main effect of K (p=0.002) was observed at EFAW21 (Table 3). Application of K increased grain Cu concentration from 1.22 to 1.34 mg kg^{-1}.

Iron

Interactive and main effects of N, P, and K had no significant effect on Fe concentration in any plant component at any growth stage. However, grain Fe concentration ranged from 26.25 to 35.39 mg kg^{-1} and 22.87 to 39.87 mg kg^{-1} at EFAW21 and at LCB22 respectively (Fig. 1).

Mineral nutrient uptake at different growth stages.

Manganese, Zn, Fe, and Cu uptake at early growth stage (V6) was affected significantly by N rate application at all locations (Table 6). There was no interaction effect of N x P x K observed for mineral nutrient uptake at V6 stage. Moreover, at all locations, application of P and K did not have any significant effect on micronutrient uptakes at V6 stage (Table 6). Nitrogen application rate increased micronutrient uptake in whole aboveground plant linearly at V6 stage. Increased N rate increased micronutrient uptake at all locations (Fig 4).

ANOVA results showed that total Zn uptake in whole aboveground plant at maturity (R6) was significantly affected by main effect of N rate (*p*=0.0005) at LCB22, while at EFAW21 interaction of N x K (*p*=0.01) was also noted along with main effect of N rate (*p*=0.0001) (Table 4). Total Mn uptake was affected by main effect of N rate (*p*=0.0007) and main effect of K (p=0.03) at EFAW21, while only main effect of N (*p*=0.008) was observed at LCB22 (Table 4). Application of K at EFAW21 increased Mn uptake from 397.5 to 453.7 g ha^{-1}. Interaction effect of N x P x K (*p*=0.01) was observed for Cu uptake at LCB22 along with main effect of N rate (*p*=0.0004). However, at EFAW21, only main effect of N rate (*p*=0.0001) was observed. Interaction effect of N x P x K (*p*=0.02) was also observed for Fe uptake at EFAW21, while main effect of N and main effect of P was observed at LCB22 (Table 4). Application of P reduced Fe uptake from 381.9 to 299 g ha^{-1}. Overall, our study showed that application of N increased all mineral nutrients uptake at both locations except Fe at EFAW21 (Fig 4).

Relationship between N and different mineral nutrients.

The stoichiometry between N and Mn, Fe, Zn, and Cu was established in whole plant at V6 growth stage and in grains at maturity. Linear correlation was observed for N and Cu concentration, as well as N and Fe concentration at V6 stage (Fig 5). However, positive linear correlation was noted for total nutrient uptake at V6 between each mineral nutrient uptake and N uptake. Correlation between Cu and N uptake was strongest followed by correlation of Fe and N uptake. However, Mn and Fe uptake had strongest correlation with N uptake in the grain. While, on grain nutrient concentration basis, Mn and N concentration had strongest relationship (Fig 6).

More detailed correlation between various parameters was estimated and presented using heatmap (Fig 7). It was observed that grain yield as well as biomass had correlation with each nutrient's uptake in plant at V6 stage as well as in grain. Nutrient concentrations in plant at V6 and nutrient concentrations in grain had no relationship with biomass and yield except grain Zn concentration. A strong linear correlation was found between Fe and Cu uptake, Mn and Zinc uptake at V6 stage. Cu concentration at V6 had linear correlation with N concentration only rather than biomass.

DISCUSSIONS

Corn plant is zinc intensive, which means it requires high amount of zinc for its growth (Mengel & Kirkby, 2001). The effect of N on Zn concentration at different growth stages was different at different site-years. At EFAW21, Zn concentration in plant components increased with increased rate of N, but at LCB22 Zn concentration decreased with application of N. At LCB22, application of P increased Zn concentration at V6 stage, but the effect of P faded out as plant matured. Some previous studies reported that increased N application increased Zn concentration in corn plants (Losak et al., 2011; Karimian, 1995), while other studies found that N application had negative effect on Zn concentration (Ozanne, 1955; Camp, 1945). This discrepancy might be due to different soil conditions, environment, and crop management practices. Moreover, decreased Zn uptake at LCB22 could be caused by the increment in Fe uptake which competed same absorption sites (Zhang et al., 2012). However, the effect of N rate on grain Zn concentration was consistent as Zn concentration in corn grain increased with increased N application rate from 0 to 133 kg N ha^{-1} at both locations. These results were consistent with Xue et al. (2014) but contradictory with the findings of Feil et al. (2005), the latter reported that grain Zn concentration declined with increased N rate. Ciampitti and Vyn (2013) found no impact of N rate on grain Zn concentration. However, in our study grain Zn concentration increased linearly with N application rate. Grain Zn concentration varied from 19.5 to 24.5 mg kg^{-1} at EFAW21 and 23.8 to 34.9 mg kg^{-1} at LCB22. Grain Zn concentration at EFAW21 corresponds with the results of Manásek et al. (2013) (range 19.20-23.19 mg kg^{-1}), Hossain et al., (2008) (range 16.5-27 mg kg^{-1}), Feil et al. (2005) (range 21.8-26.5 mg kg^{-1}), Heckman et al. (2003) (mean= 22.6 mg kg^{-1}), and higher than that reported by Li et al. (2007) (12.6-17.3 mg kg^{-1}). Grain Zn concentration at LCB22 was higher than some published studies. This can be explained by the low grain yield at this site. Li et al. (2007) also found that lower yield had higher grain Zn concentration.

Copper concentration increased as N application rate increased at V6 and VT growth stages. The effect of N in increasing Cu concentration was also observed in stover at reproductive stages. The results showed that N application rates had significant effect on increasing Cu concentration of corn stalks (Fig 3). These results were similar to the findings of Ma & Zheng (2018) and Ciampitti & Vyn (2013). They also observed that Cu concentration in stalks increased linearly with N application rates. High N application rate increased growth of corn plant thus increased the requirement of Cu. Moreover, soil available Cu increased due to greater N fertilizer application (Wei et al., 2006). Higher N application could have made Cu more available to plants due to acidification of soil by nitrification. Sufficient N fertilizer could result in higher root growth which explored more soil for uptake of immobile Cu. Enhanced root growth could have further promoted the formation of Cu^{2+} due to organic acid release from root exudates (Fageria, 2001). According to Mills & Benton (1996), higher level of N could also inhibit translocation of Cu in the plant. This could explain why N application increased stover Cu concentration only instead of increasing grain Cu concentration as well. Although grain Cu concentration increased slightly with increased N rate but this effect was not statistically significant. Similar findings were reported by Feil et al. (2005), and Xue et al. (2014), while Orosz et al. (2009) found negative effect of NPK treatment on grain Cu concentration. Grain Cu concentration ranged from 1.04 to 1.42 mg kg^{-1} at EFAW21 and 0.78 to 2.20 mg kg^{-1} at LCB22 (Fig 1). Even the maximum concentration in our study was lower than that reported by Ciampitti

& Vyn (2013) (mean=2.5 mg kg^{-1}); Heckman et al., (2003) (mean=2.7 mg kg^{-1}); Li et al., (2007) (range=2.06-2.77 mg kg^{-1}). However, grain Cu concentration was higher than that reported by Losak et al. (2011) (range=0.3-0.6 mg kg^{-1}).

Manganese concentration increased with increased N rate, while application of K also increased Mn concentration except corn ears at R6 stage. Grain Mn concentration increased linearly with N application rate at EFAW21. Increment of grain Mn concentration with increased N rate was similar with the findings of Ciampitti & Vyn (2013), and Feil et al. (2005). However, N rate had no effect on grain Mn concentration at LCB22 site (Fig 3), which was similar to the nil effect of N rate observed by Bruns & Ebelhar (2006) on grain Mn concentration. Grain Mn concentration ranged from to 6.3 to 8.4 mg kg^{-1} at EFAW21 and 3.8 to 6.8 mg kg^{-1} at LCB22, which falls within the ranges that reported by Heckman et al. (2003) (mean =1.0 to 9.8 mg kg^{-1}), Feil et al.(2005) (range=5.8 to 6.8 mg kg^{-1}) and Ciampitti & Vyn (2013) (range= 5.8 to 7.6 mg kg^{-1}).

There was no interaction and main effect of N, P, and K noted for Fe concentration at vegetative growth stages at all locations except for PRK22. Ciampitti & Vyn (2013) also observed no change in Fe concentration as N application rate increased from 0 to 224 kg N ha^{-1}. There was no significant effect observed for grain Fe concentration among N, P, and K treatments. These results are consistent with Bruns & Ebelhar (2006) who found that grain Fe concentration did not get affected by N fertility treatments. Li et al. (2007) also reported that Fe concentration in stalk did not show differences among different combinations of N, P, and K treatments. This may be the result of dilution effect due to N application which was also observed by Ma & Zheng (2018). Dilution effect occurs when plant accumulate biomass faster than nutrient uptake rate (Riedell, 2010). Bruns & Ebelhar (2006) also found that grain Fe concentration decreased with K application. Positive effect of N rate on grain Fe concentration was reported by Ma & Zheng (2018). While no effect of NPK was reported on grain Fe concentration by Li et al. (2007). However, results from our study showed that grain Fe did not respond to N, P, and K application rates. Grain Fe concentration in our study fluctuated from to 26.25 to 35.39 g kg^{-1} at EFAW21 and 22.94 to 39.87 g kg^{-1} at LCB22. Ciampitti & Vyn (2013) reported 22 mg kg^{-1} grain Fe concentration while Heckman et al. (2003) reported 30 mg kg^{-1}g.

Different response of mineral concentration at different site-years could be partially explained by different soil type. There are many factors that affects the availabity of micronutrients which includes soil pH, soil organic matter, cation exchange capacity, redox potential, and clay content. Micronutrients are present in solid phases including secondary precipitates, primary minerals, and some are adsorbed on clay surfaces from which only 10% are soluble and available to plants (Shuman 1991). Environmental factors such as temperature and moisture also affect the availabity of micronutrients (Fageria et al. 2002). According to Kabata-Pendias (2001), soil pH and redox potential determines the fate of soil metals. Even small variation in soil pH could substantially affect the uptake of Cu, Fe, and Zn (Khoshgoftarmanesh et al., 2010). EFAW21 site was slightly acidic and is an Ashport silty clay loam, while LCB22 site had neutral pH and is classified as a Pulaski fine-sandy loam (Table 1).

