



ORIGINAL ARTICLE

Soil Fertility and Crop Nutrition

Endomycorrhizal inoculant evaluation on soybean in North Carolina under varying potassium levels

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Abstract

Potassium (K^+) is a key macronutrient for plant growth. While arbuscular mycorrhizal (AM) fungi have been shown to enhance K^+ uptake in model legumes like barrel medic (*Medicago truncatula*), their effect on crop legumes such as soybean [*Glycine max* (L.) Merr.] is not well documented. A 2-year field study across three North Carolina regions assessed the impact of AM inoculation on soybean growth, nutrient uptake, yield, and seed quality. Trials targeted soils low in phosphorus and K^+ , using K^+ chloride to establish high (67 kg ha^{-1}) and low (0 kg ha^{-1}) K^+ environments. Three commercially available soybean cultivars (maturity groups IV, V, and VI) were either inoculated with AM fungi or left untreated. Measurements at 10 and 16 weeks post-planting included chlorophyll content estimation, shoot biomass, and tissue nutrient concentrations. Results indicated that environmental conditions and cultivar maturity group had the strongest influence on biomass, K^+ uptake, yield, protein, and oil content. AM inoculation had limited impact across treatments. These findings, consistent with previous studies, suggest that AM inoculation offers minimal practical benefit for soybean K^+ nutrition under field conditions in North Carolina. Growers should critically evaluate product claims and use caution when adopting AM inoculants expecting significant yield improvements.

Plain Language Summary

Potassium (K^+) is an important nutrient that helps plants grow. Some helpful fungi, called arbuscular mycorrhizal (AM) fungi, can help plants take in more K^+ . Although, we do not know much about how these fungi affect major crops like soybeans, so, we did a 2-year field study in three parts of North Carolina. We wanted to

Abbreviations: AM, arbuscular mycorrhizal; PM, Piedmont Research Station; SH, Sandhills Research Station; SPAD, soil plant analysis development; UC, Upper Coastal Plain Research Station; WAP, weeks after planting.

Danielle R. Cooney and Arjun Kafle contributed equally to this work.

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see if adding AM fungi to soybean plants would help them grow better, take in more nutrients, and produce more seeds. We tested this in soils that were low in K^+ . We added K^+ fertilizer to make some plots high in K^+ and left others low. We used three types of soybeans and either added AM fungi or did not. We measured leaf color, plant weight, and nutrient levels in the plants. The results showed that things like weather and the type of soybean had the biggest effect on how well the plants grew and how much K^+ they took in. However, adding AM fungi did not make much difference. In short, this study suggests that using AM inoculum does not help soybeans much in North Carolina fields.

1 | INTRODUCTION

Soybean [*Glycine max* (L.) Merr.] production serves a crucial role in providing a portion of the global population's food demand through the seed end uses of oil for cooking, protein sources for humans and animals, as well as biodiesel (Voora et al., 2020). Increasing extreme weather events pose a significant risk for the agricultural row-crop production environment attempting to meet the increasing food demand (Arora, 2019; Harris et al., 2018). In North Carolina, soybean producers use a wide diversity of production practices, leading to variability in yield achieved across the state and within individual farming operations (Vann et al., 2021). North Carolina soybean yields typically average between 2353 and 2690 kg ha⁻¹ compared to average yields of over 3000 kg ha⁻¹ for the United States (Vann et al., 2021). North Carolina soybean yields lag behind US averages due to the wide range of planting dates and suboptimal soils where soybeans are produced in much of the state. Thus, increased efforts to develop various technologies and methods for resilience in management practices are emerging. One proposed management strategy is to utilize beneficial soil microbes to improve resilience in crop production systems. These mutualist occurrences between soil microflora, soil nutrients, and plant roots have led to a rapidly growing subsegment of industry working to harness this beneficial symbiosis (Salomon et al., 2022a, 2022b).

Among the many symbiotic associations, arbuscular mycorrhizal (AM) fungi, specifically, serve a foundational role in the ability of plants to function on land and to this day continue to have a predominant role in natural and agroecosystems (Puginier et al., 2022; Rich et al., 2021). AM fungi are the most prevalent type of mycorrhizal symbionts since they colonize more than 80% of land plants, including many crop species (Harley & Smith, 1983; Smith & Reed, 2008; B. Wang & Qiu, 2006). The host plant is essential for AM fungal existence, as these fungi are asexual, symbiotic, obligate biotrophs (Parniske, 2008). In this mutualistic plant-fungal symbiosis, the plant provides a critical fraction (up to 25%) of the photosynthetically fixed carbon (C) in return for nutri-

ents and water collected by the fungus with mycelia extending beyond the limits of the plant roots (Fajardo Lopez et al., 2008; Garcia et al., 2016; Roth & Paszkowski, 2017; Smith & Smith, 2011). This bi-directional resource exchange is facilitated in the cortical root cells of the plant, where the fungus forms functional, densely branched structures, called arbuscules, connected to hyphae that extend externally out into the soil solution—conditionally, the fungus may also form vesicles in the cortical cells (Gutjahr & Parniske, 2013). Thus, these hyphal structures associated with the plant root result in the categorization of AM fungi as an endomycorrhizal fungus (Bonfante & Genre, 2010).

Since the early 1980s, AM fungi have been studied as a method to reduce fertilizer use in agriculture (Johnson & Hummel, 1986; Plenchette et al., 1982; Rhodes, 1980). Research demonstrates positive plant responses, particularly under stress conditions (Bücking & Kafle, 2015; Miransari, 2011; Pérez-Tienda et al., 2012; Santander et al., 2021). Findings of improved soil health (Leifheit et al., 2014), reduced fertilizer dependency (Lal, 2008), and fertilizer accession restrictions (Hassen & Bilali, 2022) have driven the development of commercial AM fungal products. However, sustainable agricultural production, particularly for staple crops like soybeans, requires extensive multi-environment field testing (Berruti et al., 2016; Srivastava et al., 2021).

Soybean studies under controlled conditions show AM associations with *Funneliformis mosseae* or *Rhizophagus irregularis* enhance water stress resilience and salinity tolerance (Bethlenfalvay et al., 1988; Jones et al., 2025). Additionally, Carling and Brown (1980) showed that while some *Glomus* sp. enhanced soybean growth in low-fertility soils, other species like *Gigaspora* sp. had neutral or negative effects. Field trials in Brazil found AM inoculation by *Rhizophagus clarus* and fertilization improved yields by around 20%, but no single treatment was superior (Cely et al., 2016). In Nigeria, commercial AM inoculants made of the fungal species *F. mosseae*, *R. irregularis*, and *Claroideoglomus etunicatum* increased root associations, plant growth, and yield by over 30% (Adeyemi et al., 2020, 2021). Positive responses

are more pronounced in low-fertility conditions, while high soil phosphorus (P) levels inhibit AM function (Balzergue et al., 2013; Gutjahr & Parniske, 2013). A meta-analysis found native AM fungi outperform commercial inoculants, whose success often depends on added nutrients or plant growth-promoting microbes (Berruti et al., 2016). Most AM research has focused on nitrogen (N) and phosphorus (P), with limited studies on potassium (K⁺), an essential element for enzymatic activities, membrane potential, plant growth, and stress responses (Cooney et al., 2025; Johnson et al., 2022; Kafle et al., 2018; M. Wang et al., 2013). Soybeans require peak K⁺ at the R2–R3 stage (Clover & Mallarino, 2013; Slaton et al., 2010), and deficiencies can cause chlorosis or “hidden hunger” with no visible symptoms (Parvej et al., 2016). K⁺ availability also influences seed quality, protein, and oil content (Anthony et al., 2012).

