

Breeding early-generation sorghum: integrating agronomic stress resilience, parent-progeny predictability, and the rhizosphere microbiome

by

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B.A., Washburn University, 2024

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Agronomy
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2026

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Abstract

Selection strategies for sorghum [*Sorghum bicolor* (L.) Moench] adapted to low-input systems require understanding quantitative traits' stability, stress response, and cross-generation predictability. This study evaluated F₂ and F₃ populations under conventional and low-input management to quantify trait responses, assess parent-progeny predictability, and identify resilient populations using a rank summation index (RSI). Twenty-nine F₂ populations were evaluated in summer 2024, and the selected eight were advanced to F₃ field testing in 2025. Low-input management imposed stronger stress in summer 2025, resulting in 26 to 68% reductions in seeds per panicle and 11 to 61% in total seed weight. In contrast, 100-seed weight increased in eight of ten populations, indicating compensatory allocation. Parent-progeny regressions had the strongest cross-generation predictability for plant height ($R^2 = 0.52$ and 0.51 under conventional and low-input management, respectively), moderate predictability for 100-seed weight ($R^2 = 0.32$ and 0.44), and weak relationships for flowering time, seeds per panicle, and total seed weight. Rank summation index (RSI) analysis identified GB07118/BTx2921 (P1) and GR04104/RTx2939 (P8) as the most resilient populations, while Check 2 (Macia) had greater sensitivity to stress. These results support direct selection under low-input environments and multi-trait ranking to improve selection for stress-resilient sorghum.

Understanding rhizosphere microbial assembly in field conditions is critical for harnessing microbiome-associated traits in sorghum breeding, particularly given proposed links between AMF and strigolactone signaling through the LOW GERMINATION STIMULANT 1 (*lgs1*) locus. A field study used phospholipid fatty acid analysis (PLFA) and neutral lipid fatty acid analysis (NLFA) profiling and root colonization assessment across V5 and reproductive stages in 131 sorghum (*Sorghum bicolor*) plants, spanning eight F_{2:3} breeding populations, two

checks (85P58 and Macia), and an LGS1-characterized genotype panel. Developmental stage was the dominant driver of rhizosphere community structure (PERMANOVA: $R^2 = 0.063$, $p = 0.002$), with the fungal-to-bacterial ratio and AMF NLFA abundance increasing from V5 to reproductive growth. Contrary to expectations under strigolactone-mediated AMF recruitment, *lgs1* loss-of-function genotypes had higher AMF NLFA abundance than LGS1-functional genotypes, though this could not be isolated from genetic background. Arbuscular mycorrhizal fungi NLFA and root colonization percentage were uncorrelated overall but significantly negatively correlated within LGS1-functional genotypes alone ($R = -0.57$, $p = 0.0021$), indicating these were not interchangeable AMF metrics. The functional RTx430 NIL produced roughly three-fold greater grain yield and higher tissue potassium than its *lgs1* counterpart ($n = 2$ /genotype, exploratory). AMF colonization had weak, largely non-significant associations with plant performance. Developmental stage was the primary driver of sorghum rhizosphere assembly and genotype-dependent AMF-LGS1 relationships need confirmation in replicated trials.

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Acknowledgements

I would like to thank my co-advisors, Dr. Ramasamy Perumal and Dr. Terry Felderhoff, for their mentorship throughout this project. Their guidance in sorghum breeding, genetics, and field-based research design shaped both the direction of this work and my growth as a scientist. They gave me the space to ask questions, make mistakes, and find my own footing as a researcher, and I am grateful for their patience and trust in me and this project. I also thank my committee member, Dr. Charles Rice, whose mentorship in soil microbiology opened an entirely new way of thinking about this system for me, and whose insight into rhizosphere microbial dynamics became one of the most rewarding parts of this thesis.

This work was made possible through the support of NIFA–USDA, through the project "Climate-smart organic sorghum partnership for grain yield and silage production," and I am thankful for the collaboration of Dr. William Rooney and Dr. Nithya Rajan at Texas A&M University, whose partnership strengthened the low-input project in ways that extended well beyond Kansas. I also thank the KSU Soil Testing Laboratory and the Soil Agroecology Laboratory for their support throughout this work.

To the members of Dr. Felderhoff's lab who spent long, hot days with me out in the low-input field: Assistant Scientists Robby McGowan and Dennis Hitz, thank you for your help with weeding and note-taking, and for the company that made those days pass faster. To my fellow graduate research assistants and post-doctoral researchers, Sabreena Parray, Neethu Francis, and Portia Osei Obeng, thank you for being there with me these past two years, through every long field day and every setback. You became so more than lab mates; you became my best friends, and I could not have done this without you.

I owe a special thank you to Dr. Rice's lab. Priscila Marchioro, thank you for patiently training a plant breeder in soil protocols and for your generosity with your time whenever I had questions. Endy Kailer, thank you for being such a genuine mentor to me in soil microbiology, for teaching me how to appreciate and understand AMF, and for training me in colonization assessment. Your guidance shaped so much how I came to understand and truly love this work.

Finally, to my husband, Zachary Stephens: thank you for your unconditional love and support throughout this journey. You have been my steady place through every long field season, every late night of writing, and every moment of doubt along the way. This thesis carries a piece of your patience and encouragement in every page, and I could not have reached this milestone without you by my side.

Dedication

I dedicate this dissertation to my family. To my parents, Debbie and Shalem Arazi. To my husband, Zachary Stephens. And to my cats, Miso and Mochi.

Chapter 1 - Introduction

Global food systems face pressure to produce sufficient grain while reducing dependence on synthetic fertilizers, herbicides, and other chemical inputs that have defined traditional agriculture since the mid-twentieth century (Tilman et al., 2002). Concerns over environmental sustainability, input costs, soil health, and consumer demand for organically produced food have expanded organic and low-input farming systems across the United States and globally (Kremen and Miles, 2012). In the United States interest in reduced-input production extends farther than certified organic systems to include a broader category of low-input management practices that reduce synthetic inputs without carrying formal organic certification. These systems share a common theme: crops must produce acceptable yields under nitrogen limitation, without synthetic herbicide protection against weed competition, and under the environmental variability that characterizes production outside the controlled conditions of high-input agriculture.

Meeting this challenge requires crop cultivars specifically adapted to low-input production conditions. Yet many commercially available cereal cultivars, including sorghum hybrids, were developed and selected under conventional, high-input management systems that do not represent the selective pressures of organic or low-input environments (Lammerts van Bueren et al., 2011). This mismatch between the environment in which cultivars are bred and the environment in which they are grown has consequences for both producers and breeders. For producers, it means that cultivar selection under low-input management is largely empirical and unreliable, because performance rankings established under conventional conditions do not always hold when inputs are reduced. For breeders, it raises fundamental questions about selection strategy: where should selection be conducted, which traits should be prioritized, and how reliably do early-generation decisions predict long-term performance under low-input stress.

Sorghum [*Sorghum bicolor* (L.) Moench] occupies an important position within this context. Its tolerance to drought, heat, and low-fertility soils makes it one of the most stress-resilient cereal crops, and its relatively low water and nitrogen requirements compared to maize make it a logical candidate for low-input production systems across the semi-arid Great Plains and other resource-limited environments (Rooney, 2004; Blum, 2011; Tuinstra et al., 1997). Despite this inherent stress tolerance, the genetic improvement of sorghum for organic and low-input systems remains limited. Most breeding efforts have been directed toward maximizing yield potential under conventional management, leaving the genetic variation relevant to low-input adaptation largely unexplored in breeding programs (Boyles et al., 2022; Muleta et al., 2018).

Addressing this gap requires understanding several dimensions of the problem. First, it requires characterizing how low-input stress affects trait expression in sorghum, and which traits are most sensitive to the combined stressors of nitrogen limitation and weed competition. Second, it requires evaluating whether selection in early segregating generations predicts performance in later generations under contrasting management systems. This is a question of cross-generation predictability that determines whether early selection decisions made under conventional conditions can identify populations adapted to low-input stress. Third, it requires identifying selection strategies that efficiently integrate multi-trait information to distinguish resilient populations from those whose performance under stress reflects compensatory responses in individual traits rather than balanced adaptation across traits.

This literature review examines the scientific foundation underlying each of these dimensions. It begins with the agronomic context of sorghum in low-input and organic production systems, proceeds through the biological challenges imposed by low-input

environments and the breeding frameworks developed to address them, examines trait responses to stress and their implications for selection, and concludes with the quantitative genetic basis of parent-progeny predictability and its consequences for early-generation selection efficiency. Together, these sections establish the conceptual and empirical foundation for the research presented in the following chapter.

Sorghum in low-input and organic production systems

Sorghum [*Sorghum bicolor* (L.) Moench] ranks fifth globally in cereal production area behind wheat, maize, rice, and barley (FAO, 2023). Its C4 photosynthetic pathway, deep root architecture, capacity for osmotic adjustment under water deficit, and ability to enter dormancy during severe stress and resume growth when conditions improve allow it to produce grain under environmental constraints that significantly reduce productivity in other cereal species (Tuinstra et al., 1997; Rooney, 2004; Blum, 2011). These characteristics have made sorghum a primary cereal crop across the semi-arid tropics, sub-Saharan Africa, and the Great Plains of the United States. (Enyew et al., 2021; Lamb et al., 2021; Carcedo et al., 2022).

Demand for organic cereal grain products has grown in recent decades, driven by expanding food and feed markets, yet cultivar development for organic sorghum production has fallen behind this demand (Boyles et al., 2022). This reflects a broader pattern across crop species in which more than 95% of organic production relies on cultivars bred for conventional systems and consequently lacking traits relevant to organic or low-input production conditions (Lammerts van Bueren et al., 2011). Most commercially available sorghum hybrids were selected under conventional management characterized by high synthetic nitrogen inputs, pre- and post-emergence herbicide applications, and mostly uniform agronomic environments. When evaluated under organic management, genotype rankings can shift as G×M interactions emerge

from changes in selective environments (Finlay and Wilkinson, 1963; Atlin et al., 2000; Murphy et al., 2007). Traits that demonstrate yield advantage under high nitrogen availability may provide little benefit under nitrogen limitation and weed pressure, where competitive ability, nutrient use efficiency (NUE), and stress tolerance become primary determinants of performance (Ceccarelli, 1994; Wolfe et al., 2008). Boyles et al. (2022) evaluated a broad range of sorghum hybrids, advanced breeding lines, and diverse accessions under certified organic management across multiple years and locations, identifying canopy closure, sugarcane aphid tolerance, and grain mold resistance as traits positively correlated with grain yield under organic conditions. Improving sorghum for low-input systems requires deliberate alignment of breeding objectives and selection environments with the challenges of the target production system (Ceccarelli, 1994; Rattunde et al., 2013). While the terms organic and low-input are sometimes used interchangeably in the agronomic literature, they are not equivalent. Certified organic production systems operate under strict regulatory frameworks that generally restrict synthetic inputs and prohibit most conventional synthetic fertilizers, herbicides, and pesticides, while permitting specific restricted synthetic substances and designated natural inputs authorized under national regulatory standards (Benbrook et al., 2021). Low-input systems represent a broader category of reduced-input management that may incorporate organically approved inputs (such as fish-based fertilizer emulsions and mechanical weed control) without carrying formal organic certification. Biological stressors imposed under both systems, however, are functionally analogous: nitrogen availability is restricted relative to conventional management, weed pressure is managed without synthetic herbicides, and genotypes must maintain productivity under reduced resource availability. The research reviewed in the sections that follow draws primarily from the organic breeding literature, where much of the foundational work on low-input adaptation has been

conducted, while recognizing that its principles apply broadly to production systems in which synthetic inputs are reduced or eliminated.

Challenges of low-input production environments

Low-input and organic production systems impose a distinct set of stressors that differ from the single-factor stress conditions most studied in crop physiology research. Nitrogen limitation, weed competition, and temporal variability in nutrient release occur simultaneously and interact in ways that complicate both crop management and breeding decisions (Jordan, 1993; Biradar et al., 2024). Understanding each of these stressors independently, and their combined effects on crop performance, is necessary for designing breeding programs that target meaningful adaptation to low-input environments.

Nitrogen is the most frequently limiting nutrient in agricultural soils and a primary determinant of grain yield in cereal crops (Biradar et al., 2024). In conventional production, synthetic nitrogen fertilizers are applied at rates sufficient to meet crop demand throughout the growing season (Snyder, 2017). In organic and low-input systems, nitrogen is supplied through organic amendments, including compost, manure, and fish-based fertilizer emulsions, which release nitrogen through microbial mineralization at rates governed by soil temperature, moisture, and biological activity rather than by agronomic timing (Jordan, 1993). This introduces temporal unpredictability in nitrogen availability that can result in deficits during critical periods of crop development even when total seasonal nitrogen inputs are adequate.

In sorghum, nitrogen availability strongly influences reproductive development. Floret initiation and grain set occur during a narrow window that coincides with peak nitrogen demand, and reductions in nitrogen supply during this period disproportionately reduce seed number relative to other yield components (Craufurd and Peacock, 1993; Sadras, 2007). Tari et al. (2022)

demonstrated across a diverse sorghum association panel that grain yield exhibited substantial genotype-specific responses to long-term nitrogen deficit stress, while many morphological and physiological traits remained stable across nitrogen regimes. This differential sensitivity (reproductive traits responding more strongly to nitrogen stress than structural traits) has direct implications for early-generation selection, as it suggests that yield component performance under nitrogen limitation is both highly variable among genotypes and difficult to predict from performance under well-fertilized conditions. Nitrogen use efficiency in sorghum varies widely among genotypes, with significant variation documented for nitrogen uptake, assimilation, and partitioning to reproductive structures under reduced nitrogen regimes (Patil et al., 2021). This genetic variation represents both a challenge and an opportunity for breeding programs targeting low-input systems: identifying genotypes that maintain grain set under nitrogen limitation, and selecting for heritable differences in nitrogen use efficiency that translate to stable yield under organic management.

Weed competition represents a second major stressor in low-input systems and one that interacts directly with nitrogen limitation to amplify crop stress. In conventional production, pre- and post-emergence herbicide applications suppress weed populations early in the season, reducing competition for light, water, and nutrients during the critical establishment period. Without herbicide inputs, weed populations can develop rapidly and impose yield penalties through resource competition that compounds nutrient deficiency already present under low-fertility management (Jordan, 1993; Dille et al., 2020).

Redroot pigweed (*Amaranthus retroflexus* L.) is among the most competitive and widely distributed annual weeds in grain sorghum production across the central United States (Knezevic et al., 1997; Dille et al., 2020). As a C₄ species, pigweed competes directly with sorghum for

light and resources with high physiological efficiency, and its rapid growth rate (often exceeding 2 to 6.4 cm per day during peak development) relative to many crop species allows it to establish competitive dominance when early-season weed control is insufficient (Knezevic et al., 1997). The timing of pigweed emergence relative to crop emergence is particularly critical. Early emerging pigweed imposes significantly greater yield penalties because competition during the vegetative establishment phase restricts canopy development and reduces the crop's ability to suppress subsequent weed flushes (Knezevic et al., 1997). Dille et al. (2020) estimated potential yield losses in grain sorghum from weed interference across U.S. production environments, documenting losses that vary with weed species composition, density, and timing of emergence. Under organic management, where mechanical cultivation and hand weeding are the primary control methods, achieving the level of early-season weed suppression needed to prevent significant yield loss is difficult and inconsistent between years, contributing to year-to-year variability in the severity of low-input stress.

Crop competitive ability against weeds is a trait of practical breeding value in low-input systems, though it has received limited attention in conventional sorghum improvement programs (Boyles et al., 2022; Peerzada et al., 2017). Traits associated with competitive ability, including early canopy closure, rapid early-season growth, and leaf area development, reduce the light available to establishing weed seedlings and can suppress weed biomass accumulation without herbicide inputs (Peerzada et al., 2017). These traits are not routinely measured or selected in conventional breeding nurseries where herbicide programs eliminate weed competition before it affects crop performance.

The simultaneous occurrence of nitrogen limitation and weed competition in low-input systems creates a stressful environment that is qualitatively different from either stressor applied

in isolation. Weed competition reduces the effective nitrogen available to the crop by diverting soil nitrogen into weed biomass, effectively intensifying nitrogen stress beyond what fertility management alone would impose (Jordan, 1993). This interaction means that yield responses to low-input management cannot be predicted by summing independent responses to nitrogen stress and weed competition. The combined effect is context-dependent and varies among genotypes based on their capacity to acquire nitrogen efficiently and compete effectively for light and space simultaneously.

This complexity is captured analytically through genotype $\times$ environment ($G \times E$), genotype $\times$ management ($G \times M$), and genotype $\times$ environment $\times$ management ($G \times E \times M$) interactions, which describe the degree to which genotype performance rankings change across environments and management systems (Finlay and Wilkinson, 1963; Cooper et al., 1995; Messina et al., 2009). When $G \times M$ interactions are large, selection decisions made under conventional management provide unreliable guidance for identifying genotypes adapted to low-input conditions, because the genotypes that perform best under high inputs are not necessarily those that perform best when inputs are reduced (Atlin et al., 2000). Ceccarelli (1994) demonstrated that realized genetic gain from selection conducted outside the target population of environments is reduced when $G \times E$ interactions are present, and that this reduction is proportional to the genetic correlation between performance in the selection environment and the target environment. When management systems differ substantially, this genetic correlation can be low enough that indirect selection under conventional conditions contributes negligible genetic gain for low-input adaptation. Messina et al. (2009) and Annicchiarico et al. (2019) further showed that $G \times E \times M$ interactions compound this problem by introducing additional unpredictability when both environment and management vary, as is the case across years and

locations in organic production systems. These interactions reflect biological differences in how genotypes respond to the selective pressures imposed by contrasting management systems, and they have direct practical consequences for how breeding programs should be structured when the target production system differs from the conventional nursery environment.