Nitrogen application rates influenced all mineral nutrient concentration at V6 stage. Higher N application rate increased N concentration and uptake in plant at early growth stage (Singh et al., 2021). Therefore, to determine the relationship between N concentration and other mineral nutrient concentrations, correlations between these nutrients were established (Fig 5-7). Each mineral nutrient uptake was linearly correlated with N uptake in whole plant at V6 stage as well as in grains. Mineral nutrient content increased as N uptake increased. There was positive association between mineral nutrient uptake at V6 stage and mineral nutrient uptake in grain. Grain N concentration had strong relationship with grain Mn concentration. However, grain Fe concentration were also positively correlated with grain Mn concentration. While grain Cu concentration was not related to any mineral nutrient concentrations, nor grain yield. Grain Cu concentration was not dependent on nutrient supply, biomass, and yield. Moreover, other nutrient concentrations in the plant at early growth stage as well as in grain did not relate to grain Cu concentration. Grain Cu might be governed by other factors such as environment instead of nutrient supply. This nil association of Cu was also observed at V6 growth stage. However, on the basis of total nutrient uptake in grain, each and every mineral nutrient was positively correlated to each other.

The effect of N rate application on nutrient uptake at V6 stage was consistent at all locations (Fig 4). Mineral nutrient uptake increased linearly with increased N rate across four site-years. Manganese uptake in our 2-year field study ranged from 17.6 to 121.2 g ha^{-1}, whereas Zn uptake across all locations varied from 10.4 to 64.5 g ha^{-1}. Fe uptake ranged from 27.3 to 219.2 g ha^{-1}, while Cu uptake varied from 1.5 to 10.18 g ha^{-1} at V6 stage. These ranges were similar to that reported by Xue et al. (2014) at V6 growth stage. Similar effect of N was observed for total nutrient uptake at R6 stage. Nutrient uptake increased with increased N application rate at both locations except Fe uptake at EFAW21. The similar total Fe uptake across treatments at EFAW21 was at least in part due to higher stover and ear Fe concentration in treatments with 67 kg N ha^{-1} and 0-N. These treatments had lower biomass and grain yield but higher Fe concentration which caused similar total Fe uptake. Application of K also increased the uptake of grain Mn, Zn, and Cu. Total mineral nutrients uptake was in the order of: Fe>Mn>Zn>Cu, with the minimum and maximum ranges of 170.1 to 882.4 g Fe ha^{-1}, 122.5 to 503.7 g Mn ha^{-1}, 86.8 to

424.1 g Zn ha^{-1}, and 2.8 to 30.3 g Cu ha^{-1}. Increased nutrient concentration and total uptake with higher N rate may be due to increase of root length in higher N treated plots resulting in more exploitable area by roots.

Biomass showed progressive enhancement due to increased N rate at V6 and R6 growth stages which further caused greater micronutrient uptake in plants. Results from our study showed that biomass is the driving force for increasing accumulation of Mn, Fe, Zn, and Cu at both V6 and R6 stage. These results were in accordance with the results reported by Xue et al. (2014) and Ma & Zheng (2018). Furthermore, application of P at LCB22 increased Mn uptake while inhibits Fe uptake.

CONCLUSION

Mineral nutrient concentration in plants is very complex. We observed in our study that fertilization rates had different effects on the uptake and concentrations of the evaluated mineral nutrients. Environment in which the study was conducted, management practices of crop, soil conditions, growth stage of crop, all had significant effect on the observed difference in plant mineral nutrient concentrations. Corn plant accumulated nutrients in the order of: Fe>Mn>Zn>Cu. It was found that grain yield and biomass had the strongest correlation with each nutrient (except for Cu) uptake rather than nutrient concentration. Cu concentration in the plant as well as in grain was unrelated to N, P and K supply, biomass or grain yield, and other nutrient concentrations. Grain Fe concentrations were positively correlated with grain Mn concentration. Micronutrient concentrations in corn grain improved with increased rate of N application. Furthermore, improved biomass due to N rate enhanced total uptake of these nutrients. With the increase in N application rate, corn plant contained more Fe, Mn, Zn, and Cu at early growth stage as well as at physiological maturity as compared to low N rate. Co-application of P with N did not have any significant effect on grain micronutrient concentration. However, application of K had significant effect on grain Mn, Fe, and Cu. Increased N application rate did not result in any negative effect on micronutrient content of corn stover and grain. These findings demonstrated that improved micronutrient nutrition for optimal production may be achieved by better N management.

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TABLES AND FIGURES

Table 4.1: Basic soil properties at Efaw in 2021, and Lake Carl Blackwell (LCB) Oklahoma in 2022.

Year	Location	pH	Organic Carbon %	NH_4-N	NO_3-N	P ǂ	K ǂ	Mn	Fe	Zn	Cu
				mg kg^{-1}							
2021	Efaw	5.6	0.83	42.5	23.3	24	201	53.1	37.5	0.35	0.6
2021	LCB	6.6	0.90	107.5	5.83	25.6	149.3	72.1	33.5	0.30	0.6
2022	LCB	6.1	0.63	3.3	<0.1	11.5	69.5	230	20	0.50	1.1
2022	PRK	6.3	0.64	2.89	2.37	20	94.5	122	28.5	1.00	0.7

ǂ P& K are plant available phosphorous (P) and potassium (K) using Mehlich 3, respectively.

Table 4.2. Nitrogen (N), phosphorous (P), and potassium (K) application rates employed at both Efaw and LCB locations.

Treatment code	Nutrient rates (kg ha^{-1})		
	N	P	K
N0P0K0	0	0	0
N0P0K1	0	0	60
N0P1K0	0	20	0
N0P1K1	0	20	60
N1P0K0	67	0	0
N1P0K1	67	0	60
N1P1K0	67	20	0
N1P1K1	67	20	60
N2P0K0	133	0	0
N2P0K1	133	0	60
N2P1K0	133	20	0
N2P1K1	133	20	60

Table 4.3. The ANOVA for main effect and interactive effect of N, P, and K on nutrient concentrations in whole plant at V6 and VT, stover and ear at R2 and R6, and grains. Plants were portioned into stover and ear at R2 and R6 different growth stages.

Site-year	Source of Variation	df	**Zn (mg kg^{-1})**								**Cu (mg kg^{-1})**						
			V6	VT	R2		R6		Grain		V6	VT	R2		R6		Grain
					Stover	Ear	Stover	Ear					Stover	Ear	Stover	Ear	
EFAW 21	N	2	**	**	*	ns	ns	ns	*		**	**	***	**	***	ns	ns
	P	1	ns	ns	ns	ns	ns	ns	ns		ns	ns	ns	ns	ns	ns	ns
	N × P	2	ns	ns	*	ns	ns	ns	ns		ns	ns	ns	ns	ns	ns	ns
	K	1	ns	ns	ns	*	ns	ns	ns		ns	ns	ns	*	ns	ns	*
	N × K	2	ns	ns	ns	ns	*	ns	ns		ns	ns	ns	ns	ns	ns	ns
	P × K	1	ns	ns	ns	ns	*	ns	ns		ns	ns	ns	ns	ns	ns	ns
	N × P × K	2	ns	ns	ns	ns	ns	ns	ns		ns	ns	ns	ns	ns	ns	ns
			Fe (mg kg^{-1})								**Mn (g kg^{-1})**						
			V6	VT	R2		R6		Grain		V6	VT	R2		R6		Grain
					Stover	Ear	Stover	Ear					Stover	Ear	Stover	Ear	
	N	2	ns	ns	ns	ns	*	ns	ns		***	**	*	ns	*	ns	**
	P	1	ns	ns	ns	ns	ns	ns	ns		ns	ns	ns	ns	ns	ns	ns
	N × P	2	ns	ns	ns	ns	ns	ns	ns		ns	ns	ns	ns	ns	ns	*
	K	1	ns	ns	ns	ns	ns	ns	ns		**	**	**	**	*	ns	ns
	N × K	2	ns	ns	ns	ns	ns	ns	ns		ns	ns	ns	ns	ns	ns	ns
	P × K	1	ns	ns	ns	ns	ns	ns	ns		ns	ns	ns	ns	ns	ns	ns
	N × P × K	2	ns	ns	ns	ns	ns	ns	ns		ns	ns	ns	ns	ns	ns	ns
LCB 22			**Zn (mg kg^{-1})**								**Cu (mg kg^{-1})**						
			V6	VT	R2		R6		Grain		V6	VT	R2		R6		Grain
					Stover	Ear	Stover	Ear					Stover	Ear	Stover	Ear	
	N	2	ns	**	**	ns	**	ns	*		**	**	***	*	ns	ns	ns
	P	1	*	ns	ns	ns	ns	ns	*		ns	ns	ns	ns	ns	ns	ns
	N × P	2	ns	ns	ns	ns	ns	ns	ns		ns	ns	ns	**	ns	ns	ns
	K	1	ns	ns	ns	ns	ns	ns	ns		ns	ns	ns	ns	ns	ns	ns
	N × K	2	ns	ns	ns	ns	ns	ns	ns		ns	ns	ns	ns	*	ns	ns
	P × K	1	ns	ns	ns	ns	ns	ns	ns		*	ns	*	ns	ns	ns	ns
	N × P × K	2	ns	ns	ns	ns	ns	ns	ns		ns	ns	ns	ns	ns	ns	ns
			Fe (mg kg^{-1})								**Mn (g kg^{-1})**						
	N	2	ns	ns	ns	ns	*	ns	ns		***	ns	*	ns	ns	ns	ns
	P	1	ns	ns	ns	ns	ns	ns	ns		ns	ns	ns	ns	ns	ns	ns
	N × P	2	ns	ns	ns	ns	ns	ns	ns		ns	ns	ns	ns	ns	ns	ns
	K	1	ns	ns	ns	ns	ns	ns	ns		ns	ns	ns	ns	ns	ns	ns
	N × K	2	ns	ns	ns	ns	ns	ns	ns		ns	ns	ns	ns	ns	ns	ns
	P × K	1	ns	ns	ns	ns	ns	ns	ns		ns	ns	ns	ns	ns	ns	ns
	N × P × K	2	ns	ns	ns	ns	ns	ns	ns		ns	ns	ns	ns	ns	ns	ns

*, **, and *** are significant at the 0.05, 0.01, and 0.001 levels respectively. ns: not significant at the 0.05 level.