Recent studies highlight AM fungi’s role in K⁺ uptake under controlled conditions using the model legume *Medicago truncatula* (barrel medic) (Garcia & Zimmermann, 2014; Garcia et al., 2017; Kafle et al., 2022; Kafle & Garcia, 2022). Enhanced K⁺ assimilation may benefit large-scale row-crop production. North Carolina’s diverse soils (Daniels et al., 1999) provide an opportunity to assess the effects of AM fungi under various conditions. To simulate real-world agricultural settings, our study applied a commercial AM inoculum with K⁺ fertilization across multiple production regions in North Carolina, evaluating soybean growth, development, and yield responses under field conditions.

2 | MATERIALS AND METHODS

2.1 | Location information

A multi-location, small-plot, field-based trial was conducted over the course of two growing seasons in North Carolina (2020–2021). The selection criteria for the three locations was based on initial field conditions that ideally had deficient or low K⁺ index values, as indicated by routine soil sample analysis, along with representing soybean production locations in North Carolina and a diversity of growing environments. Research sites included three North Carolina State University research stations (Figure 1): Upper Coastal Plain Research Station (UC) in Rocky Mount, NC (35.98 N, –77.376 W), Sandhills Research Station (SH) in Jackson Springs, NC (35.19 N, –79.68 W), and Piedmont Research Station (PM) in Salisbury, NC (35.70 N, –80.62 W). The UC work was conducted on a Roanoke silt loam (Fine, mixed, semiactive, thermic Typic Endoaquults) and a Dogue fine sandy loam (Fine, mixed, semiactive, thermic Aquic Hapludults). The SH work was conducted on Candor sand soil (Sandy, kaolinitic, thermic Grossarenic Kandiodult). Finally, the PM work was conducted on a Lloyd clay loam (Fine,

kaolinitic, thermic Rhodic Kanhapludults) (Figure S1). Composite soil samples were collected from each plot by taking six to eight soil cores at a depth of 0–20 cm. The cores were homogenized and submitted to the North Carolina Department of Agriculture & Consumer Services (NCDA&CS) Agronomic Services for analysis (Table S1). Soil pH was determined using a 1:1 soil-to-water suspension, and nutrient concentrations were extracted following the Mehlich-3 procedure (Mehlich, 1984) and quantified by inductively coupled plasma-optical emission spectroscopy. The PM field site was a no-till environment; comparatively, SH and UC were tilled each year, and UC was planted into bedded soil conditions. Chemical weed management varied across research sites and aligned with farmer standard practices, generally including a preemergence herbicide application and one to two postemergence herbicide applications at each site. At SH, supplemental irrigation was applied using a Valley 8000 linear move system. Irrigation events were scheduled weekly at a rate of 1.3–2.5 cm, but only if rainfall during the preceding week totaled less than 1.3 cm.

2.2 | Field plot design

A small plot field trial was designed to evaluate a factorial treatment combination. Each location has randomization within the design that is unique to year and site, composed of a modified split-strip plot and a randomized complete block design with replicated strips. The factors included two K⁺ regimes, two conditions of AM inoculum as a seed treatment, and three untreated soybean maturity groups. The K⁺ regime is attributed to the main plot factor and consisted of either the original condition of the field (initial selection for low K⁺ index value) (0); or fertilization with K⁺ chloride (0-0-67) as recommended by the North Carolina Department of Agriculture specifications and guidelines utilizing the index value (Table S1). The following formula is to achieve the desired rate in kg ha⁻¹: $0.0116 I_2 - 2.75 I + 150 = \text{answer} \times (2.2/2.47) = \text{kg ha}^{-1}$ (Hardy et al., 2014) (“K+”). Within an assigned K⁺ condition, we randomly assigned subplot strip of the AM seed treatment factor, which was either the presence of a commercial inoculum additive (MycoApply EndoPrime). The inoculum contained a mix of the following four fungal species: *R. irregularis*, *F. mosseae*, *Glomus aggregatum*, and *C. etunicatum* (5625 propagules g⁻¹ each), and was applied as a seed treatment at a rate of 9.88 g ha⁻¹ based on manufacturer recommendations or the absence of a commercial inoculant (control). Finally, the sub-sub plot consisted of the soybean maturity groups randomly assigned within the other factors. The three selected groups included (a) maturity group IV, Dyna-Gro, indeterminate, S43XS27; (b) maturity group V, Dyna-Gro, indeterminate, S52RS86; and (c) maturity group VI, Dyna-Gro,

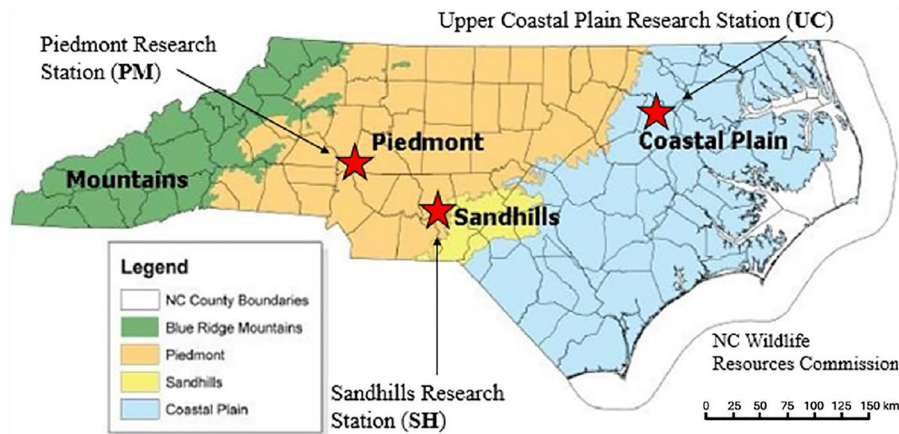


FIGURE 1 Location of the three research sites. This map of North Carolina represents the major geographical soil areas and the three locations on which this study was conducted (red stars: Piedmont Research Station [PM], Sandhills Research Station [SH], and Upper Coastal Plain Research Station [UC]).

determinate, S64XT18. Seeds (9 kg per treatment) were coated with the AM inoculant by preparing a suspension containing 185-mL autoclaved milli-Q water, 15-mL Flow-Rite adjuvant (to enhance propagule adhesion), and the inoculum additive at rates of 1.6 g (S43XS27), 1.5 g (S52RS86), or 2.1 g (S64XT18) based on seed weight. Each mixture was prepared in a 1-L glass beaker, and the corresponding seed batch was placed in a pre-cleaned cement mixer (wiped several times with paper towel, followed by two washes each with 70% and 100% ethanol). The mixer was tilted to swirl and agitate the seeds while the inoculant suspension was added incrementally in four portions over 15 min of mixing, followed by a 5-min stationary period to allow propagules to adhere to the seed coat. Treated seeds were transferred to a larger tub lined with aluminum foil to prevent inoculant loss, air-dried for 20 min, and placed into labeled cotton pillow bags for storage.

One day later, each treatment combination was planted in four rows wide by a length of 12.2 m. Data were collected from the middle two rows, with two outside rows used as borders between treatments to limit potential non-target effect from the AM inoculant. Each treatment combination was replicated six times per a location and randomized each year. Soybeans planting typically occurred at a target date of mid-May; however, PR 2020 was delayed until mid-June due to following a wheat crop, an entire K^+ block at UC 2020 was replanted in early June due to deer damage, and UC 2021 was delayed until early June due to poor early-season moisture. Seeding rates were adjusted for germination rates to achieve a target final population of 296,400 seeds ha^{-1} . Plots were established utilizing a four-row unit John Deere MaxEmerge 2 planter (John Deere) at PR on 76.2 cm rows, a four-row unit John Deere MaxEmerge (John Deere) at UC on 91.44 cm rows, and an Almaco four-row, dual-cone plot planter (Almaco) at SH on 86.36 cm rows.