Breeding frameworks for low-input adaptation

The recognition that conventional selection environments may not represent low-input production conditions has motivated the development of breeding frameworks that explicitly account for the target environment in selection decisions. These frameworks capture a foundational principle in quantitative genetics: genetic gain from selection depends not only on the heritability of the target trait and the intensity of selection applied, but also on the genetic correlation between performance in the selection environment and performance in the target environment (Falconer and Mackay, 1996). When this correlation is high, indirect selection in a convenient or controlled environment can be as effective as direct selection in the target environment. When it is low (as occurs when $G \times M$ interactions are large) indirect selection loses efficiency and direct selection within the target environment becomes necessary to capture relevant adaptive variation (Ceccarelli, 1994; Atlin et al., 2000).

The distinction between direct and indirect selection is central to debates about how organic and low-input breeding programs should be structured. Indirect selection refers to selection conducted in an environment different from the intended production system. In the context of organic breeding, this typically means selecting under conventional management with the assumption that performance rankings will be maintained when varieties are grown organically. Direct selection refers to evaluation and selection within the target environment itself. Where traits are highly heritable and genetically stable across environments, indirect

selection can be efficient and logistically advantageous, allowing breeders to evaluate large numbers of lines under controlled conditions before committing resources to more complex low-input trials (Wolfe et al., 2008). However, where traits exhibit strong $G \times E$ or $G \times M$ interactions (as yield components often do), indirect selection fails to identify genotypes carrying alleles that confer advantage specifically under low-input conditions (Ceccarelli, 1994; Murphy et al., 2007). Empirical evidence across cereal crops supports the superiority of direct selection for low-input adaptation. Przystalski et al. (2008) documented poor prediction in variety rankings between organic and conventional management systems across multiple European countries and cereal species, concluding that varieties selected under conventional conditions cannot be reliably assumed to perform well under organic management. Rivière et al. (2019) further demonstrated that breeding programs targeting low-input environments must include low-input selection environments to maximize genetic gains, and that the inefficiency of indirect selection becomes more pronounced as the contrast between conventional and low-input management intensifies. These findings collectively support the argument that selection for organic or low-input adaptation should be conducted, at least in part, within environments representative of the target production system rather than relying exclusively on conventional performance.

Organic breeding frameworks have formalized the principle of selection within the target environment through what Ceccarelli and Grando (2007) termed decentralized-participatory plant breeding, in which breeding materials are evaluated and selected within the diverse environments for which they are intended rather than in centralized, controlled nurseries. This approach recognizes that organic production environments are inherently heterogeneous (varying in soil fertility, weed community composition, and microclimate) and that adaptation to this heterogeneity requires evaluation under representative conditions rather than in uniform

experimental settings. Ceccarelli (1994) established the conceptual foundation for this argument by demonstrating that specific adaptation to marginal environments (including low-input and stress-prone systems) is a legitimate and achievable breeding target, and that selecting for broad adaptability under favorable conditions excludes genotypes carrying alleles for stress-specific performance.

Lammerts van Bueren et al. (2011) identified the traits most relevant to organic production as those associated with nutrient use efficiency, early competitive ability against weeds, disease and pest resistance without chemical protection, and yield stability across variable environments. These traits are not mutually exclusive with conventional breeding objectives, but their relative importance shifts substantially when synthetic inputs are removed. Murphy et al. (2005) described an evolutionary-participatory breeding method for inbred cereal grains specifically designed for organic and low-input farming systems, emphasizing the importance of involving end-users in selection decisions and of evaluating materials under production-relevant conditions from early generations onward. Wolfe et al. (2008) reviewed the state of organic cereal breeding in Europe and concluded that the wide range of environments within organic agriculture, combined with substantial year-to-year variability, underscores the need to select for specific adaptation in target environments rather than relying on broad adaptability criteria developed under conventional management.

In contrast to yield components, phenological traits such as flowering time and structural traits such as plant height exhibit more limited sensitivity to low-input management stress. Flowering time in sorghum is regulated primarily by photoperiod and temperature signals rather than by resource availability and is largely buffered against the nitrogen limitation and weed competition that drive reproductive trait responses (Craufurd et al., 1999). Plant height reflects

cumulative biomass accumulation over a long vegetative growth period and is determined by both genetic factors (including dwarfing gene composition) and environmental factors that influence overall growth rate. While plant height can be reduced under severe resource limitation, the effect is typically smaller in magnitude and less variable among genotypes than the effects observed for seed number and seed weight (Blum, 2011).

The relative stability of phenological and structural traits under stress has important implications for early-generation selection. Traits with high cross-environment stability are more reliably expressed across management systems, making them more useful as screening criteria in early generations where replication is limited and environmental precision is lower. Sadras and Slafer (2012) demonstrated across multiple cereal species that yield components exhibit lower heritability than structural traits across variable environments, reflecting a hierarchy of phenotypic plasticities in which reproductive traits are more environmentally modulated than morphological ones. This hierarchy suggests that early-generation selection for structural traits such as plant height can efficiently eliminate phenologically or architecturally unsuitable populations before more resource-intensive evaluation of yield components under low-input conditions is warranted.

A consistent observation under stress conditions that reduce seed number is a compensatory increase in individual seed weight, reflecting the source-sink dynamics that govern assimilate distribution within the plant. Source-sink theory describes the relationship between the supply of photoassimilates produced by photosynthetic tissues (the source) and the competing demands of developing seeds for those assimilates (the sinks) (Gambín and Borrás, 2009; Sinclair and Jamieson, 2006). When grain number is reduced under stress, fewer sinks compete for the available assimilate supply, and individual seeds receive a greater share of the total

photoassimilate pool. The result is an increase in individual seed weight that partially, but incompletely, offsets the reduction in total reproductive output caused by lower grain number. This compensatory response has been documented across multiple cereal species. Borrás et al. (2004) demonstrated through source-sink manipulation experiments in wheat, maize, and soybean that individual seed weight increases when seed number is reduced, but that these increases do not fully compensate for the loss of total yield associated with reduced grain number. The quantitative reappraisal presented by Borrás et al. (2004) confirmed that total reproductive output under stress is constrained primarily by sink number rather than individual sink capacity, consistent with earlier frameworks establishing grain number as the dominant yield determinant in cereals (Fischer, 2008; Sinclair and Jamieson, 2006). Ordóñez et al. (2018) extended this finding to maize across a wide range of field conditions, demonstrating that grain weight sensitivity to source-sink manipulations is consistent and predictable across environments, further supporting the primacy of grain number in determining total yield under stress.

In the context of low-input breeding, the source-sink framework has a practical implication that is easily overlooked: an increase in individual seed weight under stress is not evidence of stress tolerance. A genotype that maintains individual seed weight while experiencing severe reductions in seed number has not maintained total reproductive output. It has simply redistributed a smaller assimilate pool across fewer seeds. Selecting for individual seed weight in isolation under low-input conditions therefore risks identifying genotypes that are among the most stressed rather than the most resilient, because the largest compensatory increases in individual seed weight tend to occur in genotypes with the greatest reductions in grain number (Gambín and Borrás, 2009; Sadras, 2007).

Parent-progeny regression and cross-generation selection

Effective early-generation selection depends not only on understanding how traits respond to stress within a generation, but on whether that response is consistent and predictable across generations. If the phenotypic performance of a population in one generation reliably predicts its performance in the next, breeders can make advancement decisions with confidence that selected populations will retain their relative rankings as they progress through the breeding pipeline. If predictability is weak, early selection decisions may advance populations that fail to maintain their apparent advantage in later generations, reducing the efficiency of the breeding program regardless of how well the selection environment represents the target production system.

The quantitative genetic framework for assessing cross-generation predictability centers on narrow-sense heritability, defined as the proportion of total phenotypic variance attributable to additive genetic effects (Falconer and Mackay, 1996). Narrow-sense heritability determines the degree to which phenotypic differences among individuals or populations in one generation are transmitted to the next through additive gene action and governs the expected response to selection under a given selection intensity. Traits with high narrow-sense heritability respond predictably to selection because additive genetic effects dominate phenotypic expression, while traits with low heritability are more strongly influenced by non-additive genetic effects (such as dominance or epistasis) and environmental variation, reducing the reliability of selection decisions based on observed phenotype.

Parent-progeny regression provides a foundational method for estimating realized narrow-sense heritability directly from observed phenotypic data across generations (Smith and Kinman, 1965; Fernandez and Miller, 1985). In its simplest form, the regression of mean

progeny performance on mean parent performance yields a slope estimate that approximates heritability under the environmental conditions in which both generations were evaluated. Strong positive relationships between parent and progeny means indicate stable transmission of trait rankings across generations, while weak or inconsistent relationships suggest that environmental modulation of phenotypic expression is large relative to additive genetic effects. Hill and Mackay (2004) and Hill et al. (2008) established that for complex quantitative traits in outbreeding and inbreeding species alike, additive genetic variance is the primary heritable component, but that the expression of this variance, and therefore the reliability of parent-progeny prediction, is sensitive to the environments in which parents and progenies are evaluated.

A critical assumption of parent-progeny regression as an estimator of heritability is that the environments experienced by parents and progenies are independent, that is, that environmental effects in one generation do not covary with environmental effects in the next (Falconer and Mackay, 1996; Vogel et al., 1980). When parents and progenies are evaluated in the same location or under similar management conditions, environmental covariances can inflate apparent heritability by introducing a positive correlation between parent and progeny performance that reflects shared environmental effects rather than additive genetic transmission. Casler (1982) demonstrated that $G \times E$ interactions between parent and progeny evaluation environments introduce bias into parent-progeny regression estimates, with the direction and magnitude of bias depending on the nature of the $G \times E$ interaction. When the evaluation environments differ substantially between generations (as they do in multi-year studies where seasonal conditions vary), $G \times E$ interactions can reduce apparent heritability below its true value

by introducing environmental noise that obscures the genetic signal in parent-progeny relationships.

The reliability of parent-progeny prediction is not a fixed property of a trait but a context-dependent quantity that varies with the environments in which successive generations are evaluated. Under stable, controlled environments where environmental variance is small relative to genetic variance, parent-progeny relationships are strong and selection is efficient. Under variable or stressful environments where environmental variance is large, the signal-to-noise ratio for detecting genetic differences between populations diminishes, and phenotypic rankings become less stable across generations (Finlay and Wilkinson, 1963; Falconer and Mackay, 1996). This expectation has been confirmed empirically across multiple cereal species. Vanniarajan and Ramalingam (2011) reported weak and inconsistent parent-progeny relationships for yield components in F_2 and F_3 rice populations evaluated under variable environments, concluding that early-generation performance for reproductive traits was a poor predictor of later performance. Vogel et al. (1980) documented inflated heritability estimates by environmental covariances in Indiangrass parent-progeny regressions, showing that apparent heritability can be distorted when evaluation environments are not truly independent across generations.

In the context of organic and low-input breeding, these considerations have direct practical consequences. When management systems differ between generations (as when F_2 populations are evaluated under conventional management and F_3 populations are evaluated under low-input conditions), two sources of unpredictability are introduced simultaneously: year-to-year environmental variation and management-driven changes in the selective environment. The combination of these two sources of variation can reduce cross-generation predictability for

traits that are sensitive to both environmental and management effects, even when the same populations are evaluated in both generations. Under such conditions, parent-progeny relationships for yield components may approach zero, meaning that early-generation performance under conventional management provides essentially no information about later performance under low-input stress.

Not all traits are equally affected by environmental modulation of phenotypic expression. Quantitative genetics theory predicts that traits with simpler genetic architecture, higher narrow-sense heritability, and lower sensitivity to G×E interactions will exhibit more stable parent-progeny relationships across environments than traits with complex genetic control and strong environmental sensitivity (Falconer and Mackay, 1996; Hill et al., 2008). Empirical evidence across cereal crops is consistent with this prediction. Sadras and Slafer (2012) showed that across wheat, maize, sunflower, and soybean, yield components exhibit lower heritability and greater phenotypic plasticity than structural traits, reflecting a hierarchy of environmental sensitivity in which reproductive traits are the most modulated by environmental conditions and morphological traits the least.

Plant height in sorghum exemplifies a trait with relatively high heritability and low environmental sensitivity. Determined in large part by the number and identity of dwarfing genes (specifically the *dw1* through *dw4* loci that have been extensively characterized in sorghum improvement), plant height responds less dramatically to post-anthesis resource limitation than yield components that are determined during a narrow reproductive window (Rooney, 2004; Blum, 2011). Individual panicle yield occupies an intermediate position in this hierarchy: more environmentally sensitive than plant height due to its dependence on post-anthesis assimilate supply, but more heritable than seed number. Seed number is among the most environmentally

sensitive yield components in cereals, determined during a brief and irreversible developmental window that coincides with maximum vulnerability to nitrogen limitation and weed competition, and therefore among the least predictable across generations under variable management (Craufurd and Peacock, 1993; Sadras, 2007; Gambín and Borrás, 2009).

These differences in cross-generation predictability among traits have direct implications for how early-generation selection should be structured in low-input breeding programs. A practical two-stage selection strategy (using structural traits for initial screening in F₂ followed by direct evaluation of yield components under low-input conditions in F₃ and later generations) integrates the differential predictability of traits into a breeding workflow that balances efficiency with accuracy in identifying populations adapted to resource-limited production systems (Ceccarelli, 1994; Atlin et al., 2000; Muleta et al., 2018).

Study objectives

Low-input and organic production environments impose stresses that are qualitatively different from the single-factor stresses most studied in crop physiology. The occurrence of nitrogen limitation and weed competition creates a selective environment in which reproductive traits, particularly seed number, are disproportionately affected relative to structural and phenological traits. This differential sensitivity, rooted in the developmental timing of reproduction relative to peak resource demand, has direct consequences for which traits carry the most information about genotype adaptation to low-input conditions. The source-sink framework further clarifies that compensatory responses in individual seed weight, while biologically real, do not restore total reproductive output and can actively mislead single-trait selection by appearing as evidence of stress tolerance in genotypes that are among the most stressed.

The breeding framework literature is equally clear that selection conducted outside the target production environment reduces the efficiency of genetic gain for low-input adaptation. When G×M interactions are large enough to reorder genotype rankings between conventional and organic management, early selection decisions made under high-input conditions risk discarding the alleles most relevant to stress resilience while advancing populations whose performance advantage is input-dependent. Direct selection within representative low-input environments, from early generations onward, is the approach most consistently supported by both quantitative genetic theory and empirical evidence across cereal crops.

Parent-progeny predictability introduces an additional layer of complexity to this picture. Even when selection is conducted within the appropriate environment, cross-generation predictability for yield components under variable and stressful conditions is limited by large environmental variance that characterizes low-input systems. Structural traits such as plant height offer more reliable cross-generation guidance than reproductive traits.

What the literature has not addressed is how these principles operate together in early segregating sorghum populations evaluated simultaneously under conventional and low-input management across generations. The cross-generation predictability of yield components under low-input stress, the degree to which multi-trait indices outperform single-trait criteria in identifying resilient early-generation populations, and the practicality of percent reduction metrics for quantifying management sensitivity across diverse genetic backgrounds all remain underexplored in sorghum specifically. The research presented in the following chapter directly addresses these questions through a two-year evaluation of-- F₂ and F₃ sorghum populations under contrasting management systems, contributing empirical evidence to the conceptual

framework established here and informing selection strategies for sorghum breeding programs targeting organic and resource-limited production systems.

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Chapter 2 - Parent-progeny predictability and low-input adaptation in early-generation sorghum

Introduction

Organic and low-input systems impose complex and interacting stresses, including limited nitrogen availability, greater weed competition, and increased temporal variability in nutrient release (Jordan, 1993; Biradar et al., 2024). These factors reshape the selective environment and can alter genotype rankings, leading to genotype $\times$ environment ($G \times E$), genotype $\times$ management ($G \times M$) and genotype $\times$ environment $\times$ management ($G \times E \times M$) interactions that complicate breeding decisions (Finlay and Wilkinson, 1963; Cooper et al., 1995; Atlin et al., 2000; Messina et al., 2009; Annicchiarico et al., 2019). Under such conditions, traits associated with early vigor, competitive ability, and nutrient-use efficiency may become more important than traits optimized for high-input yield potential (Ceccarelli, 1994; Wolfe et al., 2008). Consequently, selection conducted outside the target population of environments can reduce realized genetic gain when genotype performance is unstable across management systems (Kang, 1993; Atlin et al., 2000).

Organic breeding frameworks increasingly emphasize system-based adaptation, where breeding materials are selected within the environments for which they are intended (Ceccarelli, 1994; Ceccarelli and Grando, 2007; Lammerts van Bueren et al., 2011). Such approaches recognize that adaptation to low-input systems often involves complex traits interactions rather than superiority for a single trait. For crops like sorghum, which are mainly grown in marginal environments with limited water, aligning breeding environments with target production systems is especially important for identifying germplasm capable of maintaining yield stability under multiple stresses (Rattunde et al., 2013).

Sorghum [*Sorghum bicolor* (L.) Moench] is recognized for its tolerance to drought, heat, and marginal soils, making it a key cereal for resource-limited production systems (Rooney, 2004; Blum, 2011; Enyew et al., 2021; Lamb et al., 2022). As sustainable and organic agriculture expands, sorghum has gained attention as a crop capable of maintaining productivity under reduced synthetic inputs (Kremen and Miles, 2012; Boyles et al., 2022; Carcedo et al., 2022). However, most currently used sorghum hybrids were developed and grown under conventional, high-input conditions, where performance in organic environments may differ substantially because of variation in nutrient availability and weed pressure. This mismatch raises concerns about whether selection decisions made under conventional management reliably translate to performance in low-input systems (Murphy et al., 2007; Wolfe et al., 2008; Lammerts van Bueren et al., 2011).

A central assumption is that parental phenotypes provide meaningful predictions of progeny performance. Parent-progeny regression has long served as a foundational tool for estimating realized narrow-sense heritability and predicting response to selection (Hill and Mackay, 2004; Fernandez and Miller, 1985). In early-generation breeding, this assumption underpins decisions about which population selection to advance. However, parent-progeny relationships are sensitive to environmental covariance and G×E interactions, which can inflate or reduce apparent heritability and distort predictions of genetic gain (Vogel et al., 1980; Hill et al., 2008). When management systems differ, as in conventional versus organic production, the reliability of parent-progeny prediction may decline if trait expression is strongly environment-dependent.