Table 4.4. The ANOVA for main effect and interactive effect of N, P, and K on nutrients uptake in whole plant at R6 and grains.

			Significance of *F* Ratio							
			Whole Plant				**Grain**			
Site-year	Source of Variation	*df*	**Mn**	**Fe**	**Zn**	**Cu**	**Mn**	**Fe**	**Zn**	**Cu**
EFAW21	N	2	0.0007	0.6475	<.0001	0.0013	<.0001	<.0001	<.0001	<.0001
	P	1	0.5535	0.1365	0.8518	0.8629	0.0832	0.1901	0.1496	0.8976
	N × P	2	0.5251	0.0772	0.7204	0.5525	0.0071	0.7125	0.012	0.8861
	K	1	0.0364	0.7954	0.6905	0.454	0.026	0.0485	0.1757	0.006
	N × K	2	0.6249	0.1615	0.0128	0.4492	0.661	0.3832	0.7396	0.6587
	P × K	1	0.1526	0.1074	0.1215	0.9679	0.3121	0.608	0.433	0.9283
	N × P × K	2	0.4044	0.0297	0.2529	0.6827	0.4822	0.4773	0.3963	0.9364
LCB22	N	2	0.008	0.0019	0.0005	0.0004	<.0001	<.0001	<.0001	0.001
	P	1	0.1273	0.0499	0.1417	0.1463	0.1001	0.0627	0.0782	0.3901
	N × P	2	0.5874	0.2368	0.0822	0.7219	0.0782	0.0258	0.0368	0.2229
	K	1	0.5829	0.4567	0.4431	0.9615	0.7675	0.6421	0.5863	0.3035
	N × K	2	0.9534	0.5863	0.1344	0.7058	0.9323	0.9908	0.6328	0.7544
	P × K	1	0.2701	0.0616	0.4809	0.7936	0.9594	0.7892	0.9334	0.9886
	N × P × K	2	0.8043	0.6656	0.6657	0.0104	0.726	0.5692	0.5523	0.3617

*, **, and *** are significant at the 0.05, 0.01, and 0.001 levels respectively. ns: not significant at the 0.05 level.

Table 4.5a. The LS-mean for mineral nutrient concentrations in plant components at different growth stages as affected by the interaction of N x P x K. At V6 and VT stage, values are based on whole aboveground plant while plants were portioned into stover and ear at R2 and R6.

Site-year	**Zn (mg kg^{-1})**						**Cu (mg kg^{-1})**					
	V6	VT	R2		R6		V6	VT	R2		R6	
			Stover	Ear	Stover	Ear			Stover	Ear	Stover	Ear
EFAW 21	24.1	20.46	14.2	43.3	23.0	20.9	4.7	3.10	1.4 d	3.0	1.9	0.17
	28.1	22.85	16.7	44.7	23.7	19.9	5.4	3.50	2.1 bdac	3.8	2.4	0.18
	26.8	22.22	17.2	44.0	24.8	20.4	4.4	3.20	1.7 dc	3.1	1.8	2.16
	25.3	22.71	17.0	55.1	20.1	19.7	5.0	2.90	1.8 bdc	4.3	2.3	1.61
	25.6	23.45	14.8	40.9	18.6	17.9	5.3	3.50	2.4 bdac	3.5	2.7	1.55
	29.8	26.48	16.7	47.3	23.4	21.6	5.5	3.80	2.7 bdac	4.2	3.1	1.82
	28.3	25.48	17.1	39.4	22.0	21.7	5.2	3.94	2.8 bdac	3.5	2.6	1.39
	30.8	27.45	17.3	45.0	21.1	18.9	5.6	4.84	2.9 bdac	4.0	3.4	1.63
	33.0	28.54	21.0	42.2	29.2	24.7	6.3	5.48	3.8 ba	4.0	4.0	1.69
	31.0	27.64	21.1	48.0	24.6	20.0	6.2	4.48	3.9 a	5.1	4.1	1.70
	34.5	28.94	19.0	45.1	29.2	22.9	5.4	4.33	3.6 bac	4.1	4.1	1.27
	33.4	29.48	16.0	41.0	16.5	22.0	6.0	4.48	3.4 bdac	4.3	4.4	1.63

	Fe (mg kg^{-1})						**Mn (mg kg^{-1})**					
	V6	VT	R2		R6		V6	VT	R2		R6	
			Stover	Ear	Stover	Ear			Stover	Ear	Stover	Ear
	108.4	100.48	104.5	24.6	133.7	32.8	77.4 b	70.2 b	59.4	37.0	75.1	8.2
	140.7	135.14	125.3	53.8	170.1	35.4	82.2 ba	75.4 ba	66.1	42.4	84.3	7.1
	107.6	099.48	88.6	36.2	107.7	25.9	78.2 b	69.8 b	59.2	37.7	81.5	7.9
	126.9	103.15	68.5	52.0	084.6	23.3	79.8 ba	73.6 ba	59.7	46.2	88.1	6.8
	113.8	094.54	89.3	60.0	115.9	23.9	79.9 ba	72.3 ba	60.2	37.8	88.1	6.9
	134.0	132.15	131.1	43.4	086.2	25.7	85.7 ba	76.8 ba	66.3	44.6	91.7	8.3
	112.9	120.40	142.1	121.5	119.2	27.5	76.9 b	70.4 b	63.1	39.7	84.2	7.8
	112.8	130.40	145.9	52.8	085.4	21.8	89.7 ba	75.9 ba	65.8	42.2	90.2	6.8
	137.5	090.54	82.6	42.9	100.0	25.5	93.7 ba	80.7 ba	66.8	38.9	92.6	9.0
	141.9	080.95	73.0	43.3	074.7	23.1	103.8 a	90.8 a	77.2	49.1	104.3	7.3
	120.4	075.35	69.9	41.4	075.4	25.4	81.4 ba	73.4 ba	58.7	37.3	88.0	7.7
	119.7	094.64	85.0	45.8	077.8	32.2	96.6 ba	82.7 ba	70.1	41.1	101.6	8.9

Table 4.5a. continued

LCB22	Treatment	**Zn (mg kg^{-1})**						**Cu (mg kg^{-1})**					
		V6	VT	R2		R6		V6	VT	R2		R6	
				Stover	Ear	Stover	Ear			Stover	Ear	Stover	Ear
	N0P0K0	33.64	35.13	35.82	26.14	32.23	29.75	4.88 b	2.59 b	2.03 b	2.27 a	1.62	2.77
	N0P0K1	41.28	31.82	34.39	29.41	24.20	33.32	5.98 ba	2.60 ba	1.77 b	1.95 ba	2.55	2.52
	N0P1K0	33.48	32.70	26.57	33.74	31.04	25.69	5.52 ba	2.30 ba	1.48 b	0.81 ba	2.39	1.08
	N0P1K1	32.85	25.91	33.07	32.37	35.59	29.96	5.06 b	2.47 b	1.18 b	0.74 b	1.09	1.05
	N1P0K0	33.26	28.59	25.14	27.41	35.15	32.77	5.63 ba	4.76 ba	3.52 ba	0.43 b	1.11	0.96
	N1P0K1	36.49	17.94	19.18	30.91	32.17	31.11	6.67 ba	2.52 ba	3.20 ba	1.00 ba	2.27	1.10
	N1P1K0	35.21	25.89	27.83	28.37	39.77	27.71	5.64 ba	4.31 ba	2.52 b	1.05 ba	2.00	0.95
	N1P1K1	34.03	21.49	17.10	29.64	21.34	26.64	6.22 ba	2.30 ba	3.90 ba	1.04 ba	0.65	0.79
	N2P0K0	37.24	23.16	19.81	27.78	16.46	24.87	6.56 ba	4.80 ba	5.60 a	1.18 ba	3.45	0.92
	N2P0K1	38.69	26.01	20.34	31.18	21.44	29.04	7.07 a	5.73 a	3.62 ba	1.35 ba	1.25	1.62
	N2P1K0	32.46	22.85	29.70	25.57	22.36	27.70	6.37 ba	4.10 ba	2.67 b	1.05 ba	2.58	1.16
	N2P1K1	35.71	21.54	20.22	29.89	14.97	28.48	5.93 ba	4.40 ba	3.89 ba	1.48 ba	2.49	1.77
		Fe (mg kg^{-1})						**Mn (mg kg^{-1})**					
	N0P0K0	114.60 a	056.88 a	47.71 a	19.49 a	092.24 a	14.79 a	62.0 d	55.4	60.3	32.5	58.8	29.8
	N0P0K1	117.00 a	061.61 a	50.68 a	20.37 a	096.48 a	15.32 a	69.7 bdc	60.3	57.5	40.8	72.0	30.8
	N0P1K0	107.21 a	042.43 a	75.08 a	17.51 a	102.47 a	16.29 a	69.2 bdc	49.2	53.0	19.9	47.1	14.9
	N0P1K1	93.92 a	042.52 a	47.78 a	19.49 a	085.18 a	16.37 a	65.6 bdc	48.4	67.9	19.2	62.0	16.2
	N1P0K0	105.56 a	116.17 a	76.89 a	17.47 a	078.23 a	15.55 a	65.2 bdc	58.2	64.1	17.8	67.5	15.6
	N1P0K1	099.10 a	046.13 a	134.07 a	20.01 a	122.73 a	18.55 a	68.1 bdc	38.3	58.8	19.7	72.7	15.7
	N1P1K0	102.74 a	042.78 a	53.10 a	18.71 a	100.17 a	17.99 a	63.4 dc	57.5	70.9	17.2	72.1	13.9
	N1P1K1	111.03 a	117.21 a	57.08 a	19.40 a	080.55 a	14.20 a	73.3 bdac	46.2	71.6	18.3	62.5	14.2
	N2P0K0	116.11 a	063.45 a	66.11 a	29.23 a	114.25 a	17.68 a	87.1 ba	55.8	78.8	18.5	68.8	13.2
	N2P0K1	104.03 a	082.16 a	61.90 a	17.84 a	118.22 a	19.89 a	85.4 bac	64.2	72.4	19.3	63.1	15.8
	N2P1K0	110.60 a	114.97 a	65.46 a	20.52 a	124.18 a	28.30 a	78.0 bdac	55.5	75.7	21.4	73.0	14.0
	N2P1K1	111.34 a	076.01 a	55.94 a	21.20 a	089.10 a	17.17 a	94.1 a	56.6	69.6	18.1	62.1	13.6

Values represent the mean of three replications. Different letter within each column denotes the significant difference at *$P<0.05$ by Tukey test*.