2.3 | Data collection

At 10 and 16 weeks post seeding, aboveground biomass was collected from four random plants found within the inner two rows of one plot. From the same plants, the roots were also harvested and collected for further analysis. Additionally, eight repetitions of tissue chlorophyll data were randomly collected within a single plot utilizing a soil plant analysis development (SPAD) 502 plus chlorophyll meter (Spectrum Technologies Inc.). The aboveground biomass samples were then dried in forced heated-air dryers at 70°C for 2 days and dry weights were recorded. Samples were ground using a Thomas–Wiley Laboratory Mill (Arthur H. Thomas Company) with a 1-mm sieve used to ensure homogeneity. Shoot samples were then sent to Waypoint Analytical (Waypoint Analytical Inc.) for tissue nutrient analysis.

To quantify soybean yield, data were collected with a Wintersteiger Plot Combine (Wintersteiger Inc.). Additionally, subsamples were collected from the combine and retained for quantification of seed protein and oil, which was evaluated using near-infrared (NIR) spectroscopy (Perten Instruments Inc.) and corrected at 13% moisture.

2.4 | Statistical analysis

Statistical analysis software (SAS, version 9.4, SAS Institute) was used to analyze the data. Assumptions of the model were evaluated using PROC UNIVARIATE. The analysis of variance (ANOVA) was performed utilizing a Type III test of fixed effects within the PROC GLIMMIX procedure; a studentized panel also confirmed the response of the residuals. For end-of-season data, a model was fit where the treatment effects of year, location, K^+ , inoculant, and maturity group

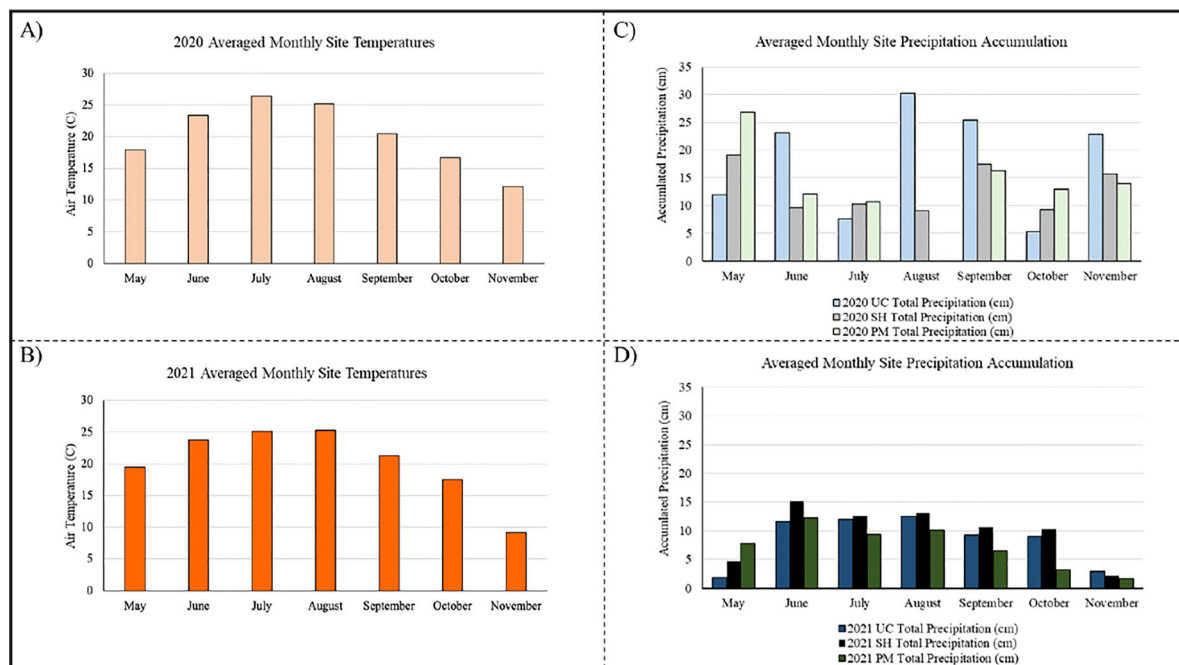


FIGURE 2 Air temperature and location precipitation for 2020 and 2021. The monthly air temperatures in °C averaged across all three locations for both 2020 (A) and 2021 (B). (C) The 2020 averaged monthly site precipitation in centimeters but separated into each site to highlight the in-season site-to-site variation in average monthly rainfall. UC—Upper Coastal Plain Research Station (light blue); SH—Sandhills Research Station (light gray); PM—Piedmont Research Station (light green). No data available for PM in August 2020. (D) The 2021 averaged monthly site precipitation in centimeters but separated into each site to highlight the in-season site-to-site variation in average monthly rainfall. UC (dark blue), SH (black), and PM (dark green).

were analyzed as fixed effects; block within site year was treated as random effects of the model. When appropriate, treatment means were separated using Tukey's honestly significant difference (HSD) at $\alpha = 0.05$. The in-season data collection included an additional repeated measure of data collected at two time points: weeks after planting (WAP) that was treated as a fixed effect.

3 | RESULTS

The initial evaluation of the model utilizing ANOVA and Type III test of fixed effects yielded highly significant effects of test year and test location. Thus, due to the interest in what is happening at the soil level, reasonable justification could be created for combining year and location together to create a model that tests the fixed effect of *environment* (Figure S1; Figure 2). Due to the limitations of the locations and experimental design parameters, the effect of K^+ treatment was nested within the *environment* factor. Fixed treatment effects of all model factors and resulting *p*-value of *F* tests for in-season data parameters are compiled in Tables 1 and 2.

3.1 | Soybean biomass production is highly responsive to environmental conditions

The conditions and treatments of *potassium (environment)*, *WAP*, *fungi*, and *maturity group* all exhibit significant responses (Table 1). Further, data show that when averaged across all environments and treatment combinations, the addition of inoculation is driving the response of aboveground biomass production (Table 1). The combined influence of environment and inoculation can be observed in Figure 3. Further, the main effect of maturity group resulted in the group VI variety producing less biomass than the groups V and IV varieties (Table S2). When assessing subplot biomass production in relation to the two-way interaction between the environment and maturity groups, the most significant differences in maturity group rankings are primarily influenced by the underlying effects of years. Additionally, location-specific factors at PM and UC contribute to these variations (Table S3). As to be expected, the average plot subsample plant biomass increased as the season progressed, with an accelerated accumulation occurring between the 10 and 16 WAP points as the plant entered the reproductive phase (Table S2).

TABLE 1 Type III fixed effects for in-season data. Statistical significance of various factors and their interactions on soil plant analysis development (SPAD) average, aboveground biomass, and nutrient concentrations (N, P, and K) in plants.

Source of variation	SPAD average	Aboveground biomass	N	P	K
Potassium (environment)	***	***	***	***	***
WAP (weeks after planting)	***	***	***	***	***
Potassium × WAP (environment)	***	***	***	***	***
Inoculum	ns	**	ns	ns	ns
Potassium × inoculum (environment)	***	**	ns	ns	*
WAP × inoculum	ns	ns	*	ns	ns
Potassium × WAP × inoculum (environment)	ns	ns	ns	ns	ns
Maturity group	***	***	***	***	***
Potassium × maturity group (environment)	***	**	*	ns	***
WAP × maturity group	***	ns	ns	ns	ns
Potassium × WAP × maturity group (environment)	***	ns	**	ns	ns
Inoculum × maturity group	ns	ns	ns	ns	ns
Potassium × inoculum × maturity group (environment)	ns	ns	ns	ns	ns
WAP × inoculum × maturity group	ns	ns	ns	ns	ns
Potassium × WAP × inoculum × maturity group (environment)	ns	ns	ns	ns	ns

Note: Potassium is nested within the effect of (environment). “ns” denotes nonsignificant effects.

* $p < 0.05$. ** $p < 0.01$. *** $p < 0.0001$.

TABLE 2 Type III fixed effects for end-of-season data. Statistical significance of various factors and their interactions on yield, and protein and oil content in plants.