These challenges are especially relevant in segregating generations such as F₂ and F₃, where genetic variance is high, but replication is limited. Early-generation selection often

prioritizes screening capacity over precision, making predictive relationships between generations critical for efficient breeding progress. If parent-progeny predictability is not perfect across management systems, early selection decisions made under conventional conditions may fail to capture alleles contributing to low-input resilience. Conversely, selection conducted within relevant management contexts may increase the likelihood of capturing adaptive trait combinations (Muleta et al., 2019; Chiaravallotti et al., 2024).

Although numerous studies have evaluated sorghum responses to individual stresses such as drought or low nitrogen, far fewer have examined segregating populations under the combined management stresses typical of organic systems. Even fewer have linked these responses to cross-generation predictability, leaving uncertainty about how early-generation selection decisions translate into later performance under contrasting management (Rooney, 2004). Quantifying adaptation to low-input stress requires metrics that capture both magnitude and stability of trait expression. Percent reduction relative to conventional performance provides a simple and interpretable measure of stress sensitivity, allowing comparison of genotype responses across contrasting environments (Sadras, 2007; Gambín and Borrás, 2010). Smaller reductions indicate greater tolerance to stress, whereas large reductions reveal susceptibility. Such metrics are particularly useful in early-generation trials where absolute yield levels may be less informative than relative stability.

Because organic environments impose multi-factor stress, selection based on single traits can overlook compensatory responses and tradeoffs among traits. Multi-trait selection indices provide a practical framework for integrating complex trait information. Rank-based indices have been widely used to identify genotypes combining stability and performance across traits and environments (Annicchiarico et al., 2019; Kang, 1993). In organic breeding contexts, such

approaches align with the need to prioritize resilience and trait balance rather than maximum yield under optimal conditions (Murphy et al., 2005; Lammerts van Bueren et al., 2011).

Based on this background, we hypothesize that: i) Low-input stress reduces reproductive traits more than phenological or structural traits ii) Parent-progeny predictability is weaker for yield components than structural traits and iii) Multi-trait indices more effectively identify resilient populations than single-trait selection.

To address these questions, this study evaluated F₂ and F₃ generation sorghum populations across conventional and low-input management systems over two years. The objectives were to 1) quantify phenological, morphological, and yield-component responses to contrasting management; 2) assess parent-progeny relationships within and across systems to evaluate cross-generation predictability; 3) quantify sensitivity to low-input stress using percent reduction metrics; and 4) identify resilient populations using a rank summation index integrating multiple traits. By linking parent-progeny predictability with stress resilience metrics, this research aims to inform breeding strategies that improve selection efficiency for sorghum adapted to organic and resource-limited production systems.

Materials and Methods

Plant materials and population advancement

Twenty-nine F₂ sorghum populations were evaluated in summer 2024 as part of a broad early-generation screening effort. Populations represented diverse genetic backgrounds developed to capture variation in phenology, plant architecture, and expression of yield components.

Eight populations (P1 to P8) were selected for advancement to the F₃ generation based on the better performance in two management systems and overall desirability (early flowering,

short to medium plant height, panicle exertion with short peduncle length and semi-compact panicle type, stay-green and non-lodging) ratings. Selection criteria also included high grain yield/panicle under both conventional and low-input conditions. Advancement decisions were based on observed phenotypic performance, consistent with standard early-generation phenotypic selection in self-pollinated crops. The eight F₃ selected populations each with randomly selected 38 families each with 2 rows following standard spacing (75 cm between rows and 7.5 cm between plants) were evaluated under head-to-row method in summer 2025. Two reference checks, 85P58 and Macia, were included to benchmark performance against widely used germplasm.

Experimental site and environmental conditions

Field experiments were conducted in summer 2024 and 2025 at the Kansas State University Ashland Bottoms Research Farm near Manhattan, KS, USA (39.14° N, 96.64° W). Weather conditions differed between years, particularly with respect to planting date and early-season temperature, allowing assessment of population responses across contrasting seasonal environments.

Field management system

In the F₂ evaluation, conventional and low-input management treatments were established in adjacent strips within the same field. Each management system consisted of a contiguous block in which all populations were planted together, characterized as a non-randomized strip design. The two treatments were separated by a 2.4 m border row, intended to reduce edge effects and limit physical drift of inputs between treatments. Although the border row reduced direct overlap between management zones, the proximity of treatments may have permitted limited nutrient or chemical drift, particularly from conventional to low-input plots. In

the F₃ evaluation, conventional and low-input management systems were planted in separate adjacent fields, allowing independent implementation of two treatments. Within each management system, populations were arranged using an augmented block design. The conventional field contained 45 blocks, with both checks replicated once per block (45 replications each). The low-input field contained 42 blocks, with Check 1 replicated in all 42 blocks and Check 2 replicated in 32 blocks. Treated seeds for conventional and untreated seeds for low-input treatment were uniformly used in all populations in both F₂ and F₃ field evaluations.

Conventional management received urea-ammonium nitrate (UAN) fertilizer at a rate of approximately 215 kg N ha⁻¹ applied prior to planting. Weed control consisted of pre- and post-emergence herbicide applications following standard regional production practices for grain sorghum. No fungicides/insecticide applications were required during F₂ and F₃ field evaluations in both treatments. Low-input management relied on organic-compliant fertility (~10 kg N ha⁻¹ fish-based organic fertilizer) and nonchemical weed control practices. Weed control was achieved through a combination of mechanical cultivation and manual weed control. No synthetic fertilizers, herbicides, or insecticides were applied.

Weed pressure in 2025 was higher than 2024, particularly within the low-input field, where red-root pigweed (*Amaranthus retroflexus* L.) became the dominant competitor. Plots were intensively managed using hand weeding and mechanical cultivation and were retained for analysis to represent biologically realistic organic stress conditions.

Trait measurements

Phenological, morphological, and yield-component traits were measured at physiological maturity. Flowering time (FT) was recorded as days after planting until tip flowering. Plant

height (PH) was measured in centimeters from the soil surface to the tip of the panicle. Yield components were quantified on a per-panicle basis. Total seed number per panicle head (SN) was determined by counting all filled seeds. Yield per panicle (SW) was measured in grams following harvest, drying, and threshing. Hundred-seed weight (HW) was calculated by weighing 100 seeds (expressed in grams) from individual selected F₂s from each population and mean of three uniform selected plants from each F₃ family.

All statistical analyses were conducted using R statistical software. Population means were used as analytical units to reflect selection-relevant performance at the population level typical of early-generation breeding. Treatment effects within each year between populations were evaluated using Welch's two sample t-tests ($\alpha = 0.05$), which accommodate unequal variances between two management systems. Tests were conducted using population means to detect directional shifts in trait expression between conventional and low-input management. Percent reduction under low-input management were intended to quantify the magnitude and consistency of management-driven changes in phenological, morphological, and yield-component traits.

Percent reduction (PR) was quantified as:

$$PR_{p,t} = \left(\frac{Control_{p,t} - Stress_{p,t}}{Control_{p,t}} \right) \times 100$$

Where, $PR_{p,t}$ represents percent reduction for population p and trait t .

Positive PR values indicate reduced trait expression under low-input management, whereas negative values indicate greater trait expression under low-input conditions. Percent reduction was used as an indicator of management sensitivity and relative stability across populations. Population-level PR values were compared across traits to identify families exhibiting low-performance penalties under low-input conditions.

Parent-progeny relationships between F₂ and F₃ generations were performed using linear regression and Pearson correlation analyses between the value of selected individual F₂ and corresponding mean of each F₃ family from eight populations for the same trait within each management system (Smith and Kinman, 1965).

$$Y_{F3,i} = \alpha + \beta Y_{F2,i} + \varepsilon_i$$

Where, $Y_{F3,i}$ = mean trait value of the F₃ population; $Y_{F2,i}$ = mean trait value of the corresponding F₂ population; α = intercept; β = regression coefficient describing parent-progeny relationship; ε_i = residual error

These analyses were used to assess cross-generation predictability of phenotypes and infer the reliability of early-generation selection. Strong positive relationships were interpreted as evidence of stable cross-generation trait expression, whereas weak or inconsistent relationships indicated reduced predictive reliability and potential environmental modulation of phenotypes. Separate analyses within conventional and low-input systems allowed evaluation of whether predictability differed between management contexts.

Pearson correlation coefficients (r) were calculated among phenological, morphological, and yield-component traits within each management system. These analyses were used to assess associations among yield components and to detect compensatory allocation patterns influencing yield performance under stress.

$$r = \frac{\sum(x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum(x_i - \bar{x})^2 \sum(y_i - \bar{y})^2}}$$

Where x_i and y_i represent paired trait values and $\bar{x}$, $\bar{y}$ are trait means.

Statistical significance of correlation coefficients was evaluated using a two-tailed t-test.

The test statistics were calculated as:

$$t = \frac{r\sqrt{n-2}}{\sqrt{1-r^2}}$$

Where r is the Pearson correlation coefficient and n is the number of observations. Degrees of freedom were calculated as $n - 2$.

Multi-trait resilience under low-input management was performed using a rank summation index (RSI):

$$RSI_i = \sum R_{ij}$$

Where R_{ij} is the rank of population i for trait j .

Earlier flowering received lower ranks, whereas higher values received lower ranks for all other traits. Lower RSI values indicate superior overall performance under low-input conditions based on percent reduction. This approach follows the non-parametric rank-based selection index described by Mulamba and Mock (1978), which is well suited for early-generation selection where variance components are difficult to estimate, and multi-trait decision-making is required (Hazel, 1943; Kang, 1993; Annicchiarico et al., 2019).

Results

Percent reduction and population effects

Twenty-nine F_2 sorghum populations were evaluated in 2024, of which eight populations were selected for advancement to F_3 evaluation in 2025 based on agronomic desirability and performance under both management systems (Table 2.1). Trait responses to contrasting management significantly differed between years and among trait classes (Fig. 2.1). In 2024, management effects were limited, with no significant differences detected between conventional and low-input systems for FT, PH, SN, SW, or HW (Fig. 2.1 A-E), and trait distributions overlapped between populations, indicating weak separation between management systems in

the F₂ populations. Population effects were significant in 2025, when low-input management significantly reduced SN and SW ($p < 0.0001$), while FT occurred slightly earlier under low-input conditions ($p < 0.05$). PH responses were smaller and less directional than those observed for reproductive traits. HW showed a slight increase under low-input conditions despite reductions in SN and SW (Fig. 2.1C–E), suggesting that individual seed weight responded differently from reproductive sink size.

Yield-component traits showed significant separation in 2025 (Fig. 2.1C, D). Under low-input management, distributions for SN and SW shifted downward relative to conventional population, indicating substantial reductions in reproductive output across populations. In contrast, FT shifted only modestly and PH responses were variable, suggesting lower sensitivity of those traits to management stress. Interestingly, HW showed a slight increase under low-input conditions despite reductions in SN and SW, suggesting that individual seed weight responded differently than reproductive sink size. Reproductive traits exhibited the strongest response to low-input management.

Population	Population ID (Selected)
(GB07118/BTx2921)-MAF1	P1
(GB11076/BTx2923)-MAF1	P2
(GBTx2926/B11076)-MAF1	P3
(GR04060/RTx2912)-MAF1	P4
(GR08303/RTx2916)-MAF1	P5
(R17161/R14047)-CSF1	P6

(GR07178/R11158)-MAF1	P7
(GR04104/RTx2939)-MAF1	P8

Table 2.1 F₂ sorghum populations evaluated in 2024 and populations advanced to F₃ in 2025.

Eight populations were selected for advancement (P1–P8), with 38 individuals advanced per population.

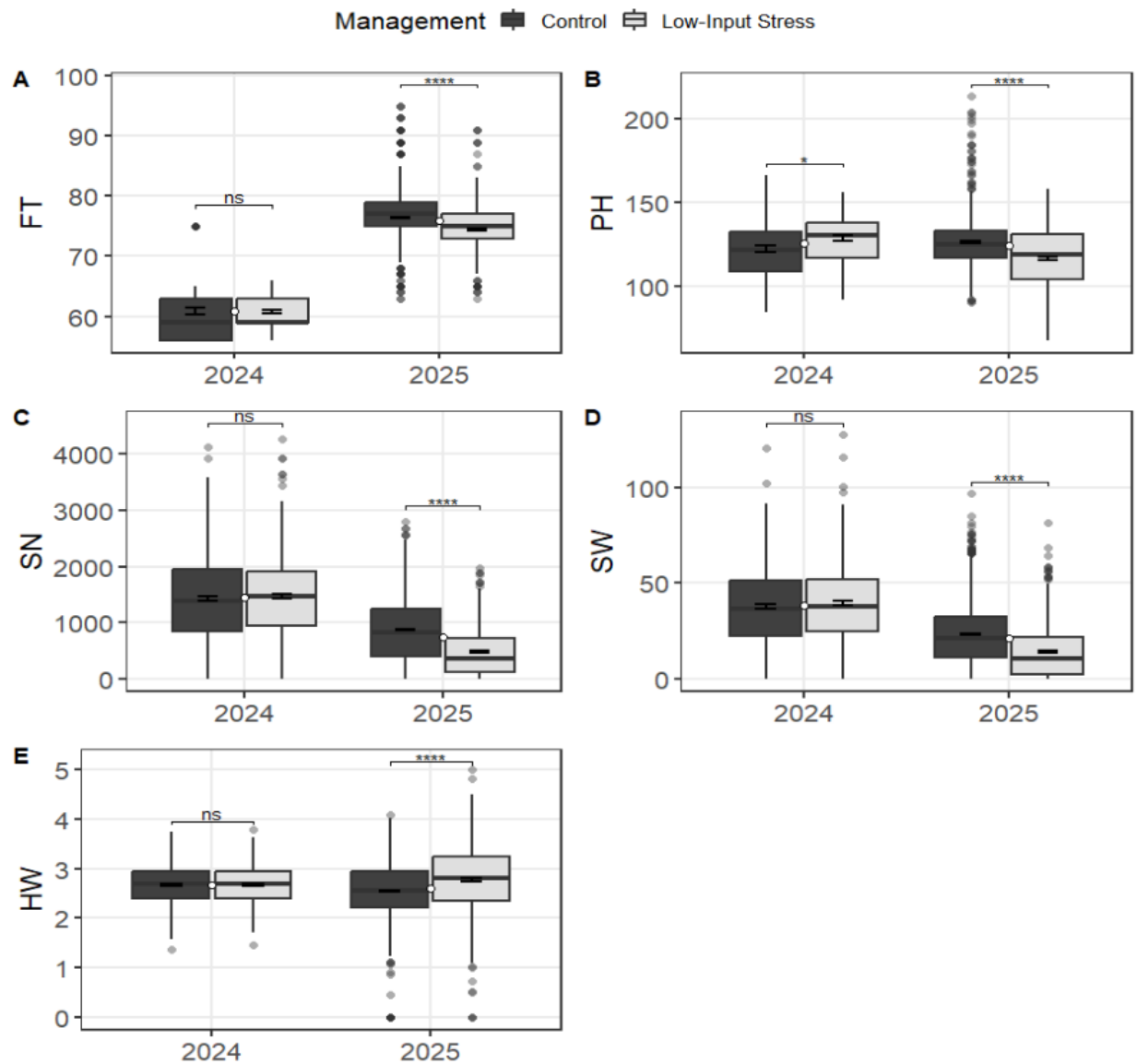


Figure 2.1 Trait responses of sorghum populations under conventional and low-input stress conditions in 2024 and 2025. Significance between treatments is indicated above panels (* $p < 0.05$; **** $p < 0.0001$; ns = not significant). Panels show (A) FT, flowering time (in days after planting); (B) PH, plant height (cm); (C) SN, seed number per head; (D) SW, yield per panicle (g); and (E) HW, 100-seed weight (g).

Percent reduction further quantified substantial differences among populations in sensitivity to low-input stress (Table 2.2). Yield-component traits had the greatest reductions, particularly SN and SW, whereas FT and PH had comparatively small shifts. Across populations, reductions in SN were larger than in SW, indicating that stress effects were often expressed most strongly through reductions in seed number. In contrast, changes in FT and PH were modest and less variable among populations. Collectively, management effects in 2025 were concentrated in reproductive traits rather than in phenological or structural traits.

SW declined in all populations, ranging from 11% in P8 to 60% in Macia (Table 2.2). Similar patterns were observed for SN, which declined from 26% in P5 to 68% in P7 and often exhibited larger reductions than SW. Populations P2, P8, and P5 consistently had the smallest reductions in both SN and SW, whereas P4, P7, and Macia showed the greatest penalties under low-input conditions.

In contrast to reductions in SN and SW, eight F_3 populations and checks exhibited negative percent reduction values for HW, indicating increased seed weight under low-input conditions (Table 2.2). The largest increases were observed in P8, P1, and P7. Although increased HW did not offset reductions in total reproductive output, the opposite directional response of this trait suggests compensatory shifts in resource allocation under stress.

PH responses were variable, ranging from moderate reductions to slight increases (Table 2.2). P2 and Check 1 (85P58) exhibited increased PH under low-input conditions, whereas the largest reductions exceeded 14%. FT shifts were small relative to yield components, most within $\pm 5\%$. Most populations flowered slightly earlier under low-input management, with the greatest advancement observed in 85P58. These modest changes indicate that phenological responses were less sensitive to management compared with yield-component traits.

Table 2.2 Percent reduction of agronomic traits under low-input stress relative to conventional conditions for F₃ (2025) populations. Values were calculated as $(\text{Control} - \text{Stress}) / \text{Control} \times 100$, where positive values indicate reduced performance under stress and negative values indicate increased performance. Populations are labeled P1-P8, with Check 1 (85P58) and Check 2 (Macia). Trait abbreviations are SW, panicle yield (g); SN, seed number per head; HW, 100-seed weight (g); PH, plant height (cm); and FT, flowering time.