Table 4.5b. The LS-means for mineral nutrient concentration in whole plant at V6 and VT growth stage as affected by the interaction of N x P x K.

Site-year	**Treatment**	**Zn (mg kg^{-1})**		**Cu (mg kg^{-1})**		**Fe (mg kg^{-1})**		**Mn (mg kg^{-1})**	
		V6	VT	V6	VT	V6	VT	V6	VT
LCB 21	N0P0K0	72.49 a	55.08 ba	4.07 a	2.27 a	72.40 a	46.39 a	75.3 a	60.3 a
	N0P0K1	43.51 ba	48.90 bac	3.83 a	2.47 a	64.27 a	51.90 a	69.4 a	60.1 a
	N0P1K0	44.76 ba	39.41 bac	3.09 a	1.96 a	53.25 a	29.94 a	56.7 a	51.3 a
	N0P1K1	57.54 ba	57.35 a	4.88 a	2.64 a	82.94 a	61.10 a	65.5 a	55.7 a
	N1P0K0	44.47 ba	28.87 c	4.39 a	1.99 a	64.17 a	25.42 a	62.1 a	46.3 a
	N1P0K1	41.10 b	37.33 bc	4.38 a	2.77 a	79.65 a	26.64 a	57.1 a	50.0 a
	N1P1K0	43.35 ba	32.05 bac	4.44 a	1.82 a	90.16 a	46.00 a	59.5 a	44.5 a
	N1P1K1	36.65 b	33.72 bc	4.42 a	3.05 a	64.87 a	43.06 a	66.7 a	53.7 a
	N2P0K0	35.45 b	27.00 c	5.64 a	3.12 a	75.65 a	63.43 a	63.7 a	48.8 a
	N2P0K1	41.63 b	31.22 bc	5.61 a	3.15 a	80.85 a	55.98 a	60.6 a	44.8 a
	N2P1K0	34.71 b	24.00 c	5.44 a	3.47 a	74.72 a	42.25 a	72.1 a	60.7 a
	N2P1K1	38.20 b	30.07 bc	5.32 a	2.50 a	80.22 a	63.63 a	56.2 a	43.3 a
PRK 22	N0P0K0	40.17 a	32.88 a	7.73 a	5.01 a	125.14 a	60.72 a	61.3 a	69.8 a
	N0P0K1	52.33 a	34.07 a	6.88 a	2.56 a	96.65 a	57.66 a	70.2 a	47.0 a
	N0P1K0	47.85 a	43.42 a	6.42 a	5.24 a	95.16 a	71.47 a	67.2 a	59.3 a
	N0P1K1	51.32 a	49.86 a	6.54 a	3.65 a	95.27 a	45.56 a	65.3 a	58.8 a
	N1P0K0	43.13 a	38.36 a	7.14 a	5.39 a	103.39 a	60.54 a	69.4 a	52.3 a
	N1P0K1	49.27 a	42.67 a	7.35 a	5.09 a	117.08 a	68.22 a	63.8 a	58.5 a
	N1P1K0	42.74 a	40.47 a	7.20 a	3.64 a	96.19 a	58.15 a	65.1 a	56.8 a
	N1P1K1	41.53 a	62.56 a	5.49 a	4.48 a	82.92 a	89.11 a	50.9 a	55.6 a
	N2P0K0	49.69 a	47.06 a	8.31 a	5.77 a	120.54 a	57.39 a	79.1 a	66.9 a
	N2P0K1	47.64 a	44.04 a	9.27 a	5.13 a	101.56 a	66.07 a	70.5 a	56.3 a
	N2P1K0	53.85 a	42.55 a	7.95 a	5.81 a	108.53 a	58.24 a	77.3 a	57.7 a
	N2P1K1	42.78 a	45.50 a	8.53 a	5.57 a	115.81 a	65.00 a	77.8 a	69.8 a

Values represent the mean of three replications. Different letter within each column denotes the significant difference at *$P<0.05$ by Tukey test*.

Table 4.6. Analysis of Variance for interaction effect and main effects of N, P, and K for total nutrients uptake at V6 growth stage by site year.

Site year	**Source of Variation**	***df***	**Cu (g ha^{-1})**	**Zn (g ha^{-1})**	**Mn (g ha^{-1})**	**Fe (g ha^{-1})**
EFAW 2021	N	2	**	**	**	*
	P	1	ns	ns	ns	ns
	N x P	2	ns	ns	ns	ns
	K	1	ns	ns	ns	ns
	N x K	2	ns	ns	ns	ns
	P x K	1	ns	ns	ns	ns
	N x P x K	2	ns	ns	ns	ns
	S.E.		1.07	5.83	12.8	25.72
	Treatments					
	N0P0K0		6.08 a	30.46 ba	73.7 a	139.29 a
	N0P0K1		6.00 a	31.34 ba	70.2 a	151.52 a
	N0P1K0		5.77 a	34.89 ba	76.8 a	138.49 a
	N0P1K1		5.76 a	28.06 b	68.3 a	139.26 a
	N1P0K0		8.63 a	40.72 ba	97.6 a	180.96 a
	N1P0K1		7.84 a	43.00 ba	98.0 a	187.07 a
	N1P1K0		6.55 a	35.57 ba	72.6 a	142.48 a
	N1P1K1		6.81 a	38.10 ba	83.1 a	136.18 a
	N2P0K0		10.18 a	52.86 ba	121.2 a	219.25 a
	N2P0K1		8.06 a	40.67 ba	109.6 a	186.26 a
	N2P1K0		9.05 a	57.99 ba	104.2 a	203.51 a
	N2P1K1		7.38 a	41.34 ba	92.6 a	149.90 a
LCB 2021	N	2	***	**	***	***
	P	1	ns	ns	ns	ns
	N x P	2	ns	ns	ns	ns
	K	1	ns	ns	ns	ns
	N x K	2	ns	*	ns	ns
	P x K	1	ns	ns	ns	ns
	N x P x K	2	ns	ns	ns	ns
	S.E.		0.82	8.17	6.7	25.23
	Treatments					
	N0P0K0		2.48 dc	43.34 ba	17.8 d	43.10 ba
	N0P0K1		2.17 d	23.23 b	28.8 dc	36.14 b
	N0P1K0		2.54 dc	37.01 ba	29.7 dc	44.09 ba
	N0P1K1		3.08 dc	37.19 ba	27.2 dc	55.08 ba
	N1P0K0		5.91 bdac	59.90 ba	46.0 bdc	87.51 ba
	N1P0K1		4.45 bdc	41.88 ba	49.2 bdac	76.16 ba
	N1P1K0		4.55 bdc	46.01 ba	47.7 bdc	99.16 ba
	N1P1K1		6.24 bac	52.69 ba	57.6 bac	96.90 ba
	N2P0K0		7.39 ba	48.71 ba	67.2 ba	100.70 ba
	N2P0K1		8.74 a	64.54 a	82.5 a	130.71 a
	N2P1K0		6.14 bac	44.28 ba	68.3 ba	84.18 ba
	N2P1K1		8.53 a	61.69 ba	70.6 ba	131.86 a

Table 4.6. continued

Site year	**Source of Variation**	***df***	**Cu (g ha^{-1})**	**Zn (g ha^{-1})**	**Mn (g ha^{-1})**	**Fe (g ha^{-1})**
PRK 2022	N	2	***	***	***	***
	P	1	ns	ns	Ns	ns
	N x P	2	ns	ns	Ns	ns
	K	1	ns	ns	Ns	ns
	N x K	2	ns	ns	Ns	ns
	P x K	1	ns	ns	Ns	ns
	N x P x K	2	ns	ns	ns	ns
	S.E.		0.63	3.00	5.06	7.61
	Treatments					
	N0P0K0		2.48 a	12.84 b	18.39 a	40.65 a
	N0P0K1		2.04 a	14.85 ba	20.19 a	29.32 a
	N0P1K0		2.13 a	15.86 ba	21.13 a	31.44 a
	N0P1K1		1.86 a	14.57 ba	17.67 a	27.38 a
	N1P0K0		3.72 a	22.76 ba	34.41 a	53.93 a
	N1P0K1		3.77 a	29.12 a	35.02 a	69.00 a
	N1P1K0		3.65 a	21.67 ba	30.76 a	48.96 a
	N1P1K1		2.90 a	21.81 ba	25.11 a	43.39 a
	N2P0K0		4.16 a	24.50 ba	38.09 a	59.45 a
	N2P0K1		4.59 a	23.36 ba	32.70 a	49.99 a
	N2P1K0		4.35 a	29.29 a	40.46 a	59.18 a
	N2P1K1		4.72 a	23.60 ba	41.05 a	64.18 a
LCB 2022	N	2	***	***	***	***
	P	1	ns	ns	Ns	ns
	N x P	2	ns	ns	Ns	ns
	K	1	ns	ns	Ns	ns
	N x K	2	ns	ns	Ns	ns
	P x K	1	*	ns	Ns	ns
	N x P x K	2	ns	ns	ns	ns
	S.E.		0.63	3.40	13.14	10.47
	Treatments					
	N0P0K0		1.52 a	10.47 c	51.8 ba	35.54 d
	N0P0K1		2.61 a	18.33 bc	45.4 b	50.36 bdc
	N0P1K0		2.57 a	15.35 bc	54.4 ba	48.58 bdc
	N0P1K1		2.21 a	14.54 bc	48.4 b	41.34 dc
	N1P0K0		4.19 a	25.16 bac	98.2 ba	81.27 bdac
	N1P0K1		5.10 a	28.22 ba	68.6 ba	76.41 bdac
	N1P1K0		4.51 a	28.16 ba	71.5 ba	83.57 bdac
	N1P1K1		5.21 a	28.40 ba	103.2 ba	93.05 bac
	N2P0K0		5.08 a	30.01 ba	102.4 ba	93.16 bac
	N2P0K1		7.20 a	39.47 a	117.4 a	105.23 a
	N2P1K0		5.81 a	29.68 ba	103.8 ba	100.87 ba
	N2P1K1		4.65 a	27.85 bac	106.5 ba	86.64 bdac

*, **, and *** are significant at 0.05, 0.01, and 0.001 levels respectively. Values represent the mean of three replications. Different letter within each column denotes the significant difference at P<0.05 by Tukey test.