Source of variation	Yield	End of season	
		Protein	Oil
Potassium (environment)	***	***	***
Inoculum	ns	ns	ns
Potassium × inoculum (environment)	**	ns	ns
Maturity group	**	*	***
Potassium × maturity group (environment)	***	***	***
Inoculum × maturity group	ns	ns	ns
Potassium × inoculum × maturity group (environment)	ns	ns	ns

Note: Potassium is nested within the effect of (environment). “ns” denotes nonsignificant effects.

Significance levels are indicated as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$.

3.2 | Leaf chlorophyll and nitrogen contents highlight seasonal and location responses

SPAD data collection is a nondestructive method to evaluate chlorophyll content and resulting crop health (Uddling et al., 2007). N tissue percentage provides a complement and approximate comparison at the plot-level randomness of collected data for the aboveground plot subsample tissue (Marschner, 2011). Further, as consistent with previous observations, SPAD and N concentration responses were both significantly driven by the environment, data collection timing (WAP), and maturity group (Table 1). Location

is a primary driver in the significant *environment* response for chlorophyll and N concentrations (Figure 4; Table 1).

3.3 | Drier conditions in 2021 highlight increased soybean potassium demands, while no impact of AM inoculation was observed

The ANOVA indicated that *potassium (environment)*, *WAP*, *inoculum*, and *maturity group* all exhibit significant responses in plant K^+ concentrations (Table S1). In evaluating our

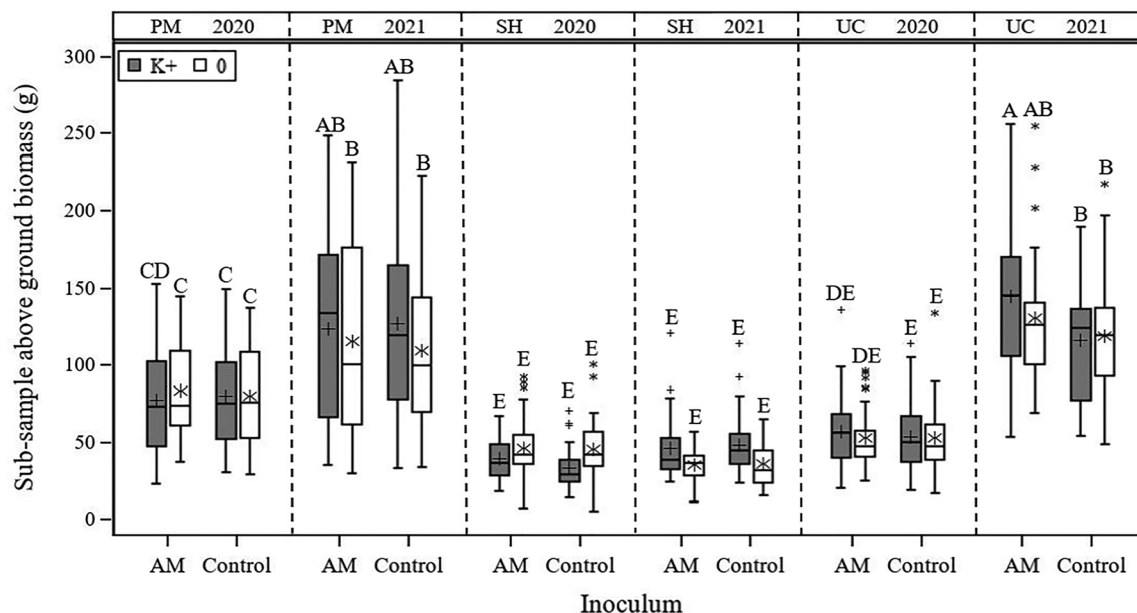


FIGURE 3 Environment and inoculum influence on produced aboveground biomass. Box plots representing the significant ($p = 0.0035$) two-way interaction between inoculation \times potassium (environment), for plot subsample aboveground soybean biomass, averaged across sampling time points (10 and 16 WAP) and all maturity group treatments. The upper X-axis represents each respective environment: PM—Piedmont Research Station, SH—Sandhills Research Station, UC—Upper Coastal Plain Research Station, and the year of field trial is denoted by 2020 or 2021. The lower X-axis represents either the addition of fungal inoculum (AM) or not (control). Dark gray boxes indicate the addition of K^+ fertilizer (K^+) and white boxes indicate no additions of K^+ fertilizer (0). (+) or (*) inside the boxes represents the mean, while the external ones represent potential data outliers. Comparisons of the means for significant differences of the treatment combinations occur across all environments. Letters are assigned based on a significance value of $\alpha = 0.05$ with Tukey's honestly significant difference (HSD).

environments, we observe that the 2021 locations exhibit an increased differential between K^+ amendments, possibly due to the drier conditions recorded (Figure 2). Tissue K^+ concentrations significantly decline for mean values as the season continues with the 10 and 16 WAP sample collection. The treatment addition of inoculum, with consideration for an environment, has limited response for K concentrations in the plant aboveground biomass. The effect of environmental conditions is driving the bulk of the response (Figure 5). Further, the treatment of the maturity group averaged across all other factors did yield an overall difference with IV and V exhibiting significantly higher tissue content relative to group VI.

3.4 | Shoot P is influenced by location rather than K^+ application, while shoot K^+ is typically more abundant when K^+ fertilizer is applied

P shoot concentrations responses were significantly driven by the environment, sampling time, and maturity group (Table 1). Environmental influence exhibits significant drivers relative to location and year as observed in Figure 6. In data sampling time relative to WAP, P-tissue concentrations mean values are significantly higher at 16 WAP compared

to 10 WAP. The treatment of the maturity group each has a significant distinction relative to others for P-tissue concentrations, as outlined in descending order of groups I, V, and VI.

3.5 | Selection of maturity group influences yield, seed protein, and oil composition across environments

The updated ANOVA revealed that yield response in the selected six environments was a key factor influencing overall yield (Table 2). Highest yields were exhibited in the 2021 UC environments for both K^+ treatments; comparatively, the lowest yields were recorded in the 2021 SH environments for both K^+ treatments as indicated in Figure 7A. Maturity group affected yield response with group V producing the highest yield, which was significantly higher than group VI, and group IV shared significance with V and VI. When accounting for the effect of the environment on the maturity group, a response was significant (Table 2) and variations in maturity group rank relative to environment (Figure 8A). Further, a significant response to inoculum applications was observed when accounting for the effects of the K^+ treatment within an environment (Figure S3).