Population	SW (%)	SN (%)	HW (%)	PH (%)	FT (%)
P1	19.3	37	-18.3	5.3	4.7
P2	18.9	28.4	1.1	-6.9	4.9
P3	42.6	47.3	-13	8.3	4.7
P4	59.7	65.3	5.1	8.8	4.3
P5	21.8	26.4	-12.2	3.9	0.8
P6	33.6	41.6	-8.3	15.6	2.1
P7	60	67.5	-15.9	14.1	0.6
P8	10.9	30.1	-26	11.2	5.3
Check 1	26.9	42.9	-14.1	-3.9	-3.3
Check 2	60.6	67.2	-0.82	9.2	2.9

Rank summation index

The rank summation index (RSI) was used to compare multi-trait performance of populations evaluated under low-input management in 2025 based on the percent reduction

values. RSI integrated FT, PH, SN, SW, and HW measured under low-input conditions, with lower values indicating superior combined performance across traits (Fig. 2.2). RSI values ranged from 17 (P1, most resilient) to 42 (Macia, least resilient), where lower values reflect consistently favorable rankings across all five traits, indicating substantial variation in multi-trait performance under low-input conditions. Among breeding populations, P1 had the lowest RSI value, indicating the greatest overall resilience, followed by P7, P6 and P4 exhibited the highest RSI values, followed by Macia.

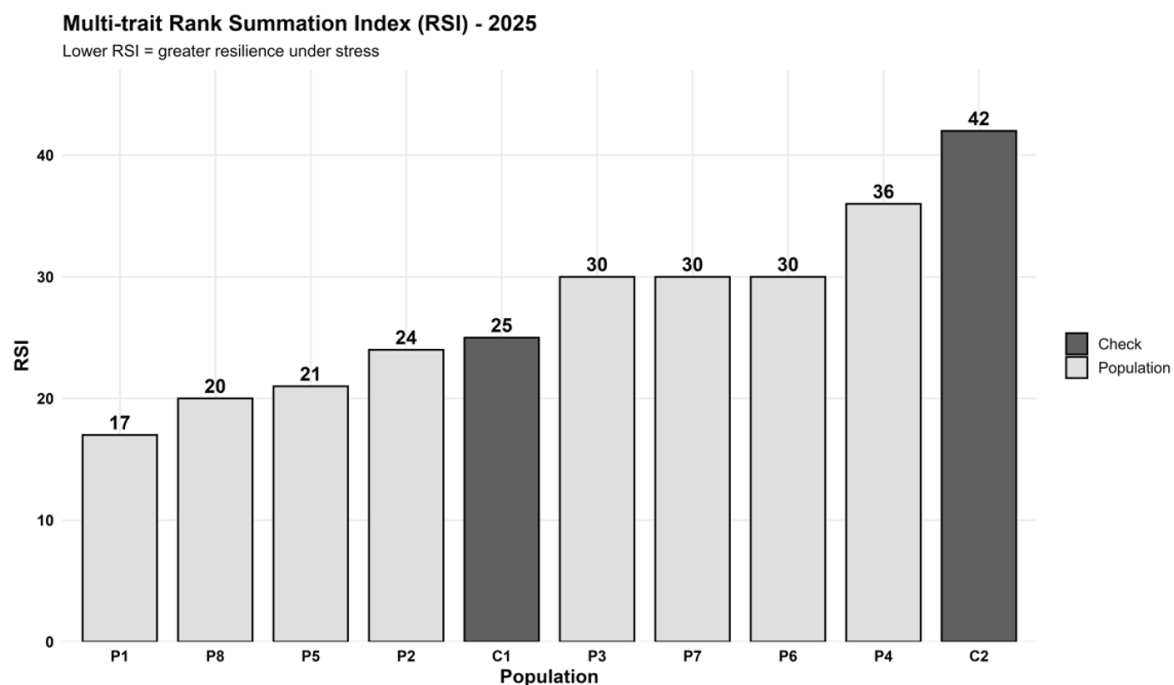


Figure 2.2 Multi-trait rank summation index (RSI) for F_3 (2025) populations based on percent reduction under low-input stress relative to control conditions. RSI was calculated as the sum of trait ranks, with lower values indicating greater resilience. Only breeding populations (P1-P8) and check (Macia) are shown. Traits: FT, flowering time (days); PH, plant height (cm); SN, total number of seeds per head; SW, total seed weight (g); HW, 100-seed weight (g).

Population comparisons relative to our inbred Macia (Check 2) further illustrated differences in trait-specific adaptation under low-input conditions (Fig. 2.3). Several populations exceeded Macia for individual traits such as HW or FT but did not rank highly for overall resilience based on RSI, indicating that superior performance for single traits did not necessarily translate into balanced low-input adaptation across traits.

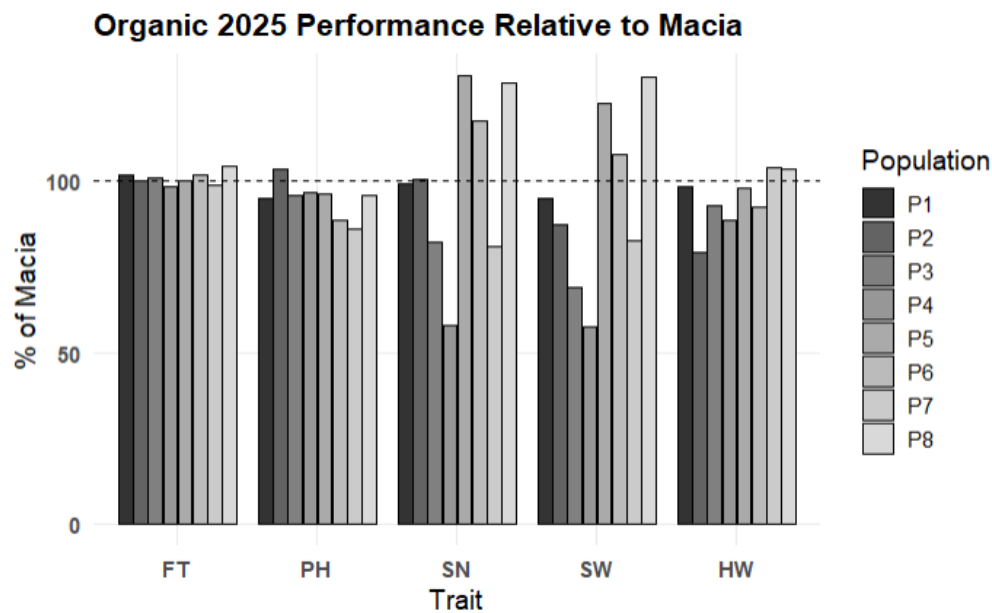


Figure 2.3 Performance of F_3 (2025) populations relative to Macia under low-input conditions. Values are expressed as a percentage of Macia (>100% indicates better population performance). Flowering time (FT) is directionally adjusted so that earlier flowering corresponds to higher values. Trait abbreviations: FT, flowering time (days); PH, plant height (cm); SN, total number of seeds per head; SW, yield per panicle (g); HW, 100-seed weight (g).

Trait relationships

Correlation analyses revealed consistent and strong relationships among yield-component traits, whereas associations involving FT and PH were weaker in magnitude and differed in direction or significance between conventional and low-input management systems. Across both

management systems, SW was strongly and positively correlated with SN ($r \approx 0.90$ - 0.95 ; Fig. 2.4), and this relationship was consistently significant, indicating that reproductive sink size was the primary determinant of yield per panicle. This association was stable across environments and represented the dominant correlation pattern in the dataset.

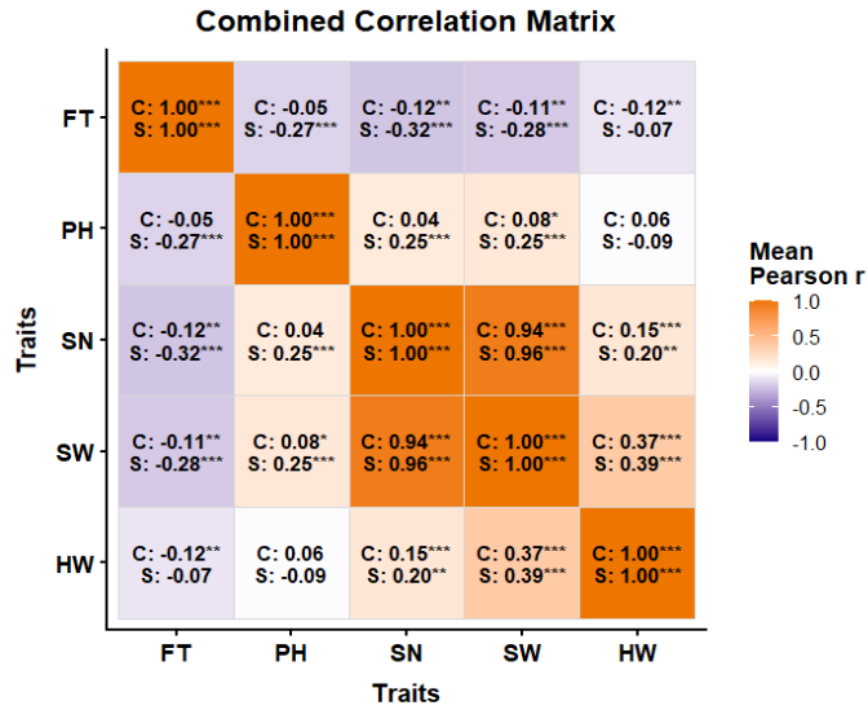


Figure 2.4 Combined Pearson correlation matrix for agronomic traits under conventional (C) and low-input stress (S) conditions, calculated across both F₂ and F₃ generations. Each cell reports correlation coefficients for both treatments, with asterisks indicating significance. Tile color represents the mean correlation coefficient across treatments. Trait abbreviations: FT, flowering time (days); PH, plant height (cm); SN, total number of seeds per head; SW, yield per panicle (g); HW, 100-seed weight (g).

HW was negatively correlated with SN under low-input management, and these relationships were not significant. This pattern is consistent with compensatory allocation, where reductions in SN are partially offset by increases in HW. Correlations between HW and SW were

also weak and inconsistent, indicating that increased seed weight did not fully compensate for reductions in seed number.

FT exhibited weak negative correlations with SN and SW, with some associations approaching significance depending on management system, suggesting that earlier flowering was modestly associated with increased reproductive output. However, these relationships were small in magnitude and not consistently significant across conditions.

PH showed weak positive correlations with SN and SW under low inputs, but the conventional correlations were weaker and insignificant, indicating that PH was not a strong predictor of yield formation under either management system.

Parent-progeny relationships

Cross-generation correlations between F₂ (2024) and F₃ (2025) populations differed between management systems (Fig. 2.5A, B). Under conventional management, several traits exhibited moderate positive correlations across generations, with PH and HW showing the most consistent associations. These relationships were positive and, in some cases, statistically significant, indicating partial retention of trait rankings from F₂ to F₃. In contrast, SN and SW displayed weaker and less consistent correlations, with several relationships not reaching significance, consistent with the low R² values observed in Table 2.3.

Under low-input management, cross-generation correlations were weaker and more variable. Relationships among SN, SW, and HW were often reduced in magnitude and, in several cases, not significant or near zero, indicating greater environmental influence on trait expression under stress conditions. Some trait combinations exhibited negative correlations, further reflecting instability in trait expression across generations.

Parent-progeny predictability differed among traits and was strongest for PH, intermediate for HW, and weak for SN and SW (Table 2.3; Fig. 2.5). Among all traits, PH exhibited the most consistent cross-generation relationships, with nearly identical correlations under conventional ($r = 0.72$; $R^2 = 0.52$) and low-input management ($r = 0.72$; $R^2 = 0.51$). These strong associations indicate substantial consistency in PH rankings across generations and management systems.

Table 2.3 Parent–progeny regression statistics describing relationships between F_2 and F_3 population means for key agronomic traits under conventional (C) and low-input stress (S) conditions. Values for b represent regression slope estimates ($h^2 = b$) reported as slope $\pm$ standard error, as a proxy for realized narrow-sense heritability. Pearson correlation coefficients (r) and coefficients of determination (R^2) are also shown. Bolded r values indicate statistically significant relationships ($p < 0.05$). Trait abbreviations: FT, flowering time (days); PH, plant height (cm); SN, total number of seeds per head; SW, yield per panicle (g); HW, 100-seed weight (g).

Trait	Management	b ($\pm$ SE)	r	R^2
HW	C	0.65 ± 0.39	0.56	0.32
HW	S	1.08 ± 0.49	0.66	0.44
FT	C	0.14 ± 0.14	0.36	0.13
FT	S	0.13 ± 0.28	0.19	0.04
PH	C	0.27 ± 0.11	0.72	0.52
PH	S	0.49 ± 0.19	0.72	0.51
SN	C	0.19 ± 0.58	0.13	0.02
SN	S	0.07 ± 0.27	0.13	0.02
SW	C	0.66 ± 0.42	0.54	0.29
SW	S	0.19 ± 0.32	0.23	0.06

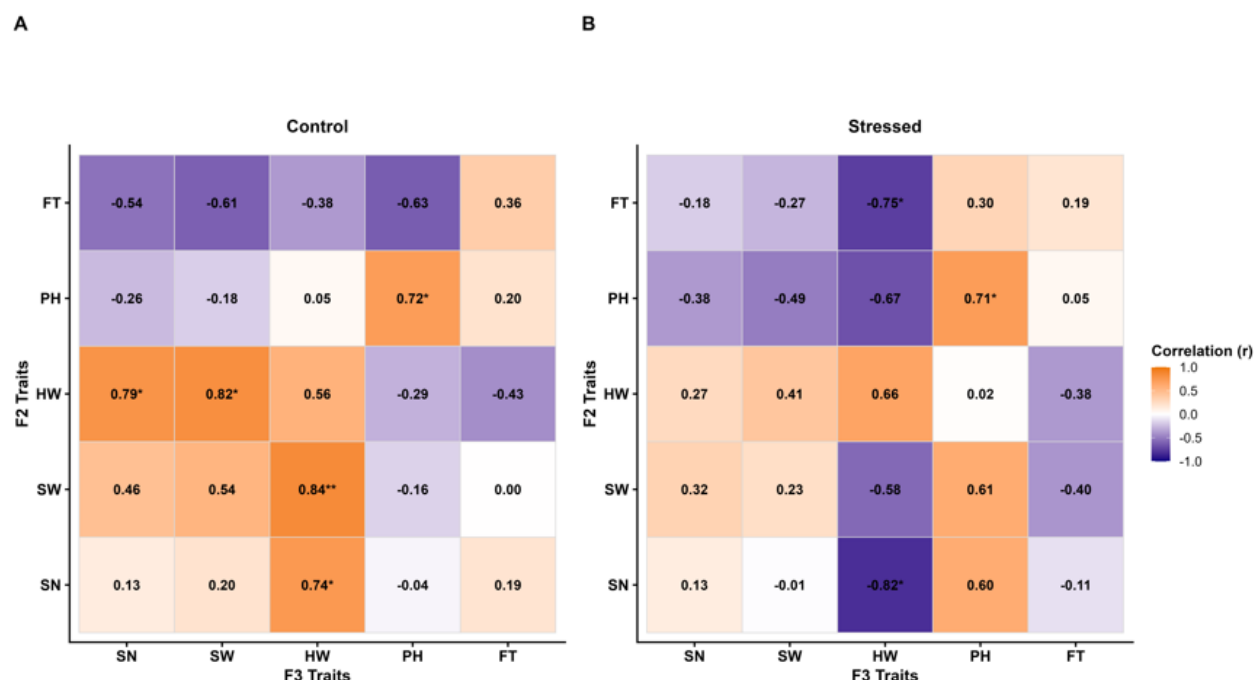


Figure 2.5 Cross-generation Pearson correlation matrices for agronomic traits between F₂ (2024) and F₃ (2025) populations under (A) conventional and (B) low-input stress conditions.

Correlations were calculated using population means for lines present in both generations and represent the consistency of trait expression across generations. Each cell shows the correlation coefficient between F₂ and F₃ trait values, with asterisks indicating significance (* $p < 0.05$, ** $p < 0.01$). Trait abbreviations: FT, flowering time (days); PH, plant height (cm); SN, total number of seeds per head; SW, yield per panicle (g); HW, 100-seed weight (g).

HW showed moderate cross-generation association, particularly under low-input management. Table 2.3 shows $r = 0.56$ and $R^2 = 0.32$ under conventional management and $r = 0.66$ and $R^2 = 0.44$ under low-input management. These values suggest that HW had moderate cross-generation predictability, although it was less consistent than PH.

Yield exhibited weak parent-progeny relationships. For SN, R^2 values were 0.02 under both conventional management and low-input management. For SW, R^2 values were 0.29 under conventional management and 0.06 under low-input management (Table 2.3). Within this

experiment, early-generation performance for SN and SW was a poor predictor of later reproductive performance, especially under low-input conditions.

FT also showed weak cross-generation relationships, with $r = 0.36$ and $R^2 = 0.13$ under conventional management and $r = 0.19$ and $R^2 = 0.04$ under low-input management (Table 2.3). This pattern suggests that FT was also strongly influenced by environmental conditions across years and management systems.

In addition to population-level regressions, cross-generation relationships were examined within individual populations using panicle-level data (Table 2.4). Most within-population relationships were weak, but several significant associations were detected. For HW, P5 showed a significant negative relationship under conventional management ($b = -0.86$, $r = -0.34$), whereas P7 showed a significant positive relationship under low-input management ($b = 0.52$, $r = 0.35$). For FT, P8 showed a significant negative relationship under conventional management ($b = -2.33$, $r = -0.83$), and P2 showed a significant negative relationship under low-input management ($b = -0.36$, $r = -0.65$). These results indicate that cross-generation consistency within populations was trait- and population-specific rather than broadly stable across the dataset.

Table 2.4 Within-population cross-generation regression slopes (b) and Pearson correlations (r) between F_2 (2024) and F_3 (2025) trait values under conventional (C) and low-input stress (S) conditions. Values are shown for populations P1–P8 present across both years and management systems. Asterisks indicate significance (* $p < 0.05$, ** $p < 0.01$). Trait abbreviations: FT, flowering time (days); PH, plant height (cm); SN, total number of seeds per head; SW, yield per panicle (g); HW, 100-seed weight (g).