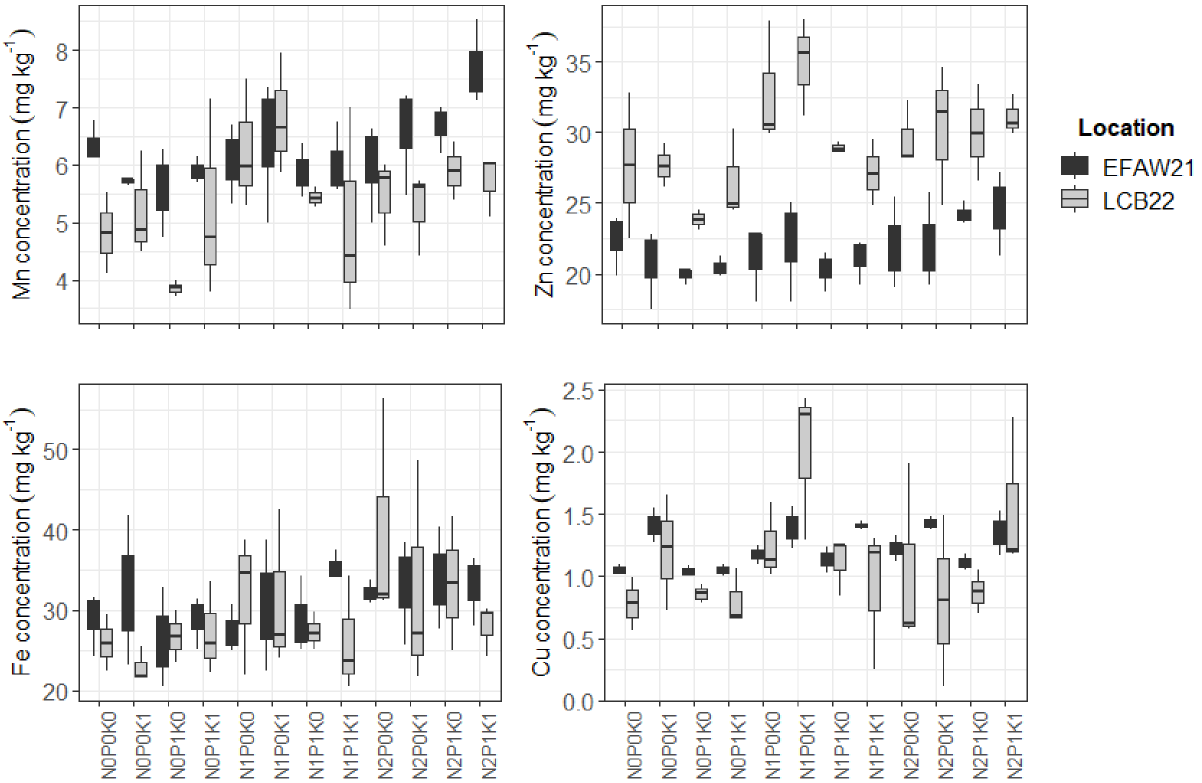


Fig. 4.1. Mineral nutrient concentration in corn grain as affected by different treatments at EFAW21 and LCB22 site.

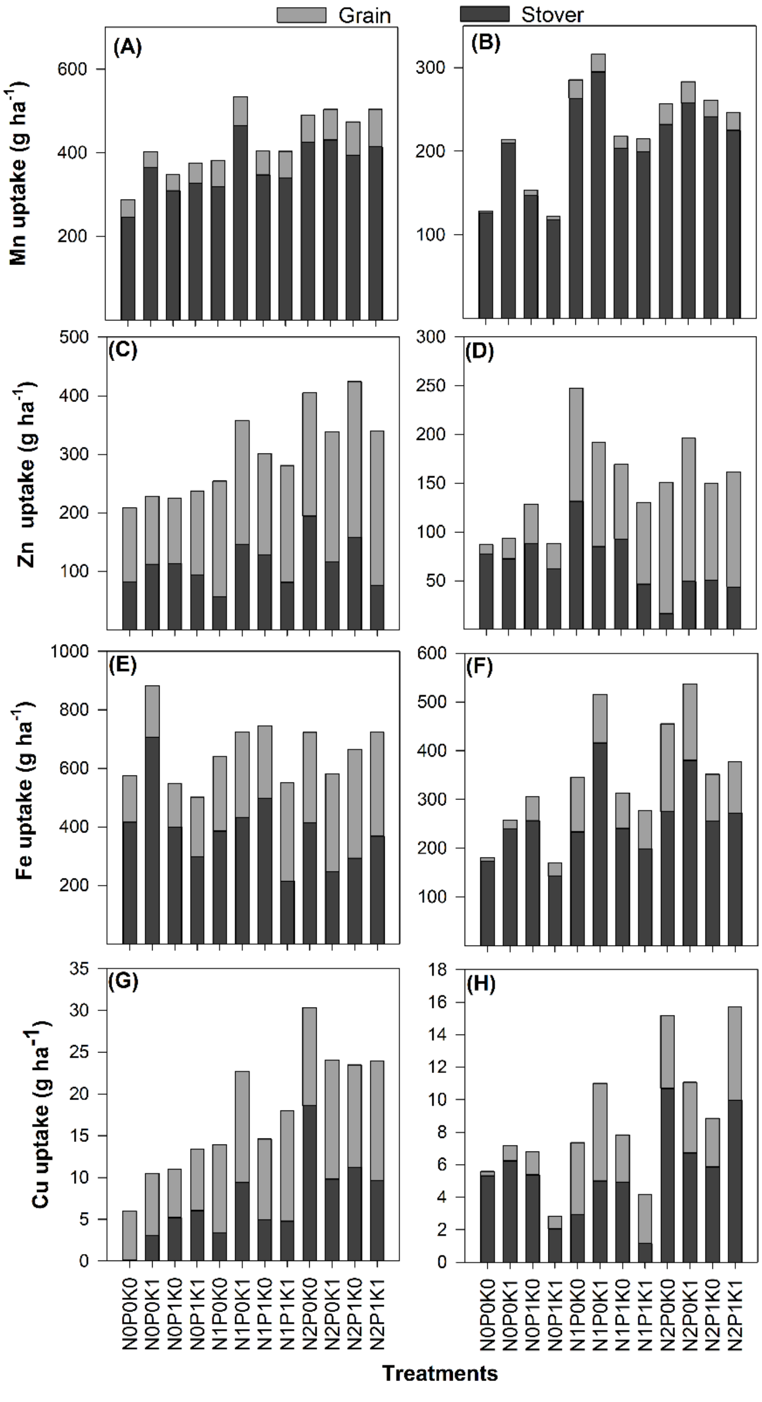
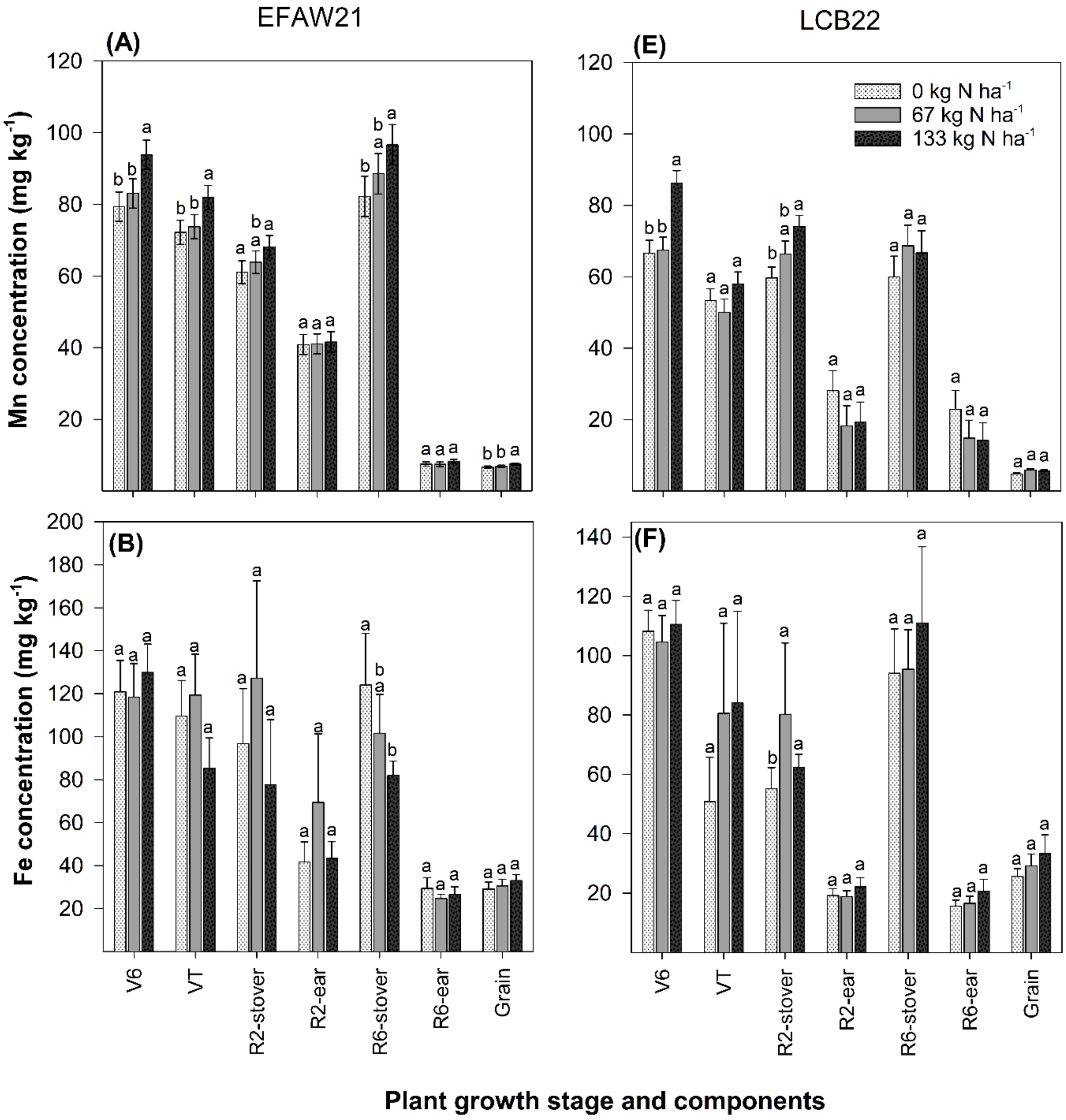