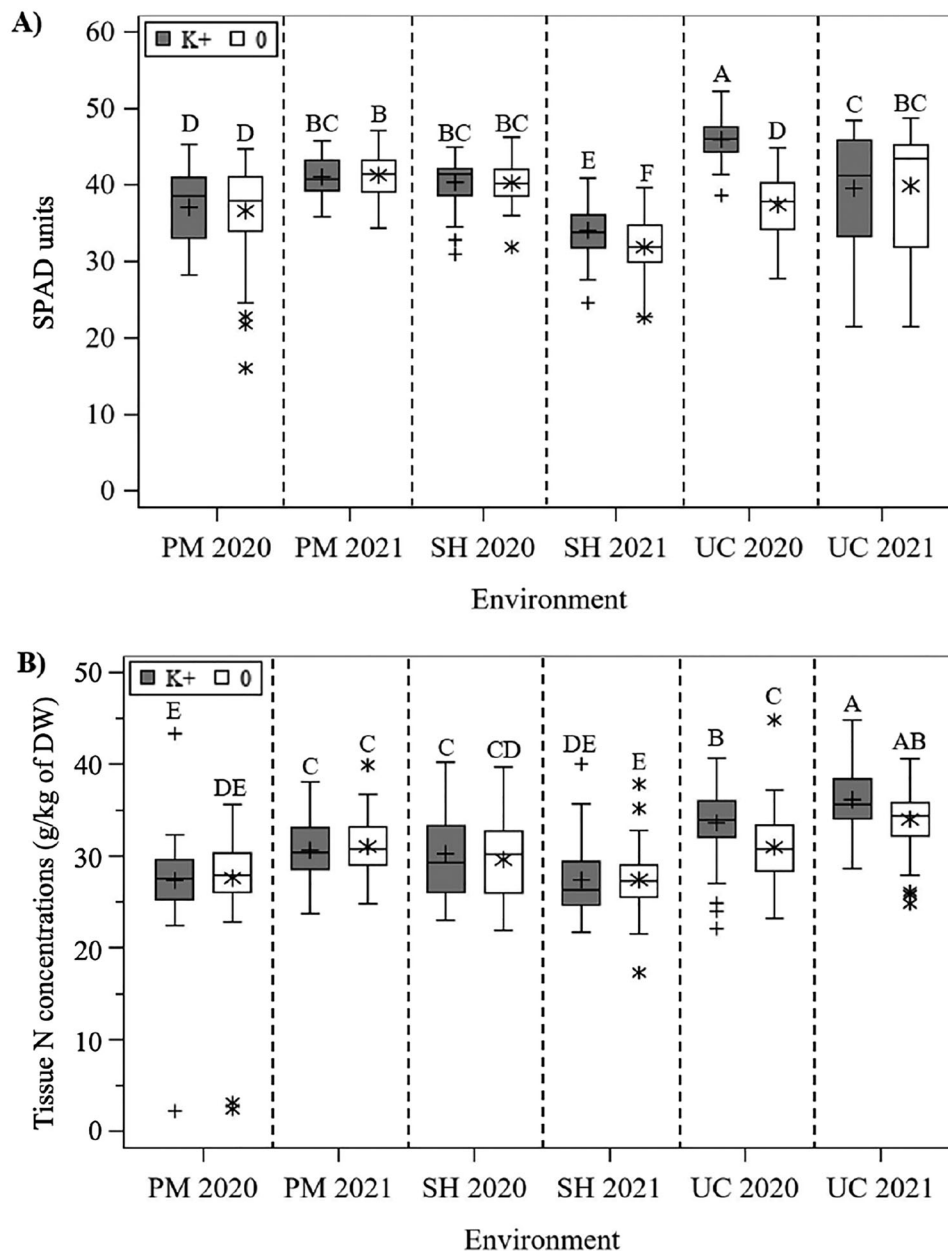


FIGURE 4 *Environment* influence on soybean health indices and growth potential. Box plots representing the significant ($p < 0.0001$) interaction of *potassium* (*environment*) for (A) chlorophyll estimation (soil plant analysis development [SPAD] units) and (B) shoot N concentration data averaged across all other treatment factors, including sampling time point (10 and 16 weeks after planting [WAP]). The X-axis represents each respective *environment*: PM—Piedmont Research Station, SH—Sandhills Research Station, UC—Upper Coastal Plain Research Station, and the year of field trial is denoted by 2020 or 2021. Dark gray boxes indicate the addition of K^+ fertilizer (K^+) and white boxes indicate no additions of K^+ fertilizer (0). (+) or (*) inside the boxes represents the mean, while the external ones represent potential data outliers. Comparisons of the means for significant differences of the treatment combinations occur across all environments. Letters are assigned based on a significance value of $\alpha = 0.05$ with Tukey's honestly significant difference (HSD).

As the six environments influenced soybean yield potential, they also had a significant influence on total seed protein production. As evidenced by the ANOVA (Table 2), higher protein levels were observed in our SH environments that had the lowest yields (Figure 7B). Maturity groups did again affect protein levels, following the same trend as yield with group V having the highest composition of protein, significantly higher

than VI, and group IV shared significance with groups V and VI. Maturity, in combination with the environment, again showed significant differences (Figure 8B).

Oil composition within the harvested seed was also notably impacted by the environment (Table 2). Variation of the oil concentration within the seed composition is smaller compared to the variation in protein, but environmental influence

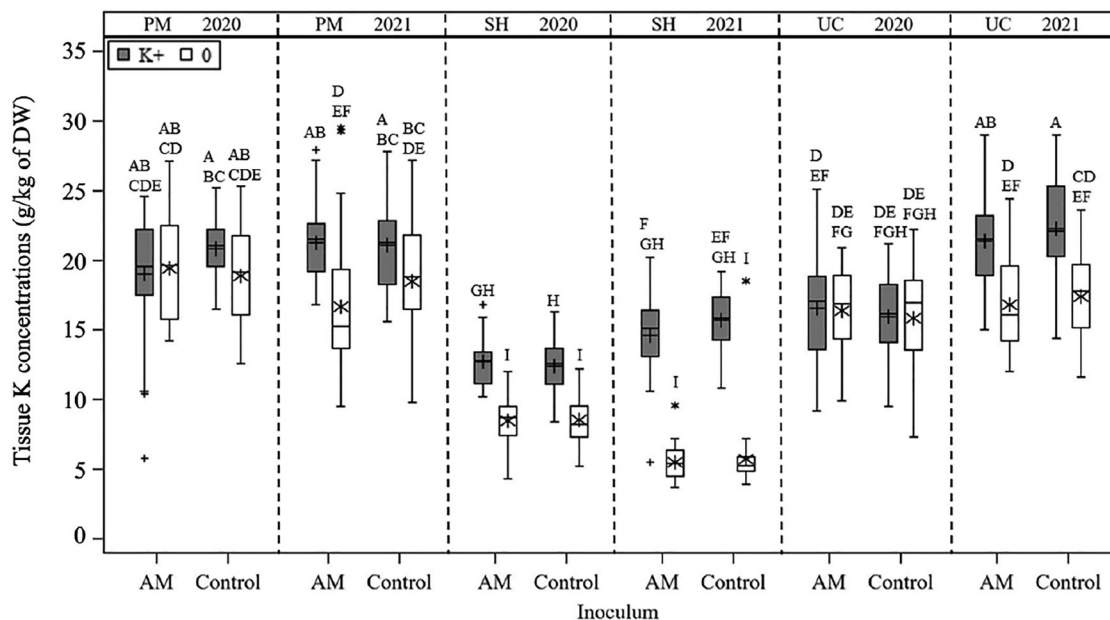


FIGURE 5 *Inoculum* by *environment* influence on percentage of K^+ in aboveground soybean tissue. Box plots representing the significant ($p = 0.0439$) interaction of *potassium* (*environment*) by *inoculum*, in potassium shoot tissue data, are averaged across all other treatment factors, including sampling time point (10 and 16 weeks after planting [WAP]). The upper X-axis represents each respective *environment*: PM—Piedmont Research Station, SH—Sandhills Research Station, UC—Upper Coastal Plain Research Station, and the year of field trial is denoted by 2020 or 2021. The lower X-axis represents either the addition of fungal inoculum (AM) or not (control). Dark gray boxes indicate the addition of K^+ fertilizer (K^+) and white boxes indicate no additions of K^+ fertilizer (0). (+) or (*) inside the boxes represents the mean, while the external ones represent potential data outliers. Comparisons of the means for significant differences of the treatment combinations occur across all environments. Letters are assigned based on a significance value of $\alpha = 0.05$ with Tukey's honestly significant difference (HSD).

is still significant, with variation from responses observed in both yield and protein (Figure 7C). Maturity group shows a definitive difference relative to oil production. Group V had the highest oil percentage, significantly higher than group IV, which was also significantly higher than group VI. The influence of the response of the maturity group by the environment further highlights the rank changes among groups and the exhibited significant responses (Figure 8C).