Trait Management Stat	P1	P2	P3	P4	P5	P6	P7	P8
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HW	C	b	0.47	-0.18	0.53	0.2	-0.86*	0.08	-0.26	0.09
HW	C	r	0.22	-0.07	0.18	0.09	-0.34*	0.08	-0.19	0.09
HW	S	b	-0.11	-0.26	-0.12	-0.22	0.34	-0.27	0.52*	-0.39
HW	S	r	-0.09	-0.09	-0.04	-0.09	0.19	-0.21	0.35*	-0.23
FT	C	b	-0.09	0.01	0.29	0.16	-0.02	NA	0.16	-2.33**
FT	C	r	-0.17	0.02	0.54	0.16	-0.05	NA	0.35	-0.83**
FT	S	b	-0.09	-0.36*	NA	-0.06	0.64	NA	-0.22	0.96
FT	S	r	-0.33	-0.65*	NA	-0.05	0.36	NA	-0.22	0.24
PH	C	b	-0.52	0.33	0.33	0.24	-0.02	0.34	-0.23	-0.15
PH	C	r	-0.45	0.22	0.21	0.34	-0.05	0.37	-0.54	-0.22
PH	S	b	0	0.08	0.33	0.82	0.48	0.5	-0.89	0.45
PH	S	r	0	0.09	0.21	0.49	0.38	0.38	-0.45	0.18
SN	C	b	0.02	-0.01	0.03	-0.04	0.07	0.02	-0.07	0.01
SN	C	r	0.03	-0.01	0.04	-0.05	0.11	0.04	-0.09	0.02
SN	S	b	-0.08	-0.11	-0.11	-0.03	0.18	0.03	0.	0.07
SN	S	r	-0.14	-0.23	-0.24	-0.05	0.27	0.03	-0.01	0.13
SW	C	b	0.14	0.02	0.06	-0.02	0.01	0	-0.03	0.04
SW	C	r	0.14	0.03	0.08	-0.03	0.02	0	-0.04	0.06
SW	S	b	-0.07	-0.13	-0.1	-0.01	0.23	0.04	0.05	0.03
SW	S	r	-0.12	-0.23	-0.21	-0.01	0.29	0.05	0.08	0.05

Discussion

Trait responses, population resilience, and multi-trait selection

The stronger suppression of SN and SW relative to FT and PH under low-input management reflects the heightened sensitivity of reproductive sink establishment to combined nitrogen limitation and weed competition. In sorghum, floret initiation and grain set occur during a relatively narrow critical window that coincides with peak nitrogen demand; when N availability is low and assimilate partitioning is further disrupted by weed competition, reductions in seed number are disproportionately large (Craufurd and Peacock, 1993; Sadras, 2007; Dille et al., 2020). In contrast, phenological traits such as FT are largely regulated by

photoperiod and temperature signals, and structural traits such as PH are canalized earlier in development and determined by cumulative biomass accumulation over a longer growth period, making both less responsive to resource limitations concentrated at or after anthesis (Craufurd et al., 1999; Blum, 2011). These differences in developmental timing help explain why reproductive traits are the most informative indicators of low-input stress and the most difficult to predict across environments and generations.

In 2024, the adjacent-strip design and limited weed pressure likely moderated the low-input treatment, compressing phenotypic differences among populations. The 2025 conditions - separate fields, substantially higher red-root pigweed (*Amaranthus retroflexus*) pressure, and lower fertility - more closely approximated realistic organic production stress and exposed population-level variation that was masked under the less severe 2024 conditions. This pattern is consistent with observations that meaningful G×M interactions emerge primarily when management stress is sufficiently strong to alter genotype rankings, and that discriminating between adapted and non-adapted genotypes requires evaluation in environments representative of the intended production system (Finlay and Wilkinson, 1963; Ceccarelli, 1994; Wolfe et al., 2008; Annicchiarico et al., 2019). The percent reduction and RSI analyses revealed that variation in reproductive performance across populations was driven primarily by differences in SN rather than HW. The magnitude of SN reductions observed, ranging from 26% (P5) to 68% in the most sensitive (P7), is comparable to responses reported under severe drought and heat stress in grain sorghum (Craufurd and Peacock, 1993), reflecting the degree to which combined nitrogen limitation and weed competition in low-input systems can approximate the reproductive impact of abiotic stress alone. The strong positive relationship between SN and SW across populations ($r \approx 0.90\text{--}0.95$; Fig. 2.4) confirms that once grain number is reduced, total seed weight declines

proportionally, because compensatory increases in HW are insufficient to offset lower grain set. This is consistent with source-sink theory, in which total reproductive output under stress is constrained primarily by sink number rather than individual sink capacity (Gambín and Borrás, 2010; Sinclair and Jamieson, 2006). These results indicate that low-input stress primarily affected reproductive sink establishment, and that populations differed substantially in their capacity to maintain grain set under these conditions.

Among breeding populations, P1 (GB07118/BTx2921) ranked as the most resilient based on its low RSI value and the smallest reductions in SN and SW under low-input conditions. P8 (GR04104/RTx2939) demonstrated similarly strong resilience through stable reproductive performance and balanced responses across all five measured traits. P5 (GR08303/RTx2916) ranked third, driven by the smallest SW reduction of any population and a strong compensatory HW response under stress. In contrast, P6 (R17161/R14047) and P4 (GR04060/RTx2912) exhibited large reductions across multiple reproductive traits and ranked among the least resilient populations. Whether these differences reflect specific allele combinations controlling nitrogen uptake efficiency, competitive ability under weed pressure, or patterns of assimilate partitioning warrants further investigation. The consistent resilience of P1 and P8 across RSI based on percent reduction analyses suggests that these populations carry trait combinations with practical breeding value for low-input systems, regardless of the underlying genetic mechanism.

Although HW increased under low-input conditions in most populations, these compensatory responses did not offset reductions in SN sufficiently to maintain total reproductive output. This pattern is consistent with the source-sink framework, in which a reduction in sink number releases greater assimilate per remaining grain, increasing individual seed weight without restoring total yield (Gambín and Borrás, 2010; Sadras, 2007). The practical

implication for selection is important: HW alone is not a reliable indicator of low-input performance. P7, for example, showed among the largest increases in HW under low-input conditions, yet simultaneously experienced the greatest reductions in SN (68%), resulting in a poor overall RSI. Selecting HW in isolation would misidentify such populations as well-adapted, highlighting the necessity of integrating multiple traits when evaluating performance under low-input conditions.

Population comparisons relative to Macia (Check 2) further illustrated that favorable performance for individual traits did not correspond to superior overall adaptation. Several populations exceeded Macia for HW or FT while remaining lower in overall RSI ranking, confirming that single-trait superiority is not equivalent to multi-trait resilience. In contrast, P1 (GB07118/BTx2921) and P8 (GR04104/RTx2939) showed stronger overall adaptation because favorable performance was distributed evenly across reproductive and supporting traits rather than concentrated in one response. The relatively poor RSI performance of Macia is notable given its status as a widely used inbred check in sorghum improvement. Its sensitivity to low-input stress, particularly in SN and SW, reflects a selection history under conventional management, suggesting that widely adopted germplasm may not carry the adaptive traits most relevant to organic or resource-limited production systems (Murphy et al., 2007; Lammerts van Bueren et al., 2011). These results establish that tolerance to low-input management in early-generation sorghum populations is characterized by minimizing reproductive stress penalties across multiple traits rather than maximizing performance under favorable conditions alone. This distinction shifts the practical breeding target from absolute yield capacity to relative stability, a distinction that single-environment conventional-management selection is poorly equipped to capture, and one that motivates the cross-generation predictability analysis addressed below.

Parent-progeny predictability

Parent-progeny relationships differed among traits and management systems, with PH showing the strongest and most consistent cross-generation predictability, HW showing moderate predictability, and reproductive traits showing comparatively weak predictability (Table 2.3; Fig. 2.5). Although parent-progeny regression is commonly interpreted as an estimate of realized narrow-sense heritability, the contrasting environments and segregating populations evaluated here make these relationships more appropriately interpreted as measures of phenotypic predictability under practical breeding conditions (Vogel et al., 1980; Fernandez and Miller, 1985; Hill and Mackay, 2004; Hill et al., 2008). It should also be noted that with only eight populations contributing to population-level regressions, statistical power is limited and R^2 estimates should be interpreted as indicative of predictability trends rather than precise heritability parameters.

PH exhibited nearly identical cross-generation relationships under conventional and low-input conditions, consistent with the expectation from quantitative genetics theory that morphological traits with higher heritability and simpler genetic architecture are less sensitive to $G \times E$ than yield components (Hill and Mackay, 2004). HW also maintained moderate predictability and notably showed slightly stronger cross-generation association under low-input management ($R^2 = 0.44$) than under conventional management ($R^2 = 0.32$). When grain set is reduced, fewer sinks compete for available assimilate, which may allow individual seed weight to be more consistently expressed and therefore more predictable across generations. These results suggest that structural traits are more stable across environments than reproductive traits, which are more sensitive to environmental variation (Craufurd et al., 1999; Blum, 2011), and that

PH and HW may serve as useful supporting criteria during early-generation selection even when yield component predictability is low.

SN and SW exhibited weak parent-progeny relationships under both management systems, with R^2 values near zero for SN (0.02 under both management treatments) and declining substantially for SW under low-input management ($R^2 = 0.06$ compared to 0.29 under conventional). Similar instability of reproductive traits across generations has been reported in other segregating cereal populations under variable environments (Vanniarajan and Ramalingam, 2011). These results indicate that ranking F_2 families based on SN or SW performance in a single management environment is unreliable for identifying populations with superior reproductive stability under low-input conditions. Advancement decisions based primarily on these traits risk discarding resilient genotypes that express stable reproductive performance only when evaluated across multiple environments representative of the target production system.

The reduction in cross-generation predictability under low-input management is consistent with theoretical expectations from quantitative genetics: when environmental variance is large relative to genetic variance, as it is under unpredictable multi-factor organic stress, the signal-to-noise ratio for detecting genetic differences between populations diminishes, and observed phenotypic rankings become less stable across generations (Finlay and Wilkinson, 1963; Vogel et al., 1980; Hill and Mackay, 2004). The inconsistency of within-population relationships in both magnitude and direction (Table 2.4) further reflects environmental modulation of trait expression rather than a systematic genetic pattern. Under conventional management, several within-population associations were significant for PH and HW, whereas under low-input management even these relationships were weakened, indicating that stress

environments impose an additional layer of phenotypic unpredictability beyond what management contrast alone would produce.

Given the differential predictability among traits, a practical two-stage selection strategy may improve efficiency in organic breeding programs. In F₂, PH and HW can serve as initial screening criteria given their greater cross-generation stability. These traits allow breeders to eliminate structurally or phenologically unsuitable populations before committing resources to extensive low-input evaluation. However, final advancement decisions targeting low-input resilience should incorporate SN and SW evaluated in representative low-input environments during F₃ or later generations, where replication allows genetic differences to be distinguished from environmental noise. Relying on SN or SW measured in a single F₂ conventional nursery to guide selection for organic adaptation is unlikely to accurately identify populations best suited to low-input systems and may systematically exclude alleles contributing to reproductive stability under stress.

Breeding implications and selection value

These findings have direct implications for how breeding programs targeting organic or low-input systems should be structured. The compressed phenotypic variation observed under conventional management in 2024, compared to the clear population differentiation under stronger low-input stress in 2025, supports allocating early-generation screening resources to environments representative of the intended production system rather than relying on conventional nursery conditions (Ceccarelli, 1994; Wolfe et al., 2008; Lammerts van Bueren et al., 2011). Selection practiced exclusively under conventional management is unlikely to capture the genotype rankings relevant to organic stress, because G×M interactions can substantially reorder population performance when management systems differ (Atlin et al., 2000; Messina et

al., 2009). Even where direct selection in low-input environments reduces phenotypic precision due to higher environmental variance, it more accurately targets the trait combinations, maintenance of SN, moderate PH, and balanced HW, that confer multi-trait resilience under resource-limited conditions.

The combined resilience and predictability analyses identified P8 (GR04104/RTx2939) and P1 (GB07118/BTx2921) as the populations with the strongest overall breeding value from this study. P1 showed the greatest multi-trait stability, combining the lowest RSI with comparatively stable SN and a compensatory HW response under low-input conditions, reflecting both maintenance of grain number and efficient grain filling. P8 demonstrated similarly balanced performance across FT, PH, SN, SW, and HW, and its stable reproductive output under low-input stress positions it as a strong candidate for continued advancement. P5 (GR04060/RTx2912), while ranked third in overall RSI, showed the smallest SW reduction of any population and strong HW compensation, suggesting complementary parental value for traits related to individual grain filling capacity. Populations combining low stress penalties with moderate cross-generation stability represent the most tractable targets for continued selection, as they are both adapted to low-input environments and sufficiently consistent across generations to support reliable genetic progress in early-generation breeding programs (Kang, 1993; Cooper et al., 1995).

Study limitations

The contrasting experimental designs employed in 2024 and 2025 provide important context for interpreting results. The non-randomized adjacent-strip layout in 2024, coupled with a 2.4m buffer row, likely permitted limited drift of nutrients or herbicides from conventional to low-input plots. Although this design constrained formal statistical inference, it effectively

supported early-generation screening by exposing populations to realistic management gradients (Ceccarelli, 1994; Wolfe et al., 2008; Lammerts van Bueren et al., 2011). Potential drift effects may have moderated low-input stress in 2024, underscoring the importance of the fully separated field design implemented in 2025. The use of an augmented block design in 2025 enabled evaluation of numerous populations while maintaining statistical control through replicated checks. This design is particularly well-suited for early-generation breeding trials, where seed availability limits replication but broad population screening is essential (Kang, 1993; Cooper et al., 1995; Atlin et al., 2000). Additionally, the small number of populations contributing to population-level regressions ($n = 8$) limits statistical power for parent-progeny analyses, and the R^2 values reported in Table 3 should be interpreted as indicative of predictability trends rather than definitive estimates of heritability. The inclusion of two reference checks provided valuable benchmarks for interpreting population performance and confirmed differential adaptation of breeding material relative to established germplasm (Murphy et al., 2007; Wolfe et al., 2008; Boyles et al., 2022).

Future directions

This research represents the initial phase of a broader breeding effort focused on improving sorghum adaptation to low-input and organic production systems. Building upon the populations evaluated in this study, additional breeding work was conducted in the 2025-2026 winter nursery season in Puerto Vallarta, Mexico, where 154 hybrid crosses were developed using two elite female parental lines and selected resilient breeding families identified from the current study.

These hybrid populations will be evaluated during the 2026 summer growing season in both Manhattan and Hays, Kansas, to assess hybrid performance across contrasting

environmental conditions representative of the U.S. Great Plains. Expanding evaluation across multiple locations will allow further characterization of G×E and G×E×M interactions while also providing insight into hybrid stability, reproductive performance, and low-input adaptation under broader production conditions.

Future work will also focus on determining whether the resilience patterns identified in early-generation populations are retained in hybrid combinations and whether hybrid performance improves stability of reproductive traits under low-input stress. Continued evaluation across environments and generations will help refine selection strategies for sorghum breeding programs targeting organic and resource-limited production systems.

Conclusions

This study evaluated sorghum population performance across contrasting management systems to assess trait expression, cross-generation predictability, and selection strategies for low-input environments. Results demonstrated that low-input conditions imposed substantial stress, particularly on reproductive traits, with SN and SW showing the greatest reductions and highest sensitivity to environmental variation. In contrast, PH exhibited consistent cross-generation predictability, while HW showed moderate stability, indicating that trait responses to stress vary in their reliability for selection.

Parent-progeny relationships further revealed that early-generation performance was a poor predictor of later performance for yield-component traits, particularly under low-input conditions, emphasizing the importance of evaluating populations within target environments. The RSI analysis showed that superior performance under low-input management was associated with balanced expression across FT, PH, SN, SW, and HW rather than maximum performance in individual traits. Populations such as P1 (GB07118/BTx2921) and P8 (GR04104/RTx2939)

demonstrated stable performance across traits, while P6 (R17161/R14047) and P4 (GR04060/RTx2912), as well as Check 2 (Macia), exhibited greater sensitivity to stress.

Overall, these findings highlight the importance of selection strategies that integrate multi-trait evaluation and prioritize stability of reproductive traits under stress. Breeding for low-input systems requires evaluation under representative conditions to effectively identify resilient populations and improve long-term adaptation in organic and low-input sorghum production systems.

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Chapter 3 - Understanding soil microbiology in agricultural systems

Introduction

Soil microorganisms are central components of agricultural ecosystems because they regulate nutrient cycling, organic matter decomposition, plant health, and soil biological functioning (Chen et al., 2024). The rhizosphere, defined as the soil region directly influenced by plant roots, is one of the most biologically active interfaces in the soil environment. Microorganisms in this zone affect carbon, nitrogen, phosphorus, and potassium cycling, while also influencing plant nutrient acquisition, growth, and responses to biotic and abiotic stress (Schlemper et al., 2017). The soil microbiome includes bacteria, archaea, fungi, and other microorganisms that interact with one another and with plant roots. Beneficial microbial groups commonly associated with crops include plant-growth-promoting rhizobacteria, arbuscular mycorrhizal fungi, plant-growth-promoting fungi, and endophytic microorganisms (Aina & Donaldson, 2025). These microorganisms can increase nutrient availability, produce plant-growth-promoting compounds, improve stress tolerance, suppress pathogens, and modify root development.

The rhizosphere microbiome is shaped by both environmental and biological factors. Soil pH, texture, moisture, nutrient availability, organic matter, land-use history, and fertilization influence the microbial pool from which rhizosphere communities are assembled. At the same time, plant species, genotype, root architecture, developmental stage, and root exudate chemistry determine which members of the soil microbiome are selectively enriched near roots (Schlemper et al., 2017). Agricultural management influences microbial community structure through changes in soil chemistry and indirectly through effects on plant growth (Hartmann et al., 2014).

Fertilization impacts nutrient availability and can alter microbial competition, while crop identity and rotation determine the type and quantity of carbon entering the rhizosphere.

Root exudates and rhizosphere community assembly

Plant roots actively shape the rhizosphere through rhizodeposition (Badri & Vivanco, 2009). Root-derived compounds include sugars, amino acids, organic acids, vitamins, hormones, flavonoids, terpenoids, and other secondary metabolites that provide substrates for microbial growth and act as chemical signals between plants and microorganisms (Akiyama et al., 2005; van Dam & Bouwmeester, 2016). Root exudates can enrich microorganisms that possess the metabolic traits required to use carbon compounds. This process creates a selective environment in which microbial taxa from the surrounding soil are recruited to the rhizosphere. The plant actively participates in the assembly of a root-associated microbial community through the quantity, composition, and timing of root-derived resources.