Fig. 4.2. Total nutrient uptake by whole plant (stover + grain) at physiological maturity as affected by different treatments. Mg uptake at (A) EFAW21 (B) LCB22; Zn uptake at (C) EFAW21 (D) LCB22; Fe uptake at (E) EFAW21 (F) LCB22; Cu uptake at (G) EFAW21 (H) LCB22.


EFAW21
LCB22
(A)
(E)
(B)
(F)
Mn concentration (mg kg^{-1})
Fe concentration (mg kg^{-1})
0 kg N ha^{-1}
67 kg N ha^{-1}
133 kg N ha^{-1}
120
100
80
60
40
20
200
180
160
140
V6
VT
R2-stover
R2-ear
R6-stover
R6-ear
Grain
Plant growth stage and components

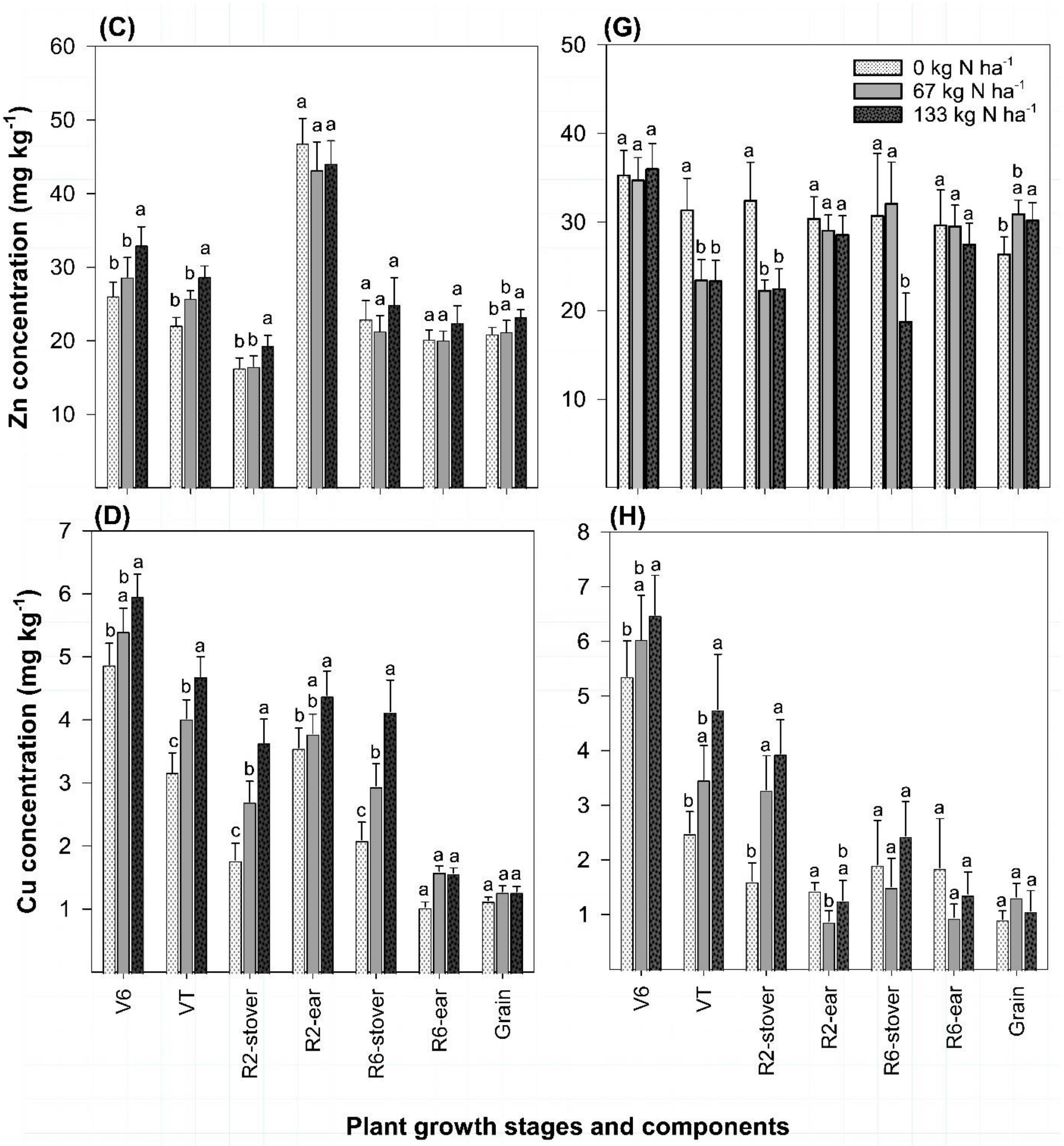
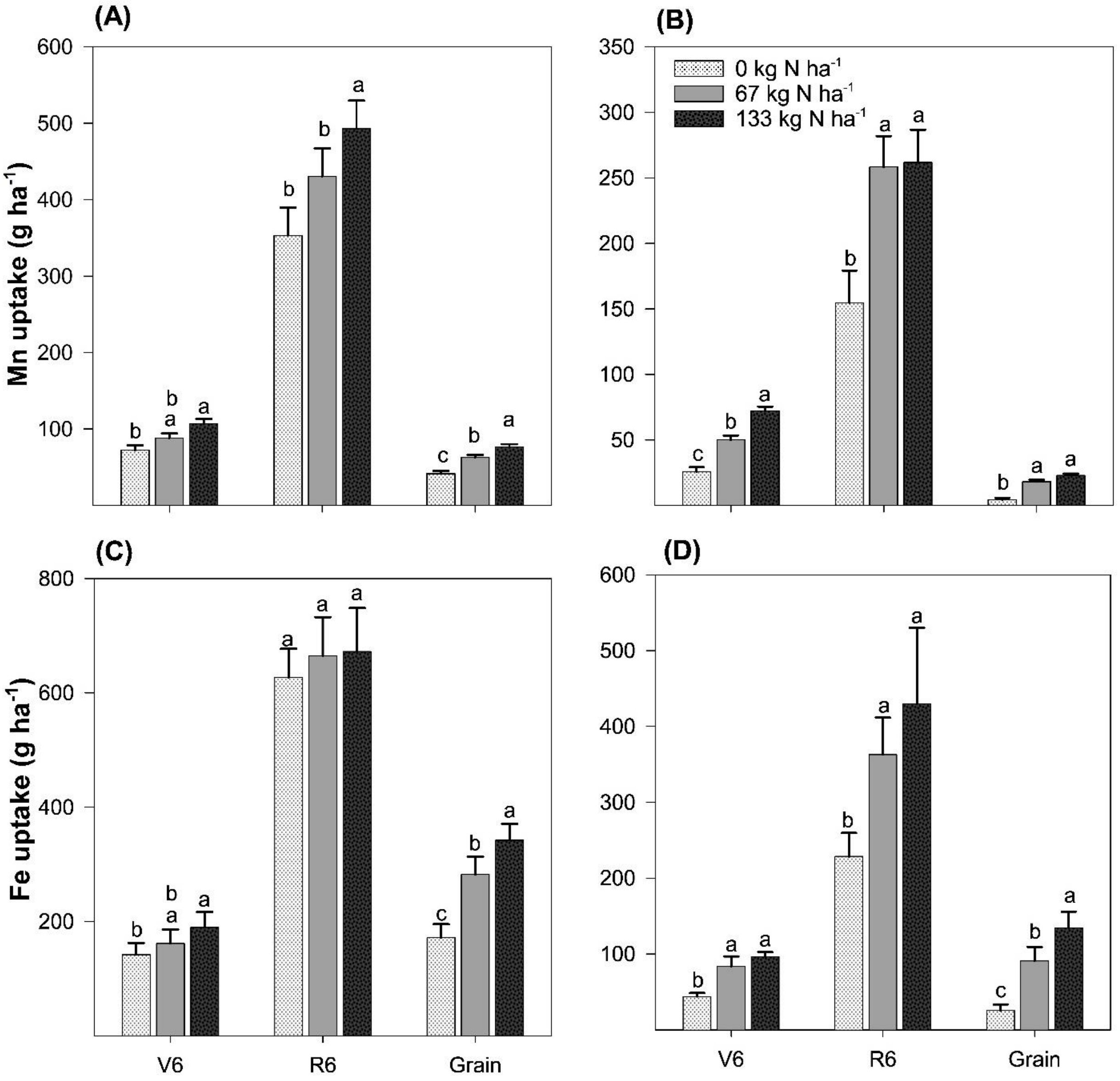


Fig. 4.3. Mineral nutrient concentrations in plant components at different growth stages as affected by different N rates. Concentration values were calculated in whole plant at V6 and VT growth stage, while plant was portioned into stover and ear at R2 and R6 stage. (A), (C), (E) and (G) on the left are for EFAW21; (B), (D), (F) and (H) on the right are for LCB22. Values are mean+ standard error (n=3). Within each growth stage, different letters are significantly different by *Tukey* test ($P<0.05$).