4 | DISCUSSION

4.1 | Environmental conditions and maturity group set vegetative growth potential in soybean in North Carolina with minimal influence of the AM inoculum

Responses were primarily driven by environmental factors, WAP, and maturity group, with no significant influence from AM inoculum. Biomass production nearly doubled between the 10 and 16 WAP data points (Table S2). These time points were selected based on the typical response of mycorrhizal products. The first sampling was delayed to allow for seed germination, while the second assessed the product's longevity. The 10 WAP sampling coincided with the VX–R1 growth

stage, and 16 WAP aligned with R3–R4, a critical phase for seed development and plant health. Biomass production was consistently lower at the SH location, despite sufficient rainfall and supplemental irrigation, likely due to the soil's limited water-holding capacity. This site is frequently used for drought-tolerant soybean breeding (Lee G. et al., 2021). Additionally, in 2020, nematode pressure in the front portion of the SH field eliminated some plots. In contrast, PM and UC locations showed higher biomass production in 2021 than in 2020 (Figure 3), despite lower rainfall (Figure 2). Site-specific factors influenced these variations. The PM location had a delayed planting in 2020 due to a preceding wheat crop, reducing biomass—a trend consistent with Morris, Vann, Collins, et al. (2021) and Morris, Vann, Heitman, et al. (2021), who reported yield declines in mid-June-planted soybeans due to limited biomass before reproductive development. Both UC and SH locations faced significant deer pressure, which has been shown to negatively impact early-season soybean growth (Begley-Miller & Cady, 2015; Rogerson et al., 2014; VanGorder et al., 2021). In 2020, deer damage at UC required replanting the low-K field area in early June. However, this delay had minimal impact on biomass production, potentially due to soil variation between fertilized and unfertilized sections. Soybean biomass strongly influences nutrient accumulation, with research showing higher nutrient uptake in

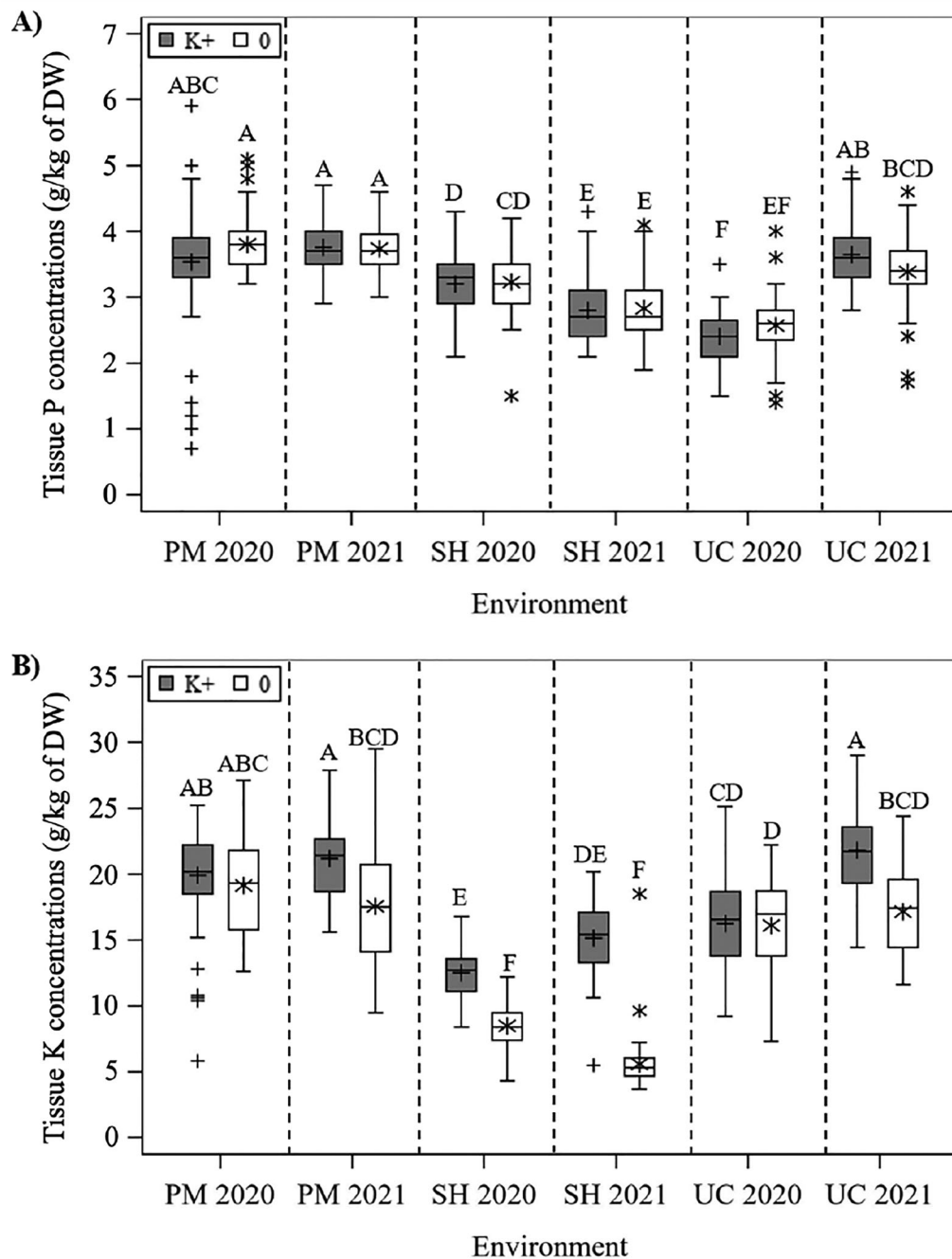


FIGURE 6 *Potassium (environment)* influence of plant tissue P and K concentrations. Box plots representing the significant ($p < 0.0001$) interaction of *potassium (environment)* for shoot P (A) and K (B) concentrations, averaged across all other treatment factors. The X-axis represents each respective environment: PM—Piedmont Research Station, SH—Sandhills Research Station, UC—Upper Coastal Plain Research Station, and the year of field trial is denoted by 2020 or 2021. Dark gray boxes indicate the addition of K^+ fertilizer (K^+) and white boxes indicate no additions of K^+ fertilizer (0). (+) or (*) inside the boxes represents the mean, while the external ones represent potential data outliers. Comparisons of the means for significant differences of the treatment combinations occur across all environments. Letters are assigned based on a significance value of $\alpha = 0.05$ with Tukey's honestly significant difference (HSD).

soybeans compared to corn (Bender et al., 2013; Hanway & Weber, 1971). Biomass production was primarily influenced by environmental factors, WAP, and maturity group, with no

significant effect from AM inoculum, highlighting the importance of site-specific conditions and growth stages in soybean development.