Root exudation is not constant throughout the growing season. As plants develop, changes in root growth, nutrient demand, photosynthetic activity, and reproductive allocation alter the chemical composition of exudates. These changes can produce developmental shifts in the microbial community and may explain why microbial communities associated with young plants differ from those associated with flowering or grain-filling plants (Vives-Peris et al., 2020; Schlemper et al., 2017). The chemical composition of root exudates can also vary among genotypes. Differences in primary metabolites, organic acids, flavonoids, sorgoleone, and strigolactones (specifically in sorghum) can modify the microbial taxa that are enriched around individual plants. Genotype-associated microbiome variation may arise from differences in root morphology, chemical resources, and signals released into the soil.

Developmental stage as a driver of the rhizosphere microbiome

Plant developmental stage is one of the most consistent factors associated with changes in rhizosphere microbial communities (Chaparro et al., 2013). As plants progress from emergence through vegetative growth, flowering, and grain development, their root systems expand and their downward patterns of carbon and nutrient allocation change. These developmental transitions alter the physical and chemical conditions experienced by rhizosphere microorganisms.

Evidence from sorghum indicates that developmental stage interacts with soil conditions and plant genotype to shape microbial community composition. Schlemper et al. (2017) evaluated seven sorghum cultivars grown in two soil types and sampled the rhizosphere at four developmental stages. At early stages, corresponding to the emergence of the second and third leaves, soil type was the primary factor influencing rhizosphere bacterial composition. At later stages, including the transition toward reproductive growth and the final visible leaf stage, cultivar effects became more apparent. When soil type, growth stage, and cultivar were evaluated simultaneously, soil type explained the greatest proportion of community variation, followed by plant growth stage and cultivar. Soil type, growth stage, and cultivar accounted for 68.3%, 14.2%, and 9.7% of the covariation with rhizosphere bacterial community composition. The contribution of genotype also increased later in plant development. On day 35, both soil and cultivar significantly affected the sorghum rhizosphere bacterial community, while at day 50, soil, cultivar, and their interaction were significant. These results indicate that the influence of genotype may be difficult to detect during early plant development because the microbial community is strongly influenced by the soil environment and because root-derived selection may become more deterministic as the plant matures. The developmental pattern was not

uniform across cultivars or soils. In one soil, the Striga-resistant cultivar SRN-39 was associated with higher relative abundances of several bacterial groups, including members of Acidobacteria, Burkholderia, Cupriavidus, Acidovorax, and Albidiferax. However, the cultivar effect was not observed in the more fertile soil, demonstrating that genotype effects can depend on the environmental context in which plants are grown (Schlemper et al., 2017).

These findings are relevant for sorghum breeding because the developmental stage at which plants are sampled may determine whether genotype-associated microbiome variation is detectable. A genotype that appears microbiologically similar to other genotypes at an early vegetative stage may exhibit a more defined rhizosphere community later in development.

Functional groups in the sorghum rhizosphere

The sorghum rhizosphere is a dynamic environment governed by continuous carbon allocation from the plant root system. Through rhizodeposition, which includes root exudates, mucilage, sloughed cells, and lysates, plant roots release a substantial fraction of their photosynthetically fixed carbon into the surrounding soil matrix (Badri & Vivanco, 2009; Haichar et al., 2008). This carbon flux acts as the primary energy source driving microbial proliferation, shaping a selective niche where specific functional groups thrive based on their metabolic strategies and nutrient requirements.

Bacterial communities represent a major fraction of the rhizosphere microbiome, with dominant phyla including Proteobacteria, Actinobacteria, and Acidobacteria, whose relative abundances vary according to soil properties, plant genotype, and developmental stage (Deng et al., 2021). Within these phyla, functional groups such as Gram-negative (G-) bacteria often exhibit copiotrophic characteristics, responding rapidly to labile carbon inputs in fresh root exudates. Gram-positive (G+) bacteria frequently display more stress-tolerant traits, persisting

under fluctuating moisture and nutrient conditions by utilizing more complex organic substrates (Finney et al., 2017). Actinobacteria, including actinomycete bacteria, constitute another essential group recognized for their capacity to decompose recalcitrant organic polymers, produce bioactive secondary metabolites, and suppress soil-borne pathogens (Hayat et al., 2010; Islam et al., 2016). Plant-growth-promoting rhizobacteria within these groups further enhance crop performance through biological nitrogen fixation, phosphate solubilization, production of phytohormones such as indole-3-acetic acid, and siderophore-mediated iron acquisition (Hayat et al., 2010).

Fungal communities complement bacterial roles by mediating both organic matter decomposition and symbiotic nutrient exchange. Saprophytic fungi drive the breakdown of complex plant residues and soil organic matter, facilitating carbon turnover and nutrient mineralization. In contrast, symbiotic arbuscular mycorrhizal fungi (AMF) form direct intracellular associations with root cortical tissues, effectively expanding the spatial volume of soil explored by the plant root system (Smith & Read, 2008; Wang & Qiu, 2006). AMF are critical for acquiring diffusion-limited nutrients such as phosphorus and improving plant water relations under stress, drawing carbon allocations from the host plant to maintain their extraradical mycelium and storage structures (Andrino et al., 2020).

To quantify these microbial groups and assess their responses to environmental factors, phospholipid fatty acid (PLFA) and neutral lipid fatty acid (NLFA) profiling serve as essential biochemical tools. PLFA analysis provides an integrative snapshot of living microbial biomass and structural community composition, distinguishing functional groups such as G⁺ bacteria, G⁻, actinomycetes, saprophytic fungi, and AMF based on signature lipid biomarkers (Finney et al., 2017). However, PLFA limitations include its inability to resolve taxonomic shifts at the species

level and potential overlap of specific fatty acid signatures across different microbial taxa. To specifically track arbuscular mycorrhizal fungi, NLFA profiling targeting the neutral lipid fatty acid 16:1 ω 5c is widely used because neutral lipids function primarily as energy storage molecules within AMF structures like spores and vesicles. Tracking NLFA 16:1 ω 5c alongside PLFA profiles allows researchers to differentiate between active vegetative fungal biomass and fungal energy storage reserves, offering deeper insight into carbon allocation and mycorrhizal physiological status under varying nutrient regimes (Andrino et al., 2020; Yao & Wu, 2010).

Arbuscular mycorrhizal fungi

Arbuscular mycorrhizal fungi form symbiotic associations with the roots of 80-90% of terrestrial plant species (Smith and Read, 2008). AMF penetrate the root cortex and form structures including arbuscules, vesicles, and intraradical hyphae. Arbuscules provide interfaces for nutrient exchange, while vesicles and spores function in fungal storage and propagation. In exchange for plant-derived carbon, AMF can improve the acquisition of phosphorus, nitrogen, potassium, zinc, iron, water, and other nutrients (Diagne et al., 2020).

AMF are very relevant to sorghum because the crop is frequently cultivated in environments characterized by low phosphorus availability, drought, and other stresses. Sorghum can respond to low phosphorus through changes in root architecture, release of root exudates, and association with AMF. These mechanisms can function together to improve phosphorus acquisition and phosphorus-use efficiency (de Oliveira et al., 2025). However, the benefits of AMF are not uniform among sorghum genotypes. Watts-Williams et al. (2018) evaluated diverse sorghum accessions and found considerable variation in growth, nutrient responses, transcriptional responses, and mycorrhizal dependence. Plant responsiveness depended not only on the sorghum accession, but also on the AMF species colonizing the plant. This genotype-by-

fungus interaction has significant implications for breeding. A genotype that responds positively to one AMF species may respond weakly or negatively to another. Therefore, AMF colonization percentage alone may not predict the functional benefit of the symbiosis unless fungal identity, soil conditions, phosphorus availability, and plant genotype are also considered.

In a study of diverse sorghum genotypes, AMF-colonized plants generally produced greater grain yield and accumulated more phosphorus, zinc, and iron in the grain than non-mycorrhizal controls (Watts-Williams et al., 2021). These effects were relevant under low-phosphorus conditions, although responses varied among genotypes and soil phosphorus treatments.

Field studies also demonstrate variation in AMF root colonization. Leiser et al. (2015) evaluated West African sorghum genotypes at an early developmental stage and at flowering under low- and high-phosphorus conditions. AMF colonization was higher in low-phosphorus field conditions than in high-phosphorus field conditions, and significant genotypic variation was observed at both developmental stages. The timing of AMF measurements is critical. Root colonization reflects fungal structures within the sampled roots at a specific developmental stage, while biochemical markers such as NLFA 16:1 ω 5 can reflect AMF storage lipids associated with spores, vesicles, and other fungal structures within soil (Ngosong et al., 2012).

Strigolactones and LGS1 genotype

Strigolactones are plant hormones and rhizosphere signaling compounds that regulate aspects of shoot and root development (Gomez-Roldan et al., 2008; Umehara et al., 2008; Waters et al., 2017). They also function as signals in interactions between plants and a broad range of micro and macroorganisms. Strigolactones released by roots stimulate AMF hyphal branching and fungal activity before the fungus physically contacts the root (Akiyama et al., 2005; Besserer et al., 2006).

Strigolactone production is often enhanced under nutrient-limited conditions, particularly when phosphorus or nitrogen availability is low (Yoneyama et al., 2010). This response may increase the probability of establishing AMF symbiosis and improving nutrient acquisition. However, the same compounds that recruit beneficial fungi can also act as germination signals for root-parasitic plants. The parasitic weed *Striga hermonthica* exploits strigolactones released by sorghum roots. *Striga* seeds can remain dormant in the soil for up to 20 years until they detect host-derived germination stimulants (Makaza et al., 2023; Shimels et al., 2024). After germination, the parasite forms a haustorium that penetrates the host root and establishes a vascular connection. This creates a biological conflict in which strigolactones can simultaneously support beneficial AMF symbiosis and increase the risk of *Striga* infection. The ecological importance of this conflict is particularly high in sub-Saharan Africa, where *Striga* is a major constraint to sorghum production (Yali, 2022).

To mitigate this threat, host genetics play a critical role, particularly natural variation at the LOW GERMINATION STIMULANT 1 (LGS1) locus in sorghum (Gobena et al., 2017). The LGS1 gene encodes a sulfotransferase enzyme that modifies strigolactone biosynthesis, shifting the dominant exudate profile from 5-deoxystrigol, which is a highly potent germination stimulant for *Striga*, toward orobanchol-type compounds with significantly reduced stimulatory activity on parasite seeds (Gobena et al., 2017; Shimels et al., 2024). Because these altered strigolactones maintain essential internal plant functions and support beneficial mycorrhizal associations without triggering parasite germination, LGS1 variation provides a vital genetic mechanism for conferring resistance without completely disrupting belowground chemical communication (Bellis et al., 2020). The ecological and agricultural impact of this host-parasite dynamic remains particularly severe across sub-Saharan Africa, where nutrient-depleted soils

and high *Striga* infestation levels pose a major constraint to sustainable sorghum production (Degebasa et al., 2022; Yali, 2022).

AMF, striga, and the soil microbiome

AMF can also influence the interaction between sorghum and *Striga*. Inoculation or colonization by AMF has been associated with lower *Striga* germination, attachment, and emergence on sorghum roots. These effects may result from changes in host nutrition, root exudate chemistry, plant defense responses, or competition among microorganisms for root colonization sites. The broader soil microbiome can also influence *Striga* infection independently of AMF. Kawa et al. (2024) demonstrated that sorghum grown in natural soil had fewer *Striga* attachments than plants grown in sterilized soil. The suppressive soil microbiome was associated with changes in sorghum root anatomy, including increased endodermal suberization and cortical aerenchyma formation. The same study found that soil microorganisms reduced the formation of *Striga* haustoria by modifying host-derived haustorium-inducing factors. Specific bacterial taxa, including *Pseudomonas* and *Arthrobacter*, were associated with degradation of host signals or induction of structural changes that reduced parasite penetration. Soil microorganisms such as AMF can affect parasitic plant infection by altering root development, root chemistry, and host-parasite signaling. They also suggest that the effect of a sorghum genotype on *Striga* resistance may depend partly on the microbial community with which that genotype interacts.

Genotypic effects on the rhizosphere microbiome

Plant genotype can influence the rhizosphere microbiome through differences in root exudation, root architecture, nutrient acquisition, immune responses, and developmental timing. Although soil environmental conditions often explain a large proportion of microbial variation, genotype-associated differences can be detected in both bacterial and fungal communities.

Deng et al. (2021) evaluated rhizosphere bacterial communities from 200 sorghum genotypes grown under field conditions. Their analysis identified bacterial lineages with heritable associations with plant genotype and detected host genomic loci associated with the abundance of specific rhizosphere microorganisms. In a field experiment involving 600 sorghum plants, phylogenetic relationships among genotypes were more strongly associated with microbiome similarity in the rhizosphere than in leaf or root-endosphere samples. This study also demonstrated that sorghum genotypic information could predict aspects of rhizosphere microbiome composition. A genomic region on chromosome 4 was associated with variation in bacterial community structure, and allele groups at this locus formed distinct rhizosphere microbiome clusters. However, the magnitude of genotype effects depends on soil and developmental context.

AMF responses show similar genotype dependence. Sorghum accessions vary in AMF colonization, nutrient acquisition, growth response, and transcriptional response, and the direction of the response can depend on the AMF species present (Watts-Williams et al., 2018). These findings support genotype-associated microbial traits may be valuable for crop improvement, but only when evaluated in the environmental and developmental context in which they are expressed.

The need for field-based research

Much of the evidence connecting strigolactones, AMF, and plant microbiomes has been generated under controlled conditions. Controlled experiments are valuable, as they allow researchers to isolate nutrient treatments, microbial inoculation, plant genotype, and specific mutations. However, greenhouse experiments do not reproduce the spatial heterogeneity, microbial complexity, weather variability, and root development observed in agricultural fields.

Field conditions contain diverse native microbial communities and fluctuating environmental conditions. Soil moisture, nutrient availability, temperature, root age, plant density, and field history can affect both plant development and microbial community composition. These factors may also modify the expression of genotype-associated traits.

The current study

The available evidence identifies several unresolved questions. First, although developmental stage is known to influence sorghum rhizosphere communities, few studies have evaluated microbial functional groups at both an early vegetative stage and a reproductive stage within the same field experiment. Second, the relationship between genotype and rhizosphere microbial composition may become stronger later in development, but this relationship has not been fully evaluated in early-generation sorghum breeding materials. Third, the relationship between *LGS1* genotype, strigolactone-associated physiology, AMF abundance, and broader rhizosphere community structure remains uncertain under field conditions. Previous studies have demonstrated that *LGS1* affects strigolactone stereochemistry and *Striga* resistance, while more recent work suggests that strigolactone pathway mutations may also affect AMF establishment, root development, and plant performance. However, these effects may depend strongly on genetic background.

The study tests whether developmental stage is a stronger driver of rhizosphere microbial variation than breeding population or genotype identity, whether AMF abundance and root colonization change between V5 and reproductive development, and whether *LGS1* status is associated with detectable differences in AMF abundance or broader rhizosphere microbial community structure. By combining developmental-stage comparisons, early-generation breeding materials, *LGS1*-characterized genotypes, AMF measurements, and field-based

microbial profiling, this work provides a framework for evaluating whether rhizosphere microbial traits can be incorporated into sorghum breeding research.

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Chapter 4 - Rhizosphere Microbial Dynamics and AMF in Sorghum

Introduction

Soil microbial communities mediate critical ecosystem functions including nutrient cycling, organic matter decomposition, and plant health (Trivedi et al., 2020). In the rhizosphere, plants actively shape these communities through root exudates, establishing feedback loops that influence plant growth and fitness (Vives-Peris et al., 2020). Understanding which factors most strongly govern rhizosphere community assembly under field conditions, including host genetics, soil environment, or plant development, remains a central question with direct relevance to breeding programs seeking to harness microbiome-associated traits.

Plant developmental stage has repeatedly been identified as one of the strongest drivers of rhizosphere microbial community assembly. As plants transition from vegetative growth to reproduction, changes in root architecture, nutrient demand, and carbon allocation alter both the quantity and composition of root exudates entering the soil, and these developmental shifts function as ecological filters that regulate microbial succession throughout the growing season (Vives-Peris et al., 2020). For sorghum specifically, 16S rRNA gene sequencing of seven cultivars sampled across four growth stages showed that rhizosphere bacterial community composition shifted with developmental stage, with genotype-linked effects becoming detectable only once plants approached the vegetative-to-reproductive transition (Schlemper et al., 2017). A similar age-driven restructuring has been documented outside cereal systems: host genetic control over community composition was evident in leaf tissue but not root tissue of a wild perennial mustard sampled across multiple growth stages (Wagner et al., 2016).

These taxonomic-resolution studies establish who is present in the rhizosphere at a given developmental stage. Phospholipid fatty acid (PLFA) and neutral lipid fatty acid (NLFA)

determine how living microbial biomass is allocated among broad functional groups at the time of sampling. Because phospholipids are essential, non-storage components of living cell membranes that degrade rapidly following cell death, PLFA profiles capture currently living microbial biomass, partitioned into major functional groups (White et al., 1996; Frostegård & Bååth, 1996). The fatty acid 16:1 ω 5 in the neutral lipid fraction specifically indicates AMF storage structures such as spores and vesicles (Ngosong et al., 2012). Despite extensive sequencing-based characterization of sorghum rhizosphere community membership across developmental stages (Schlemper et al., 2017), comparatively little work has applied PLFA/NLFA profiling to track functional-group-level carbon allocation across sorghum developmental stages under field conditions, leaving the magnitude and timing of stage-driven shifts in living microbial biomass largely unknown.