(A)
(B)
(C)
(D)
Mn uptake (g ha^{-1})
Fe uptake (g ha^{-1})
0 kg N ha^{-1}
67 kg N ha^{-1}
133 kg N ha^{-1}
V6
R6
Grain
600
500
400
300
200
100
350
300
250
200
150
100
50
800
600
400
200
600
500
400
300
200
100
b
b a
a
b
b
a
c
b
a
c
b
a
b
a
a
b
a
a
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a
b a
a
a
c
b
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a
a
b
a
a
c
b
a

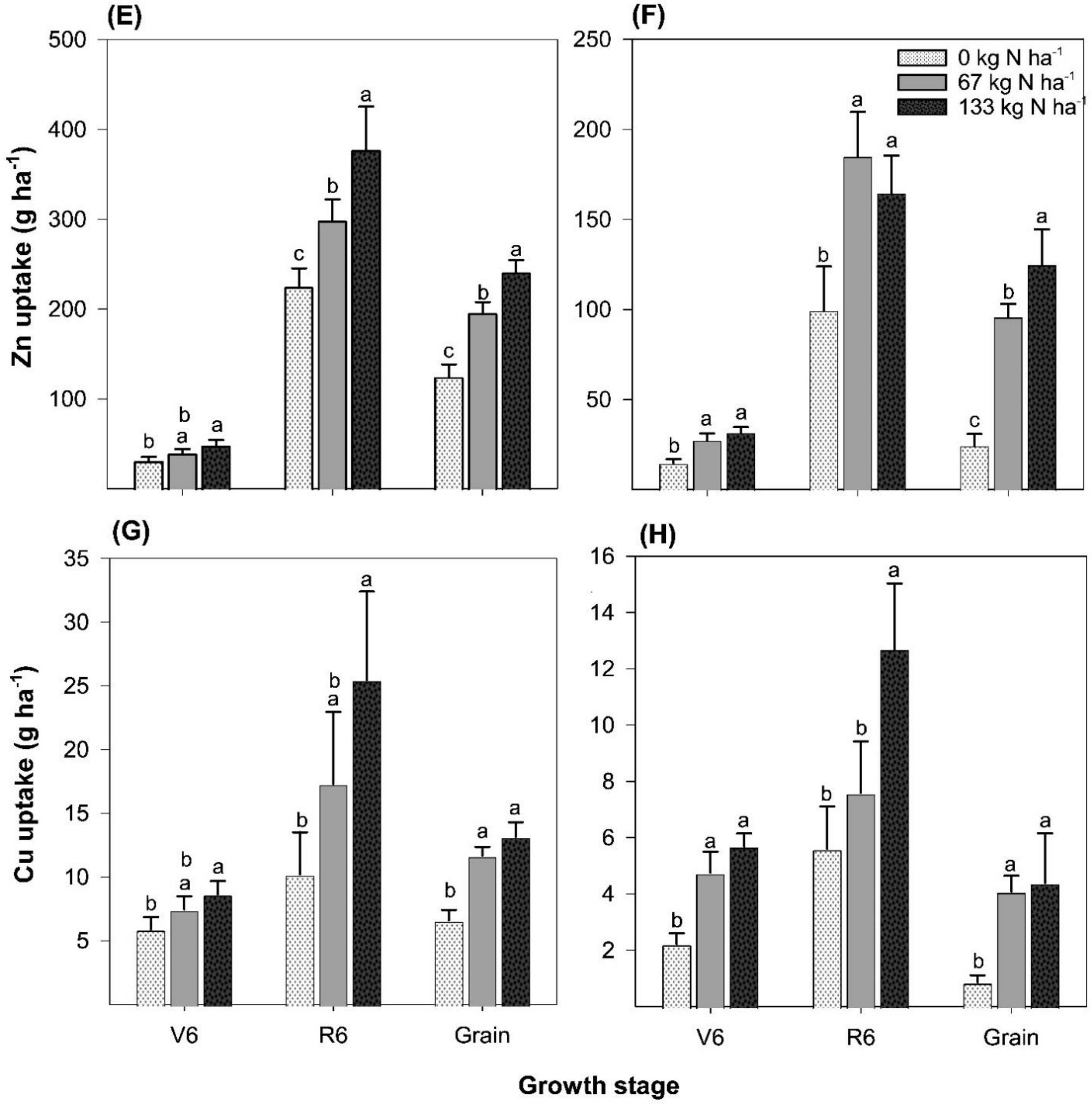


Fig. 4.4. Mineral nutrient uptake by plant components at different growth as affected by different N rates. (A), (C), (E) and (G) on the left are for EFAW21; (B), (D), (F) and (H) on the right are for LCB22. Values are mean+ standard error (n=3). Within each growth stage, different letters are significantly different by *Tukey* test ($P<0.05$).

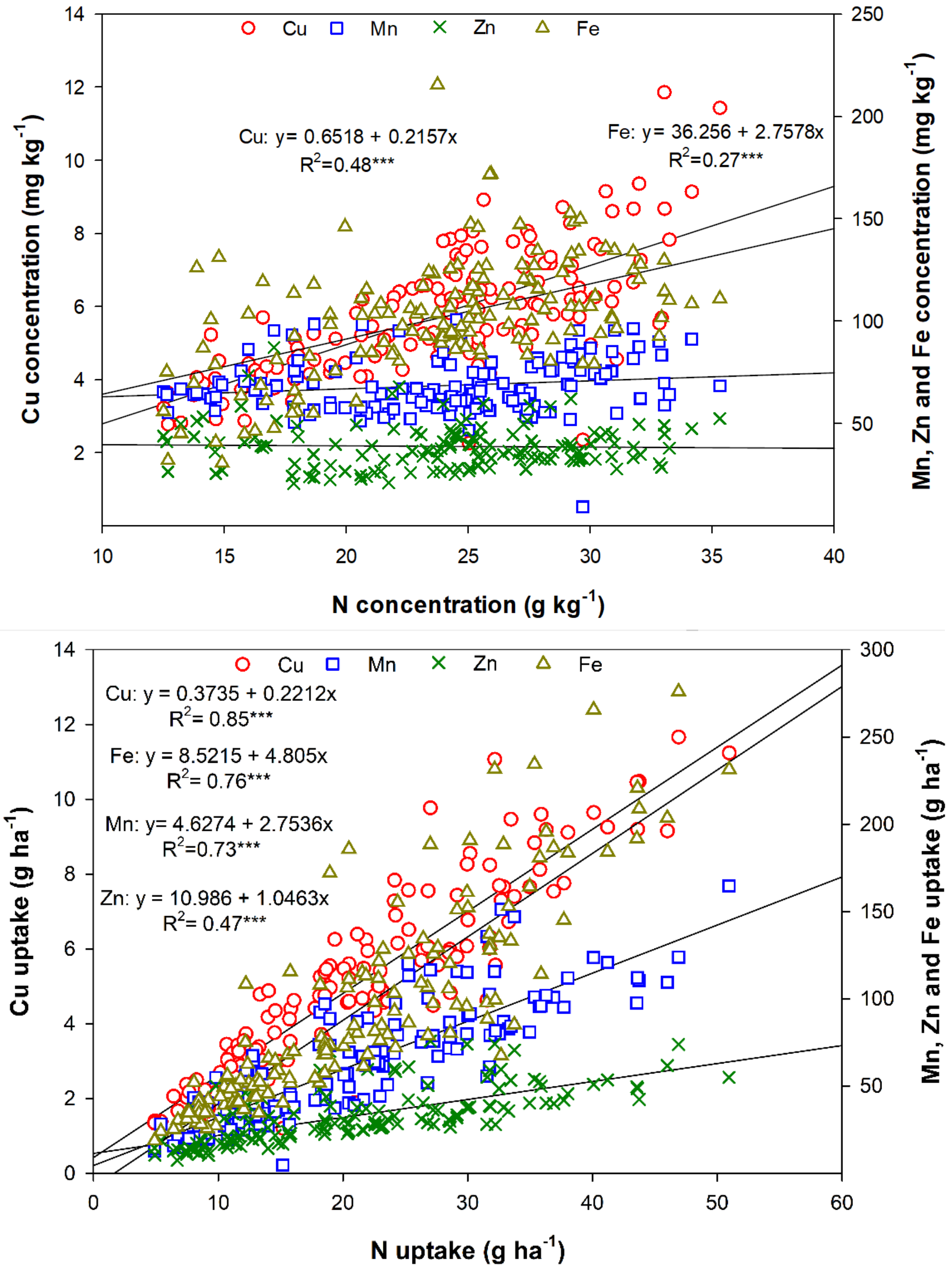


Fig. 4.5. Relationships of (A) whole plant Mn, Cu, Zn, and Fe concentrations with whole plant N concentration (B) whole plant Mn, Cu, Zn, and Fe uptake with N uptake at V6 growth stage. Values from all plots from all locations were used (n= 144).