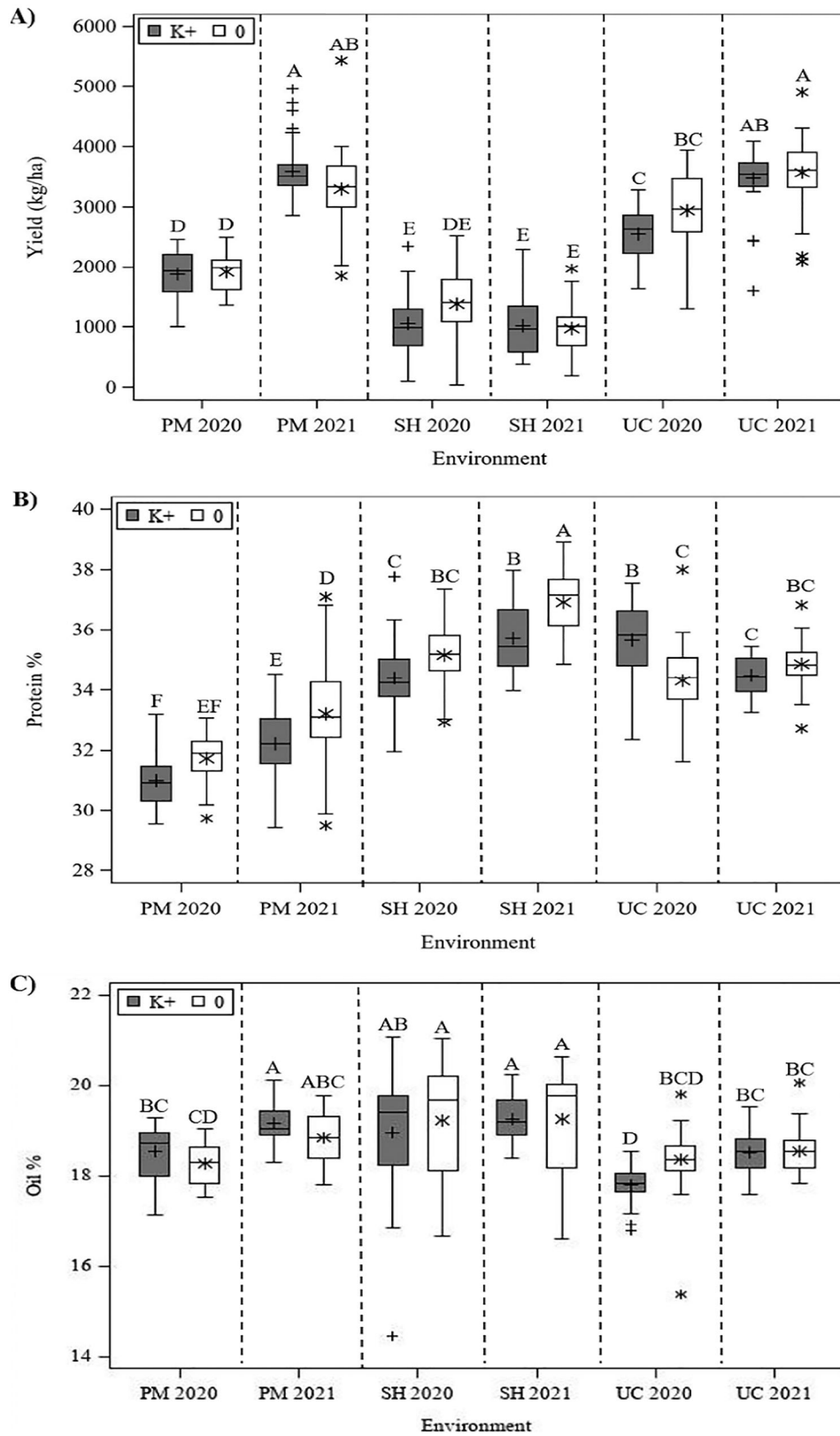


FIGURE 7 Potassium (environment) influences soybean yield, and seed protein and oil content. Box plots representing the significant ($p < 0.0001$) interaction of potassium (environment) for (A) yield (kg ha^{-1}), (B) protein %, and (C) oil %, averaged across all other treatment factors. The X-axis represents each respective environment: PM—Piedmont Research Station, SH—Sandhills Research Station, UC—Upper Coastal Plain Research Station, and the year of field trial is denoted by 2020 or 2021. Dark gray boxes indicate the addition of K^+ fertilizer (K^+) and white boxes indicate no additions of K^+ fertilizer (0). (+) or (*) inside the boxes represents the mean, while the external ones represent potential data outliers.

(Continues)

FIGURE 7 (Continued)

Comparisons of the means for significant differences of the treatment combinations occur across all environments. Letters are assigned based on a significance value of $\alpha = 0.05$ with Tukey's honestly significant difference (HSD).

4.2 | Inoculation and K⁺ fertilization had minimal influence on soybean nutrition and function

In-season tissue nutrient concentrations showed no influence of AM inoculum on N, P, or K⁺ uptake, despite studies in wheat and soybeans reporting positive effects (Al-Karaki et al., 2004; Meena et al., 2015). P levels were sufficient for soybean growth in the southern United States (Campbell & Plank, 2011), while N concentrations at UC in 2020 were lower than recommended, potentially limiting yield (Campbell & Plank, 2011), though other research suggests increased N applications do not always enhance biomass or yield (Burns et al., 2022). K⁺ fertilization influenced tissue concentrations only at SH, where initial soil K⁺ was low (Hardy et al., 2014), indicating a potential cation exchange limitation and K⁺ leaching in sandier soils. Soybean tissue K⁺ concentrations at PM and UC fell within sufficiency ranges (Campbell & Plank, 2011), though whole-plant sampling may have diluted K⁺ levels relative to recommendations based on upper mature leaves. Tillage management may have also played a role, as PM (no-till) differed from UC and SH (tilled). While a no-till soybean study in Illinois found increased tissue K⁺ with fertilization, it also reported limited yield benefits (Farmaha et al., 2011). Tillage can disrupt mycorrhizal networks, affecting AM fungal populations (Kabir et al., 1998), potentially reducing natural AM prevalence and altering plant–microbe interactions, though commercial inoculum could mitigate these effects. Additionally, IV soybeans showed greater early-season vigor with higher estimated chlorophyll content (SPAD values) at 10 WAP, likely due to favorable climate conditions in May/June, while later sampling coincided with increased heat and water stress. Chlorophyll content generally declines after R2 before peaking at R4 and tapering through R6 (Thompson et al., 1996), and water stress can further reduce readings (Ahmed et al., 2010; Schepers et al., 1996). These findings highlight the complex interactions between environmental factors, nutrient management, and plant physiological responses in soybean production. Previous studies using the same AM fungal species contained in the commercial inoculant we used here have reported variable effects on legume performance. For instance, *R. irregularis* inoculation has been shown to enhance biomass and photosynthesis in soybean under drought and high salinity conditions (Begum et al., 2023; Jones et al., 2025), and *F. mosseae* inoculation improved soybean resistance to root rot disease (Yang et al., 2023; Zhang et al., 2020). However, Carrara et al. (2024) found no

yield improvement in black beans inoculated with *F. mosseae*. Our field results also showed no significant improvement in soybean biomass, nutrient concentration, or yield with inoculation, suggesting a limiting impact of this inoculum under the well-managed conditions of North Carolina's production environments. Altogether, our results showed that soybean nutrient uptake and biomass production were primarily influenced by soil properties, fertilization, tillage practices, and environmental conditions, while AM inoculum showed no significant effect, highlighting the complexity of plant–soil interactions in optimizing soybean growth.

4.3 | Additional management strategies were negligible in improving yield potential

Yield achieved across the study was within the yield ranges commonly seen in these counties across North Carolina (USDA National Agricultural Statistics Service & Dee Webb, 2021). The specific yield achieved across the sites varied in their relationship to the county average yields for 2020 and 2021 with some sites being below, at and above the average yields achieved for the counties in those years. Yield variation in relationship to county average yields is driven largely by soybean planting date and soil type of the area. Environmental conditions and maturity group were the primary drivers of soybean yield, with little to no impact from AM inoculum across locations (Figure 8). The PM and UC environments in 2021 produced the highest yields, aligning with North Carolina's state average of 2690 kg ha⁻¹, suggesting that this particular endomycorrhizal inoculant is not a viable management strategy for improving soybean yield in these locations. The lack of yield response to the commercial inoculum could stem from various factors, including competition with native AM fungi (Niwa et al., 2018) or low viability of propagules in the commercial product (Corkidi et al., 2004; Wiseman et al., 2009). Soil fertility also played a role in yield variability. A North Carolina soybean study found no yield response to K⁺ fertilization in the coastal plain, though some response was observed in the Piedmont region (Morales et al., 2022), contrasting with our findings at PM. Similarly, an Iowa study in low-K⁺ soils with sufficient P reported little to no yield response across 14 sites (Boring et al., 2018). Initial soil fertility data (Table S1) suggested minimal response to K⁺ amendments except at SH, yet despite soil pH adjustments with lime to an optimal range of 6.3–6.5, yield improvements were not observed, indicating that an unidentified limiting factor influenced SH's yield potential. Across all locations,