The ability of plants to recruit beneficial microorganisms is mediated in part through root-derived signaling compounds, among which strigolactones are central. Strigolactones function both as endogenous plant hormones and as rhizosphere communication signals that stimulate fungal development prior to root physical contact (Akiyama et al., 2005; Besserer et al., 2006). In sorghum, natural variation at the LOW GERMINATION STIMULANT 1 (LGS1) locus alters the stereochemistry of exuded strigolactones. LGS1 encodes a sulfotransferase-like enzyme that acts on the strigolactone precursor; loss-of-function mutations shift exudate composition from predominantly 5-deoxystigol toward orobanchol-type compounds, reducing germination of the parasitic weed *Striga hermonthica* (Yoneyama et al., 2010; Gobena et al., 2017; Yoda et al., 2023). The consequences of LGS1 loss-of-function extend beyond strigolactone chemistry. LGS1 loss-of-function genotypes exhibit slower growth rate, developmental delay, altered gibberellin precursor accumulation, and enhanced lateral root

growth relative to LGS1-functional genotypes (Kawa et al., 2021). CRISPR-Cas9-edited sorghum lines show systemic down-regulation of photosystem-related genes in both root and shoot tissue, indicating that the Striga-resistance benefit of *lgs1* alleles is paired with a measurable cost to photosynthetic gene expression (Bellis et al., 2020). Population genomic analysis shows that *lgs1* loss-of-function alleles are broadly distributed in Striga-prone regions but largely absent elsewhere, a pattern of balancing selection consistent with a fitness cost that restricts fixation of the resistant allele (Bellis et al., 2020). Whether this fitness cost translates into a measurable yield consequence has not been directly established.

Direct evidence linking strigolactone variation to microbiome assembly has emerged only recently and largely from controlled-environment systems. In rice, altered strigolactone production influenced recruitment of root-associated microbial communities under controlled conditions (Kim et al., 2022). In sorghum, CRISPR-Cas9 deletion of *SbCCD8b*, a gene upstream of LGS1 in the strigolactone biosynthesis pathway, diminished both the establishment and host benefit of arbuscular mycorrhizal symbionts under controlled growth conditions (McLaughlin et al., 2025). Restoring functional LGS1 into a normally non-functional background produced inconsistent phenotypic effects across independent mutant alleles, suggesting that genetic background can buffer or modify the physiological consequences of LGS1 status (McLaughlin et al., 2025). Whether LGS1-specific, genotype-dependent effects on rhizosphere microbial communities remain detectable under field conditions, where plant development, soil heterogeneity, and seasonal variation all vary at the same time remains largely untested, particularly within early-generation breeding populations.

Among the microbial groups most closely linked to strigolactone signaling are arbuscular mycorrhizal fungi (AMF), which form mutualistic associations with 80-90% of terrestrial plant

species and play central roles in phosphorus acquisition and plant stress tolerance (Diagne et al., 2020). In sorghum, substantial genotypic variation exists in both growth and transcriptional responsiveness to AMF colonization (Watts-Williams et al., 2018), and AMF colonization has been shown to enhance sorghum grain yield and nutritional quality across a range of soil phosphorus availabilities (Watts-Williams et al., 2021).

Relatively few studies have simultaneously evaluated developmental stage, host genetic background, and LGS1 status within field-grown breeding populations. Most direct evidence supporting strigolactone-mediated microbial recruitment originates from controlled-environment or non-sorghum systems (Kim et al., 2022; McLaughlin et al., 2025), leaving uncertainty regarding the extent to which genotype-dependent effects can be detected under production-scale field conditions. While prior work indicates that developmental stage strongly influences microbial community assembly in sorghum (Schlemper et al., 2017), its influence relative to population identity and LGS1-mediated variation specifically has not been evaluated within the same field experiment. Addressing this gap is relevant for breeding programs because early-generation populations represent the stage at which selection decisions are initiated, yet little is known about whether microbiome-associated variation can be reliably detected at this point in the breeding process, before residual heterozygosity has been reduced by further selfing.

The objectives of this study were to (i) determine whether rhizosphere microbial biomass and community composition differ between vegetative and reproductive developmental stages, (ii) quantify developmental changes in AMF abundance and root colonization within the LGS1 genotype panel, (iii) evaluate the influence of F_{2:3} population identity and LGS1 genotype status on rhizosphere microbial communities, and (iv) examine relationships among microbial

characteristics, plant nutrient status, and agronomic performance under conventional field management.

Materials and Methods

Study site

Field experiments were conducted during the 2025 growing season at the Kansas State University Ashland Bottoms Research Farm near Manhattan, Kansas, USA (39.14 degrees N, 96.64 degrees W). The experimental field is located on a Eudora silt loam (coarse-silty, mixed, mesic Fluventic Haplustoll). Soil pH ranged from 8.1 to 8.4 across the field, indicating uniformly alkaline conditions. Complete soil chemical and physical properties are reported in Supplementary Table 4.1.

Table 4.1 Soil chemical and physical properties at the Ashland Bottoms Research Farm field site prior to planting (Kansas State University Soil Testing Laboratory). Four composite samples were analyzed for macronutrient, micronutrient, organic matter, and particle-size composition to characterize the conventional-management field used in Chapter 2.

Property	Quadrant #1	Quadrant #2	Quadrant #3	Quadrant #4
<i>Reaction & Organic Status</i>				
pH	8.1	8.4	8.3	8.4
Sikora pH	7.5	7.6	7.6	7.6
Organic Matter, LOI (%)	1.6	1.1	1.2	0.9
Total Organic C (%)	0.78	0.52	0.52	0.37
Total N (%)	0.08	0.06	0.06	0.03
<i>Macronutrients</i>				
P, Mehlich-3 (ppm)	41.8	36.7	25.0	21.8
Total P (ppm)	523	444	472	369

Property	Quadrant #1	Quadrant #2	Quadrant #3	Quadrant #4
K (ppm)	220	179	157	146
Ca (ppm)	2008	2144	2186	2321
Mg (ppm)	64.1	53.5	54.9	48.8
Na (ppm)	7.9	6.8	9.6	10.8
S (ppm)	1.3	0.8	1.4	1.5
<i>Micronutrients & Trace Elements</i>				
Zn (ppm)	0.9	0.7	0.8	0.4
Fe (ppm)	5.0	4.7	4.7	3.8
Mn (ppm)	9.8	7.5	8.5	4.2
Cu (ppm)	0.8	0.9	1.0	0.9
Al (ppm)	0.59	0.51	0.10	0.10
As (ppm)	1.19	1.31	1.21	1.25
Cd (ppm)	0.34	0.31	0.30	0.31
Pb (ppm)	6.73	6.26	6.49	6.21
Cr (ppm)	6.99	6.42	6.10	5.87
<i>Soil Texture</i>				
Sand (%)	42	52	46	58
Silt (%)	48	38	44	32
Clay (%)	10	10	10	10

The field received a pre-plant fertilizer application of 299 L ha⁻¹ urea-ammonium nitrate (UAN, 28-0-0) and 47 L ha⁻¹ ammonium thiosulfate (12-0-0-26S), supplying a total of approximately 115 kg N ha⁻¹ (107.5 kg N ha⁻¹ from UAN and 7.5 kg N ha⁻¹ from ATS) along with 16 kg S ha⁻¹. No herbicides or arbuscular mycorrhizal fungal inoculants were applied during the study.

Plant material

Eight F_{2:3} populations derived from Kansas State University sorghum breeding crosses were included to represent early-generation breeding material (Table 4.2).

Table 4.2 Sorghum plant materials comprising eight F_{2:3} breeding populations and two check varieties characterized for rhizosphere microbial community structure, and a panel of LGS1-characterized genotypes, including the RTx430 near-isogenic line pair, used to test the influence of LGS1 status on rhizosphere dynamics.

Label	Pedigree / Identity	LGS1 Status	Generation
P1	(GB07118/BTx2921)-MAF1	—	F _{2:3}
P2	(GB11076/BTx2923)-MAF1	—	F _{2:3}
P3	(GBTx2926/B11076)-MAF1	—	F _{2:3}
P4	(GR04060/RTx2912)-MAF1	—	F _{2:3}
P5	(GR08303/RTx2916)-MAF1	—	F _{2:3}
P6	(R17161/R14047)-CSF1	—	F _{2:3}
P7	(GR07178/R11158)-MAF1	—	F _{2:3}
P8	(GR04104/RTx2939)-MAF1	—	F _{2:3}
C1	Macia	—	Inbred
C2	85P58	—	Hybrid
BTx398-1-2	(BTx398*2/2/(BTx398ms3/Macia)-1)-1-2	lgs1-1 (loss-of-function)	F ₂ :BC ₂ F ₃
RTx430	RTx430	lgs1-2 (loss-of-function)	Inbred
RTx430 NIL	RTx430*3/4/(RTx430*4/3/((Tx430/IRAT204)-6-1/2/RTx430)-3)-1-1-1	LGS1 (functional)	BC ₈ F ₃
Tx7000	Tx7000	LGS1 (functional)	Inbred
BTx3042-2-4	(BTx3042*2/2/(BTx3042ms3/Macia)-1)-2-4	LGS1 (functional)	F ₂ :BC ₂ F ₃

Label	Pedigree / Identity	LGS1 Status	Generation
BTx3042-2-10	(BTx3042*2/2/(BTx3042ms3/Macia)-1)-2-10	LGS1 (functional)	F ₂ :BC ₂ F ₃
BTx3042-2-13	(BTx3042*2/2/(BTx3042ms3/Macia)-1)-2-13	LGS1 (functional)	F ₂ :BC ₂ F ₃
BTx398-1-1	(BTx398*2/2/(BTx398ms3/Macia)-1)-1-1	LGS1 (functional)	F ₂ :BC ₂ F ₃
BTx398-1-3	(BTx398*2/2/(BTx398ms3/Macia)-1)-1-3	LGS1 (functional)	F ₂ :BC ₂ F ₃
BTx398-1-5	(BTx398*2/2/(BTx398ms3/Macia)-1)-1-5	lgs1-7 (loss-of-function)	F ₂ :BC ₂ F ₃

Populations were planted in unreplicated plots within an augmented block design. Two check varieties were included as agronomic and biological references and were replicated across all 45 blocks. 85P58 (Pioneer Hi-Bred International), a grain sorghum hybrid, was positioned at the center of each block. Macia (SDS 3220), our inbred check was placed at a random position within each block, yielding 45 plots per check variety distributed across the full field area.

Ten sorghum genotypes with confirmed LOW GERMINATION STIMULANT 1 (LGS1) status were included to evaluate whether strigolactone exudate profile differences linked to LGS1 function produce detectable shifts in rhizosphere microbial community structure. LGS1 status for each genotype was confirmed by PCR-based genotyping prior to field planting. The panel comprised seven genotypes carrying functional LGS1 alleles and three genotypes carrying confirmed loss-of-function *lgs1* alleles (Table 4.2).

Experimental design and developmental stage sampling

The experiment was arranged as an augmented incomplete block design with 45 blocks distributed across 688 total plots. Each block contained one plot of each check variety, with F_{2:3} populations and LGS1-characterized genotypes occupying unreplicated plots within blocks. This

design allowed spatial field variation to be accounted for through check variety performance while maximizing the number of unreplicated entries evaluated.

The sorghum field was planted on 23 May 2025 at a seeding rate of 30.5 seeds m⁻² in plots measuring 2.59 by 0.76 m with 0.91 m alleys between plots. Within each plot, individual plants were randomly chosen for destructive rhizosphere sampling at V5, destructive rhizosphere sampling at the reproductive stage, and agronomic performance measurements. Five plants per plot were designated for agronomic measurement. One plant per plot was designated for destructive harvest at V5, and a separate individual for destructive harvest at the reproductive stage. Buffer plants were maintained between sampled and measured individuals to minimize soil disturbance effects.

Destructive sampling was conducted at two developmental stages. The first targeted the V5 vegetative stage (five leaf collars fully visible), completed on 14 July 2025 (52 days after planting). The second targeted the reproductive stage, encompassing anthesis through grain fill, completed on 12 September 2025 (112 days after planting). All plants at the reproductive stage were confirmed to have reached or surpassed anthesis.

In total, 66 plants were chosen for destructive sampling at each developmental stage, for 132 plants designated across the full experiment. Of these, 65 V5-stage plants and all 66 reproductive-stage plants were successfully collected, yielding 131 plants from which rhizosphere soil samples were obtained for PLFA/NLFA.

Plant tissue sampling and aboveground biomass

At the V5 stage, only rhizosphere soil and root samples were collected. At the reproductive stage, the entire aboveground portion of each sampled plant was severed at the soil surface, dried at 60⁰ C for 48 h, and weighed to obtain aboveground dry biomass per plant. Dried

tissue was ground and submitted to the Kansas State University Soil Testing Laboratory for elemental analysis. Total nitrogen and total carbon were determined by dry combustion using a LECO TruSpec CN combustion analyzer (LECO Corp.; St. Joseph, MI). Total phosphorus and potassium were determined following sulfuric acid/hydrogen peroxide digestion, with analysis by inductively coupled plasma (ICP) spectrometry (Kansas State University Soil Testing Laboratory). Of the 66 reproductive-stage plants sampled, 65 were analyzed for tissue nutrients; one plant was inadvertently not harvested during tissue collection.

Rhizosphere soil sampling and PLFA/NLFA analysis

At each sampling date, the root system of each designated plant was excavated, and rhizosphere soil was collected by shaking dislodged soil from root surfaces. A 5 g subsample per plant was immediately frozen at -80⁰ C until processing. Prior to lipid extraction, subsamples were freeze-dried. PLFA and NLFA analyses were conducted in the Soil Agroecology Laboratory, Department of Agronomy, Kansas State University. Lipids were extracted from 5 g freeze-dried rhizosphere soil and fractionated by silicic acid chromatography. The phospholipid and neutral lipid fractions were separately transesterified to fatty acid methyl esters (FAMES) and analyzed by gas chromatography-mass spectrometry following the protocol of White & Burton (2007). FAME peaks were identified by retention time comparison with a bacterial acid methyl ester mix and quantified using 19:0 FAME as internal standard. A total of 30 biomarkers were identified across all samples. PLFA abundance was reported as nanomoles per gram of dry soil.

Microbial groups were assigned based on established biomarker characteristics (Bonini Pires, 2023; White et al., 1996): iso and anteiso branched fatty acids (i15:0, a15:0, i16:0, i17:0, a17:0) to gram-positive bacteria; monounsaturated and cyclopropyl fatty acids (16:1 ω 9,

16:1 ω 11, cy17:0, 18:1 ω 7, cy19:0) to gram-negative bacteria; 10Me18:0 to actinomycetes; and 18:2 ω 6,9 to saprophytic fungi. The NLFA 16:1 ω 5c biomarker was used as an indicator of AMF storage lipid associated with spores and vesicles (Ngosong et al., 2012). Total microbial biomass (TMB) was estimated as the summed concentration of all PLFA biomarker fatty acids. Total bacteria (TB) were calculated as the sum of gram-positive, gram-negative, and actinomycete biomarkers. Total fungi (TF) were calculated as the sum of AMF and saprophytic fungi. The fungal-to-bacterial (F:B) ratio was TF/TB, and the gram-positive-to-gram-negative (GP:GN) ratio was the sum of gram-positive divided by gram-negative biomarkers.

AMF root colonization assessment

Fine lateral root segments were excised from each excavated root system at the time of rhizosphere sampling and preserved in 50% ethanol. Root clearing and staining followed a trypan blue procedure: roots were submerged in 10% KOH at 70⁰ C for 45 minutes, acidified in 2% HCl for 15 min., and stained overnight at room temperature in lactoglycerol supplemented with trypan blue. Stained root segments were mounted on glass slides and AMF colonization was quantified using the gridline intersect method (McGonigle et al., 1990) at 100-200x magnification across at least 100 intersections per sample. Total percent AMF colonization was calculated as the proportion of root intersections at which any AMF structure (hyphae, arbuscules, or vesicles) was observed.

Agronomic performance data

Agronomic performance traits were measured on five designated companion plants per plot carried through to physiological maturity. Plant height was measured at physiological maturity as the distance from the soil surface to the tip of the panicle. Days to flowering were recorded as the number of days from planting to tip-flowering anthesis per plot. As each plant

reached tip-flowering, panicles were individually bagged to enforce self-pollination. At physiological maturity, panicles were hand-harvested, dried, and threshed, and we characterized yield as total seed weight per panicle. Plot-level means were calculated across five designated plants per plot.

Because agronomic traits were measured on companion plants rather than the same individuals used for destructive rhizosphere sampling, agronomic data are represented as plot-level means. For each PLFA observation, the agronomic values reported reflect the mean across all specific plots from which the rhizosphere sample was collected.

Statistical analysis

All statistical analyses were performed using R (R Core Team, 2024). Linear mixed effects models were fit using lme4 (Bates et al., 2015) with significance testing via lmerTest (Kuznetsova et al., 2017) using Satterthwaite's method. Estimated marginal means were obtained using emmeans (Lenth, 2023), and compact letter displays were generated using multcomp (Hothorn et al., 2008) following Tukey-adjusted pairwise contrasts. Multivariate community composition was analyzed using vegan (Oksanen et al., 2024) and ape (Paradis & Schliep, 2019). Sum-to-zero contrasts were applied prior to analysis to facilitate Type III hypothesis testing.

Multivariate Community Analysis. Rhizosphere microbial community composition was represented as the five PLFA functional group totals. Bray-Curtis dissimilarities were visualized by principal coordinates analysis (PCoA) and evaluated by permutational multivariate analysis of variance (PERMANOVA) using adonis2 with 999 permutations. The effect of developmental stage was evaluated across the combined dataset for each material class, and the effect of population or genotype identity was tested separately within each developmental stage.

Univariate Analysis of F_{2:3} Populations and Check Varieties. Response variables were analyzed using linear mixed effects models with developmental stage and population identity as fixed effects, their interaction, field block as a random effect, and residual error. Fixed effects were evaluated using Type III ANOVA. Significant effects were explored using Tukey-adjusted pairwise comparisons among entries within developmental stage, and stage contrasts within entries were obtained from estimated marginal means using unadjusted pairwise comparisons.

Univariate Analysis of LGS1-Characterized Genotypes. Each genotype was represented by two plants per developmental stage ($n = 2 \text{ genotype}^{-1} \text{ stage}^{-1}$). Response variables were analyzed using a two-way ANOVA with genotype, developmental stage, and their interaction as fixed effects, without a block random effect.

AMF-Agronomic Relationships. Relationships between rhizosphere AMF abundance (NLFA 16:1 ω 5) and plant characteristics were evaluated at the reproductive stage using linear mixed effects regression models with standardized NLFA abundance as the predictor and population and block as random grouping factors.