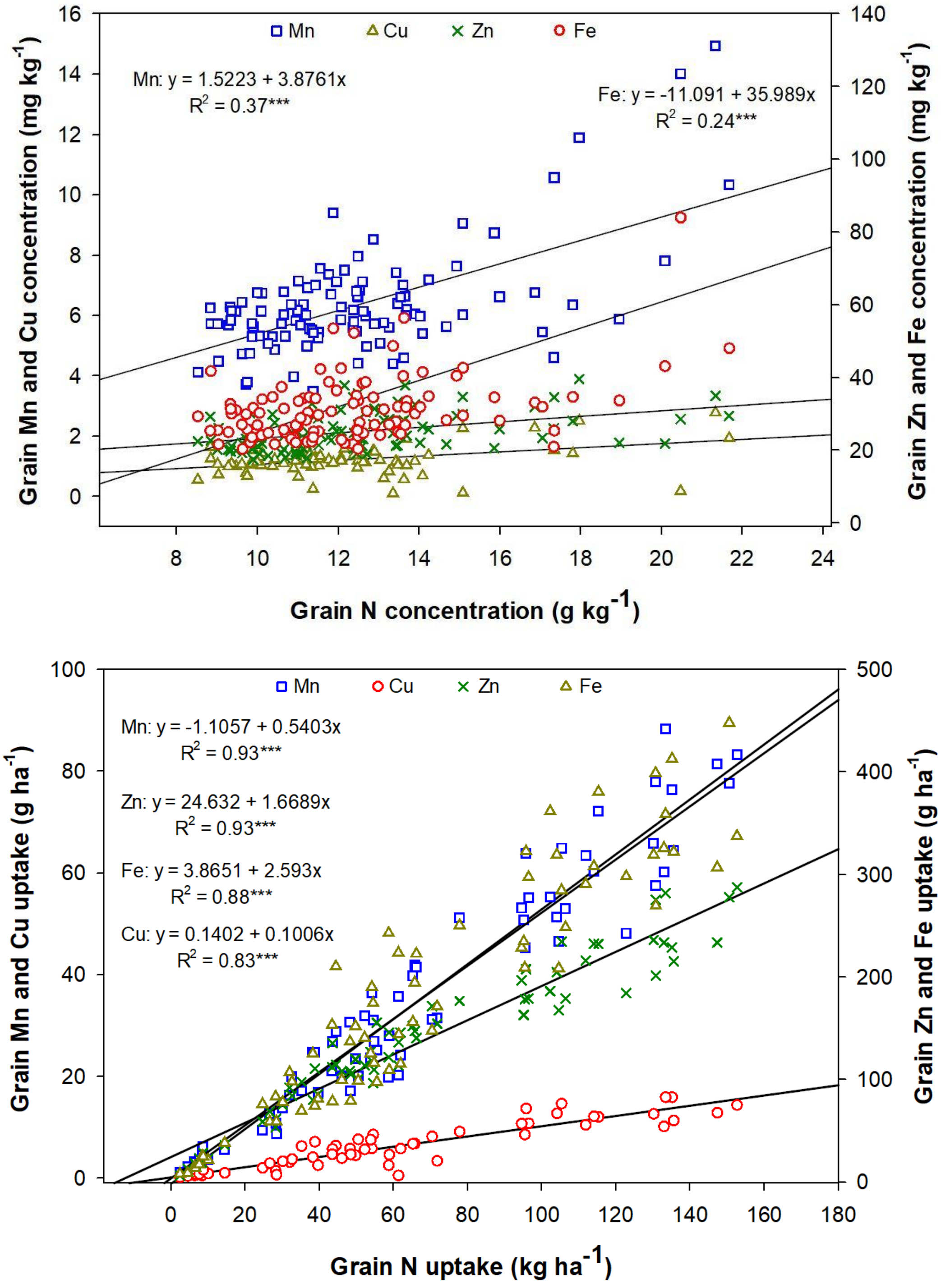
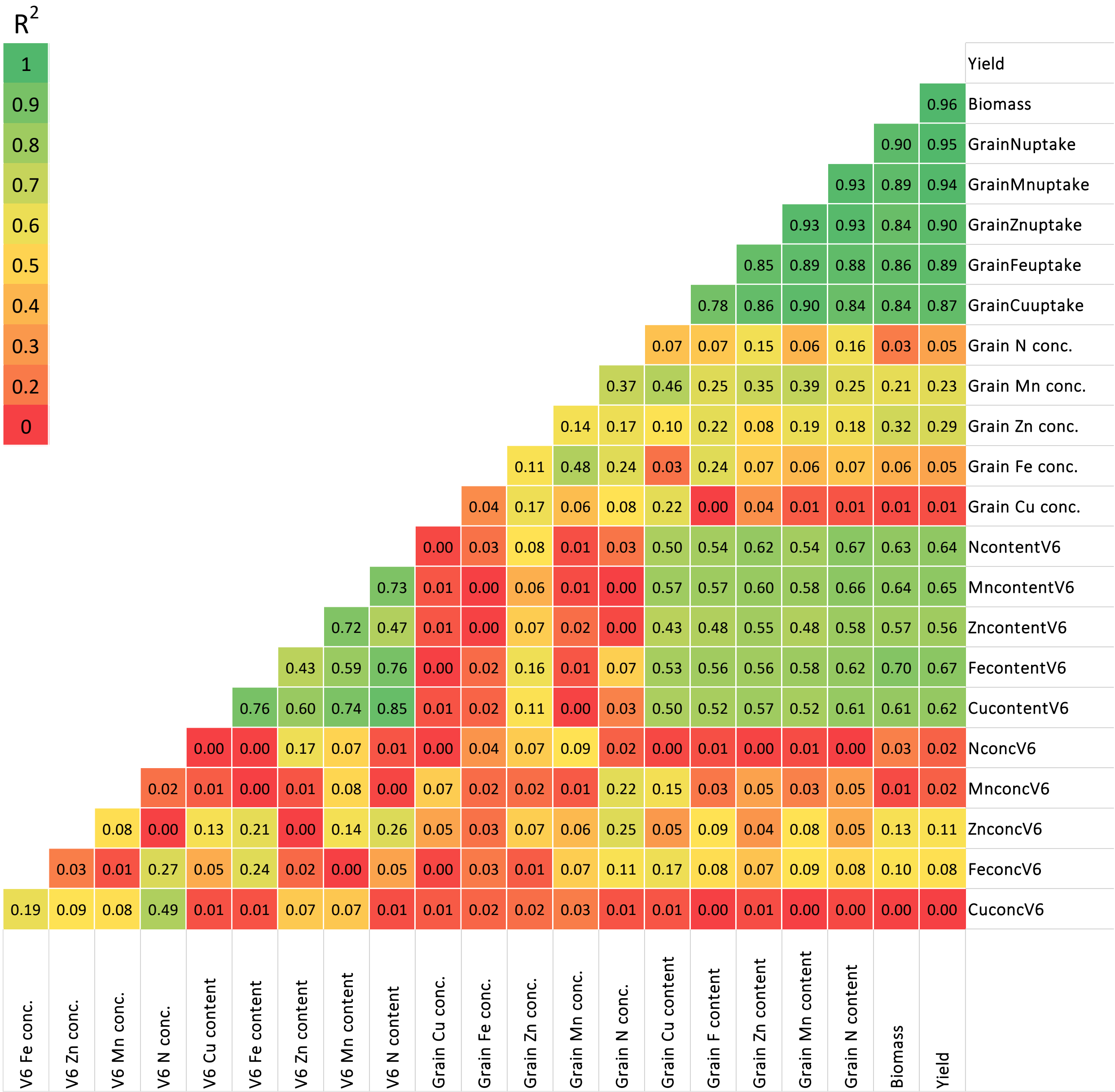


Fig. 4.6. Relationships of (A) grain Mn, Cu, Zn, and Fe concentrations with grain N concentration (B) grain Mn, Cu, Zn, and Fe uptake with grain N uptake. Values from all plots from EFAW21 and LCB22 were used (n= 72).



V6 Fe conc.	V6 Zn conc.	V6 Mn conc.	V6 N conc.	V6 Cu content	V6 Fe content	V6 Zn content	V6 Mn content	V6 N content	Grain Cu conc.	Grain Fe conc.	Grain Zn conc.	Grain Mn conc.	Grain N conc.	Grain Cu content	Grain F content	Grain Zn content	Grain Mn content	Grain N content	Biomass	Yield	
																					Yield
																				0.96	Biomass
																			0.90	0.95	GrainNuptake
																		0.93	0.89	0.94	GrainMnuptake
																	0.93	0.93	0.84	0.90	GrainZnuptake
																0.85	0.89	0.88	0.86	0.89	GrainFeuptake
															0.78	0.86	0.90	0.84	0.84	0.87	GrainCuuptake
														0.07	0.07	0.15	0.06	0.16	0.03	0.05	Grain N conc.
													0.37	0.46	0.25	0.35	0.39	0.25	0.21	0.23	Grain Mn conc.
												0.14	0.17	0.10	0.22	0.08	0.19	0.18	0.32	0.29	Grain Zn conc.
											0.11	0.48	0.24	0.03	0.24	0.07	0.06	0.07	0.06	0.05	Grain Fe conc.
										0.04	0.17	0.06	0.08	0.22	0.00	0.04	0.01	0.01	0.01	0.01	Grain Cu conc.
									0.00	0.03	0.08	0.01	0.03	0.50	0.54	0.62	0.54	0.67	0.63	0.64	NcontentV6
								0.73	0.01	0.00	0.06	0.01	0.00	0.57	0.57	0.60	0.58	0.66	0.64	0.65	MncontentV6
							0.72	0.47	0.01	0.00	0.07	0.02	0.00	0.43	0.48	0.55	0.48	0.58	0.57	0.56	ZncontentV6
						0.43	0.59	0.76	0.00	0.02	0.16	0.01	0.07	0.53	0.56	0.56	0.58	0.62	0.70	0.67	FecontentV6
					0.76	0.60	0.74	0.85	0.01	0.02	0.11	0.00	0.03	0.50	0.52	0.57	0.52	0.61	0.61	0.62	CucontentV6
				0.00	0.00	0.17	0.07	0.01	0.00	0.04	0.07	0.09	0.02	0.00	0.01	0.00	0.01	0.00	0.03	0.02	NconcV6
			0.02	0.01	0.00	0.01	0.08	0.00	0.07	0.02	0.02	0.01	0.22	0.15	0.03	0.05	0.03	0.05	0.01	0.02	MnconcV6
		0.08	0.00	0.13	0.21	0.00	0.14	0.26	0.05	0.03	0.07	0.06	0.25	0.05	0.09	0.04	0.08	0.05	0.13	0.11	ZnconcV6
	0.03	0.01	0.27	0.05	0.24	0.02	0.00	0.05	0.00	0.03	0.01	0.07	0.11	0.17	0.08	0.07	0.09	0.08	0.10	0.08	FeconcV6
0.19	0.09	0.08	0.49	0.01	0.01	0.07	0.07	0.01	0.01	0.02	0.02	0.03	0.01	0.01	0.00	0.01	0.00	0.00	0.00	0.00	CuconcV6

Fig 4.7. Relationships between grain yield, biomass, grain micronutrient concentration and uptake, whole plant micronutrient concentration and uptake at V6 st

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