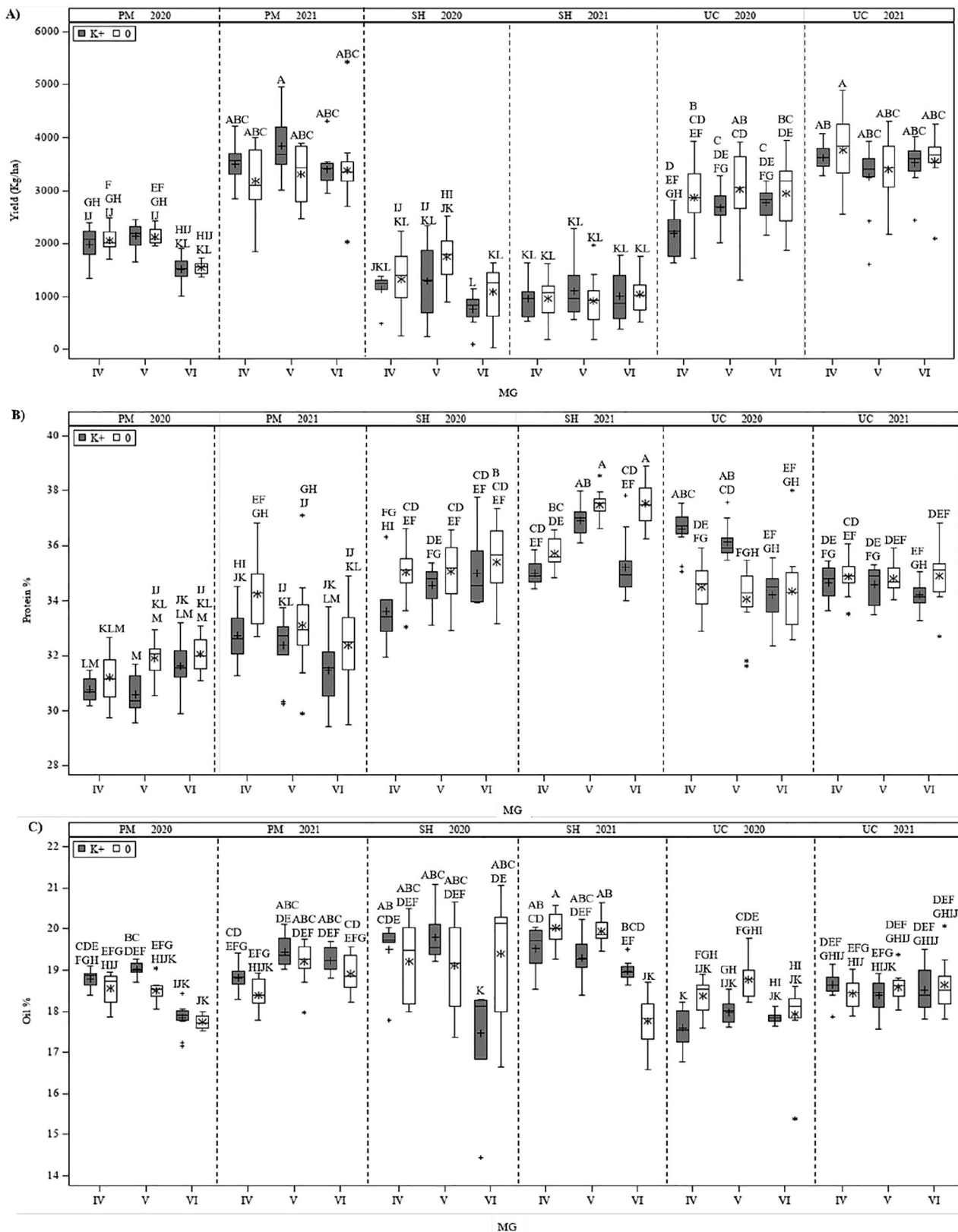


FIGURE 8 Influence of *potassium (environment)* interaction with *maturity group* on yield, and protein and oil content. Box plots representing the significant ($p < 0.0001$) interaction of *potassium (environment)* by *maturity group* for (A) yield (kg ha^{-1}), (B) protein %, and (C) oil %, averaged across all other treatment factors. The upper X-axis represents each respective environment: PM—Piedmont Research Station, SH—Sandhills Research Station, UC—Upper Coastal Plain Research Station, and the year of field trial is denoted by 2020 or 2021. The lower X-axis represents the maturity group for each environment. Dark gray boxes indicate the addition of K^+ fertilizer (K^+) and white boxes indicate no additions of K^+

(Continues)

FIGURE 8 (Continued)

fertilizer (0). (+) or (*) inside the boxes represents the mean, while the external ones represent potential data outliers. Comparisons of the means for significant differences of the treatment combinations occur across all environments. Letters are assigned based on a significance value of $\alpha = 0.05$ with Tukey's honestly significant difference (HSD).

maturity group V outperformed maturity group VI in yield, aligning with findings from Vann et al. (2021), who identified maturity group as the strongest predictor of soybean yield in North Carolina. While AM fungi play a critical role in nutrient acquisition and soil health, their impact on yield is highly variable and influenced by site-specific conditions. Without a consistent yield benefit and based on this study, the use of AM inoculants as a management strategy for soybean production in these North Carolina environments remains unsupported.

4.4 | Additions of K^+ fertilizers resulted in a decrease in seed protein content

Across environments, the amount of seed protein exhibited a negative trend that correlated with the addition of commercial fertilization; the only instance of improvement was utilizing a location with already excessively high fertility levels, warranting no further addition of fertilizer. K^+ fertilization had no influence on protein and oil quality when grown on an Oxisol (Zambiazzi et al., 2014). Comparatively, Burns et al. (2022) found N fertilization positively influenced protein content at their PM location. Protein content did vary across environments. The environment with the lowest yield, consequently, also had the highest protein content. Comparatively, oil content was also more pronounced at the lower yielding environment, but the variability across locations was reduced. These results are interesting as typically soybeans exhibit an inverse relationship with protein and oil, in which a 1% decrease in oil typically yields a 2% increase in protein (Clemente & Cahoon, 2009). This inverse relationship is more evidenced when accounting for the significant influence of maturity groups on protein and oil (Table 2; Figure 8). In which, maturity group VI produces the highest protein content but significantly lower oil content at the SH location. Comparatively, at the PM location, maturity group performance rankings differ between the 2 years, and UC had minimal differences beyond those driven by soil difference in 2020. Another North Carolina study looking across differing maturity groups found that later groups (V and VI) had significantly higher protein content and lower oil content across all environments, and quality continued to drop with later planting dates (Morris, Vann, Collins, et al., 2021), which correlates to our lower overall protein observed at PM in 2020.

5 | CONCLUSION

Despite being central to our objectives, the AM fungal inoculum produced negligible observable responses overall, with only modest effects at the SH location. This outcome highlights a critical consideration for producers: the effectiveness of inoculants remains highly variable. While some studies have demonstrated yield improvements in crops like soybean and cotton, particularly in low-productivity environments, a growing body of field-based research reports inconsistent or even negative results (Koziol et al., 2024; Leifheit et al., 2014; Salomon et al., 2022a, 2022b). Environmental conditions—especially soil texture, fertility, water-holding capacity, pH, and microbial community interactions—strongly influence outcomes. In our case, chosen sites may not have been K^+ deficient enough, contributing to the lack of response, yet it accurately reflects current production conditions in North Carolina. These findings underscore the importance of site-specific recommendations. Inoculant use should be guided by localized soil and fertility profiles to ensure economic viability and avoid generalized assumptions about efficacy.

AUTHOR CONTRIBUTIONS

Danielle R. Cooney: Conceptualization; data curation; formal analysis; investigation; methodology; writing—original draft. **Arjun Kafle:** Conceptualization; formal analysis; methodology; writing—review and editing. **Carl Crozier:** Methodology; writing—review and editing. **David Suchoff:** Data curation; writing—review and editing. **Luciano C. Gatiboni:** Writing—review and editing. **Rachel A. Vann:** Funding acquisition; writing—review and editing. **Kevin Garcia:** Conceptualization; funding acquisition; investigation; supervision; writing—original draft; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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