Results

F_{2:3} populations and check varieties

Total rhizosphere microbial biomass did not differ significantly by developmental stage among F_{2:3} populations and checks ($p = 0.14$), ranging from 39.0 to 58.5 nmol PLFA g⁻¹ dry soil at V5 and 41.2 to 60.6 nmol PLFA g⁻¹ soil at the reproductive stage (Fig. 4.3A). No significant population or stage $\times$ population interaction effects were detected, and all entries shared the same Tukey grouping letter within each growth stage.

Among individual functional groups, actinomycetes increased significantly from V5 to the reproductive stage ($p = 0.0007$), with significant within-population increases in Population 1

($p = 0.02$), Population 3 ($p = 0.0008$), and Population 4 ($p = 0.008$) (Fig. 4.3B). Saprophytic fungal abundance differed by growth stage at the model level ($p = 0.033$), though no significant stage contrast was detected within any individual population (Fig. 4.3B). Gram-positive and gram-negative bacterial abundances were not affected by growth stage, population, or interaction effects (Gram⁺: $p = 0.303$; Gram⁻: $p = 0.518$) (Fig. 4.2B), except for Population 3, which had a significant stage-driven increase in both gram-positive ($p = 0.020$) and gram-negative ($p = 0.031$) bacterial abundance. The fungal-to-bacterial (F:B) ratio increased significantly from V5 to the reproductive stage ($p = 0.0007$). The gram-positive-to-gram-negative (GP:GN) ratio declined significantly over the same interval ($p = 0.019$). Entry-level F:B and GP:GN ratio means are reported in Table S2.

Table 4.3 Fungal-to-bacterial (F:B) and gram-positive-to-gram-negative (GP:GN) ratios of rhizosphere microbial communities at V5 and reproductive stages across F_{2:3} sorghum populations and LGS1-characterized genotypes (lgs1, nonfunctional; LGS1, functional). Values are mean $\pm$ standard error. Bold values indicate a statistically significant stage contrast within that specific entry (unadjusted pairwise comparison, $p < 0.05$).

		F:B ratio		GP:GN ratio	
Entry	LGS1 Status	V5	Reproductive	V5	Reproductive
F2:3 Populations and Checks					
Population 1	—	0.43 ± 0.09	0.46 ± 0.09	3.19 ± 0.47	3.33 ± 0.47
Population 2	—	0.60 ± 0.09	0.67 ± 0.09	2.9 ± 0.47	2.9 ± 0.47
Population 3	—	0.48 ± 0.09	0.42 ± 0.09	3.03 ± 0.47	2.91 ± 0.47
Population 4	—	0.29 ± 0.09	0.42 ± 0.09	2.73 ± 0.49	3.08 ± 0.49
Population 5	—	0.50 ± 0.09	0.75 ± 0.09	2.79 ± 0.49	3.60 ± 0.49

		F:B ratio		GP:GN ratio	
Entry	LGS1 Status	V5	Reproductive	V5	Reproductive
Population 6	—	0.44 ± 0.09	0.623 ± 0.09	2.59 ± 0.54	2.63 ± 0.54
Population 7	—	0.41 ± 0.09	0.517 ± 0.09	2.84 ± 0.45	2.94 ± 0.45
Population 8	—	0.47 ± 0.09	0.77 ± 0.09	3.03 ± 0.47	5.13 ± 0.47
Check 1 (Macia)	—	0.36 ± 0.12	0.65 ± 0.12	2.29 ± 0.59	3.32 ± 0.59
Check 2 (85P58)	—	0.58 ± 0.12	0.79 ± 0.12	2.16 ± 0.62	2.44 ± 0.62
<i>Stage effect p-value</i>			p = 0.0007		p = 0.019
LGS1-Characterized Genotypes					
<i>RTx430</i>	lgs1	0.41 ± 0.07	0.58 ± 0.11	2.69 ± 0.01	2.51 ± 0.14
<i>BTx398-1-2</i>	lgs1	0.28 ± 0.03	0.63 ± 0.02	2.76 ± 0.09	3.26 ± 0.95
<i>BTx398-1-5</i>	lgs1	0.37 ± 0.03	0.48 ± 0.06	3.64 ± 0.82	2.93 ± 0.74
<i>Tx7000</i>	LGS1	0.35 ± 0.01	0.57 ± 0.134	2.84 ± 0.17	3.26 ± 0.93
<i>BTx3042-2-4</i>	LGS1	0.28 ± 0.02	0.35 ± 0.01	2.97 ± NA	2.41 ± 0.03
<i>BTx3042-2-10</i>	LGS1	0.24 ± 0.01	0.58 ± 0.09	2.78 ± 0.07	2.47 ± 0.16
<i>BTx3042-2-13</i>	LGS1	0.30 ± 0.07	0.41 ± 0.09	2.82 ± 0.04	3.04 ± 0.52
<i>BTx398-1-1</i>	LGS1	0.26 ± 0.03	0.49 ± 0.01	2.70 ± 0.03	1.78 ± 0.50
<i>BTx398-1-3</i>	LGS1	0.36 ± 0.09	0.59 ± 0.02	2.98 ± 0.08	3.18 ± 0.95
<i>RTx430 NIL</i>	LGS1	0.31 ± 0.02	0.53 ± 0.01	2.74 ± 0.11	2.24 ± 0.09
<i>Stage effect p-value</i>			p < 0.001		p = 0.449

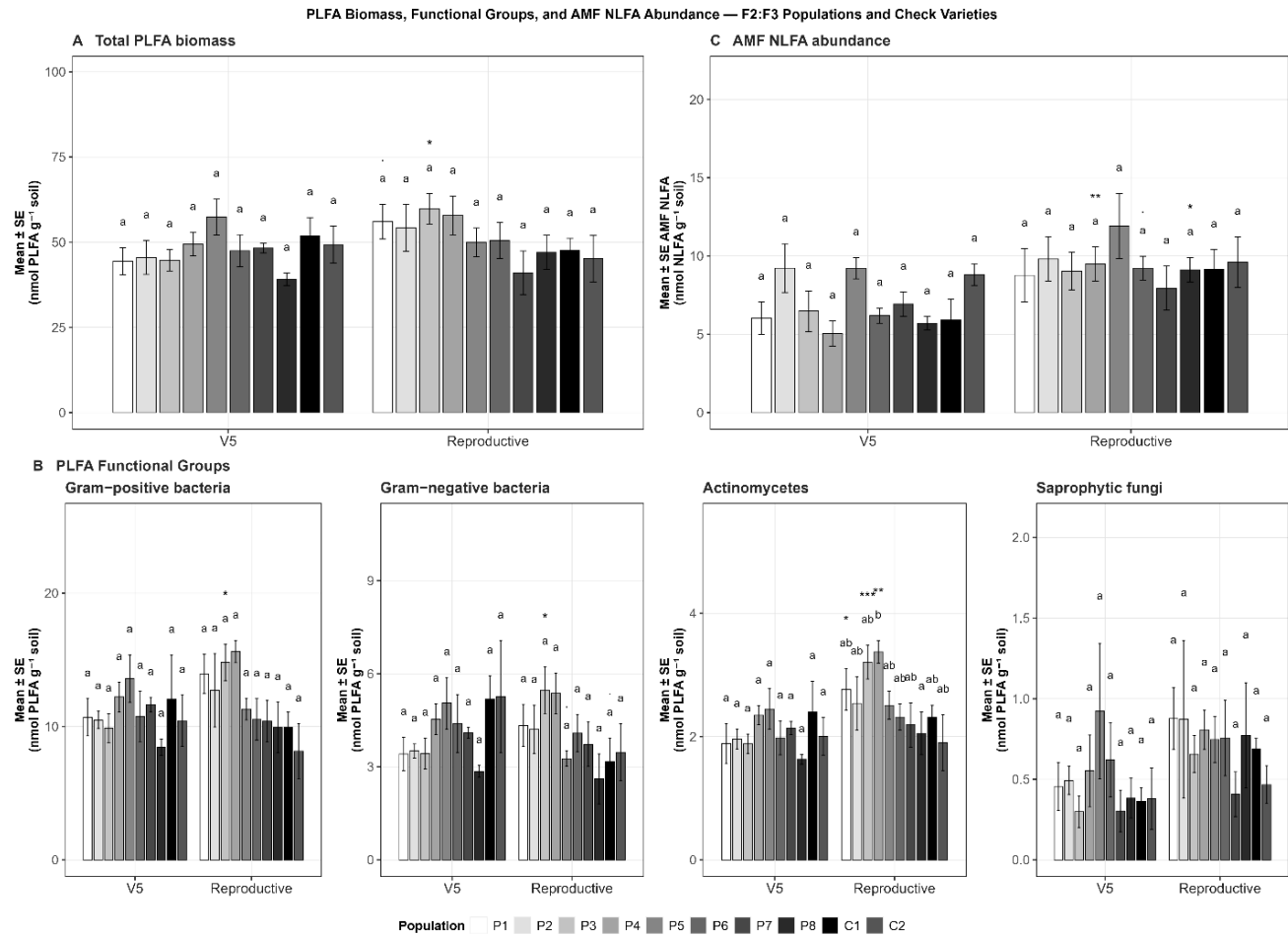


Figure 4.1 Soil microbial biomass and community composition across sorghum growth stage (V5, Reproductive) for eight F_{2:3} breeding populations (P1-P8) and two check varieties (C1, C2). (A) Total PLFA biomass. (B) PLFA functional group abundance for Gram-positive bacteria, Gram-negative bacteria, actinomycetes, and saprophytic fungi. (C) Arbuscular mycorrhizal fungi (AMF) abundance, estimated from the NLFA 16:1 ω 5c biomarker. Bars represent population means $\pm$ SE (nmol PLFA g⁻¹ dry soil for panels A and B; nmol NLFA g⁻¹ dry soil for panel C). Letters above bars indicate significant differences among populations within a stage (Tukey-adjusted, $\alpha = 0.05$). Stars above bars in the Reproductive stage indicate a significant change from V5 within that population (unadjusted): • $p < 0.1$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Arbuscular mycorrhizal fungal storage lipid, quantified as NLFA 16:1 ω 5, increased significantly from V5 to the reproductive stage across F_{2:3} populations and checks ($p < 0.001$), indicating greater AMF storage activity in the rhizosphere during reproductive growth (Fig. 4.1C). Significant within-population stage increases were detected in Population 4 ($p < 0.01$) and Population 5 ($p < 0.05$). Population identity showed only a marginal trend for AMF NLFA ($p = 0.071$), and no population $\times$ stage interaction was detected ($p = 0.849$).

LGS1-characterized genotype panel

Within the LGS1-characterized genotype panel, total PLFA biomass was significantly affected by growth stage ($p < 0.001$) and genotype $\times$ stage interaction ($p = 0.001$), indicating that the magnitude and direction of the stage-driven biomass shift depended on genotype (Fig. 4.2A). Gram-positive bacteria had a significant growth stage effect ($p = 0.011$) not mirrored by gram-negative bacteria, actinomycetes, or saprophytic fungi (Fig. 4.2B). The F:B ratio increased significantly from V5 to the reproductive stage ($p < 0.001$). The GP:GN ratio did not change significantly with stage within the LGS1 panel ($p = 0.449$). All pairwise comparisons among genotypes within each stage were non-significant for the PLFA functional groups (gram-positive bacteria, gram-negative bacteria, actinomycetes, and saprophytic fungi; Tukey-adjusted, all $p > 0.05$). Entry-level F:B and GP:GN ratio means are reported in Table 4.3.

Genotype identity significantly explained total PLFA biomass across the panel ($p = 0.002$), though it did not significantly explain variation in any individual functional group beyond the stage effect on gram-positive bacteria. AMF NLFA also increased significantly from V5 to the reproductive stage within the LGS1-characterized genotype panel (Fig. 4.2C). When genotypes were grouped by LGS1 functional status, *lgs1* loss-of-function genotypes showed higher AMF NLFA than LGS1 functional genotypes at both developmental stages. At the

reproductive stage, RTx430 NIL had the highest AMF NLFA of any genotype within the panel, while BTx398-1-1 was the lowest, with Tukey-adjusted pairwise comparisons detecting a significant difference between these two entries ($p < 0.05$). The remaining genotypes occupied intermediate positions with overlapping confidence intervals.

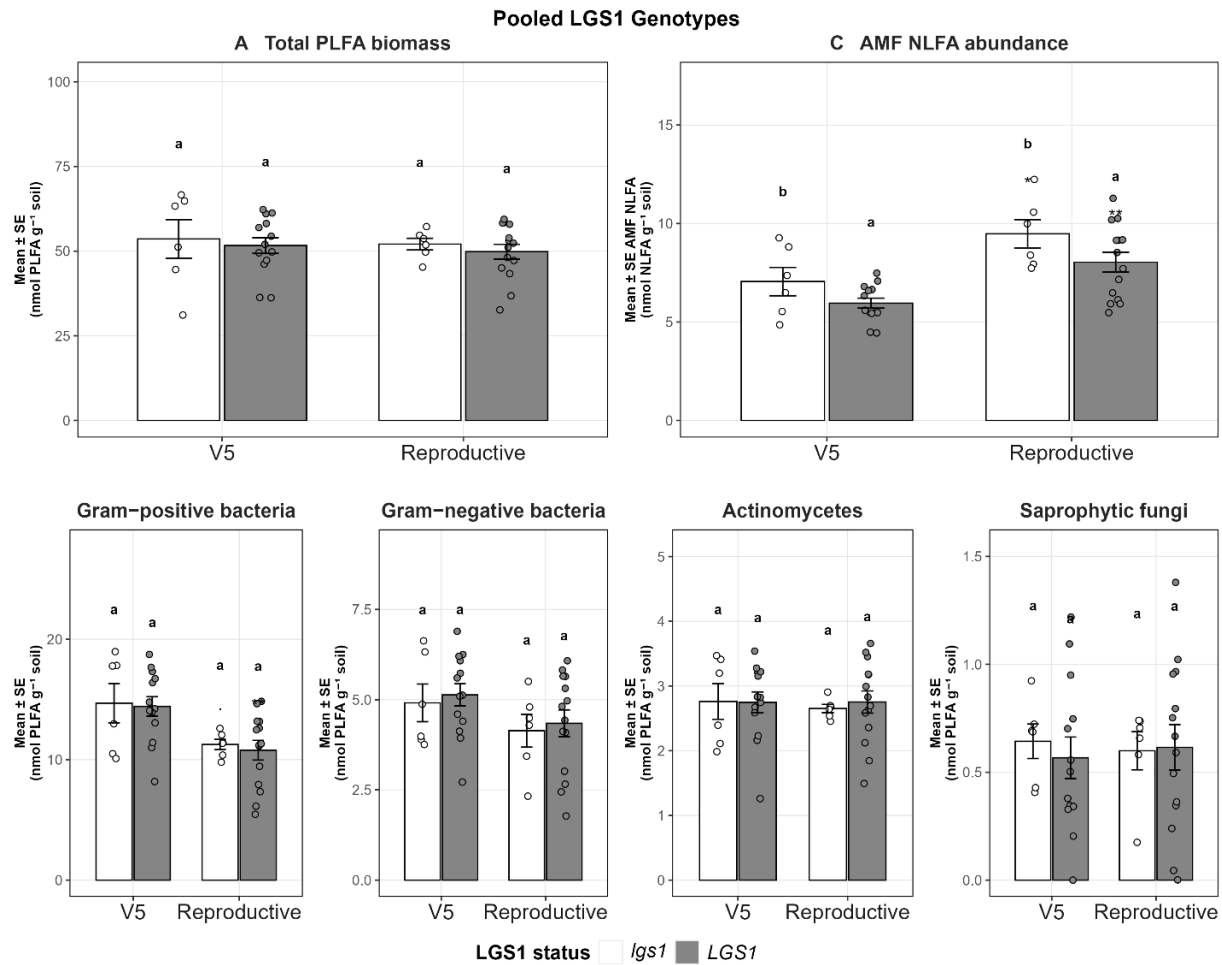


Figure 4.2 PLFA microbial functional group abundance and total PLFA biomass (nmol PLFA g⁻¹ dry soil) for pooled LGS1-status groups (*lgs1* loss-of-function vs. *LGS1*-functional) within the LGS1-characterized sorghum panel at V5 and reproductive developmental stages. Panels show total (A) PLFA biomass, (B) gram-positive bacteria, gram-negative bacteria, actinomycetes, and saprophytic fungi, and (C) AMF NLFA abundance. Bars represent estimated

marginal means for the distinct LGS1-status groups; error bars represent 95% confidence intervals. Letters indicate Tukey-adjusted pairwise comparisons between LGS1-status groups within each stage ($\alpha = 0.05$). Stars indicate significant stage contrasts within each LGS1-status group (*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, . $p < 0.1$).

Developmental changes in microbial biomass and functional groups

Principal coordinates analysis (PCoA) of Bray-Curtis dissimilarities calculated from PLFA/NLFA biomarker profiles demonstrated that developmental stage was the dominant structuring force in sorghum rhizosphere microbial communities. Across the full dataset of 131 plants, PERMANOVA confirmed a significant stage effect on overall community composition ($R^2 = 0.063$, $p = 0.002$; 999 permutations).

This separation was consistent when evaluated independently within each material class. In the F_{2:3} population and check variety panel, V5 and reproductive-stage communities formed partially overlapping but distinguishable clusters along PCoA1 (Fig. 4.3A; PERMANOVA: $R^2 = 0.08$, $p = 0.001$), with permutations constrained within experimental block. PCoA1 and PCoA2 explained 53% and 18% of total variation, respectively. Within the LGS1-characterized genotype panel, the same stage-driven separation was evident (Fig. 4.3B; PERMANOVA: $R^2 = 0.17$, $p = 0.001$), with PCoA1 and PCoA2 accounting for 54% and 21% of variation.

At the within-stage level, population identity did not explain community composition at either V5 ($R^2 = 0.28$, $p = 0.46$) or the reproductive stage ($R^2 = 0.26$, $p = 0.95$). In the full model, neither population identity ($R^2 = 0.14$, $p = 0.79$) nor the stage $\times$ population interaction ($R^2 = 0.11$, $p = 0.25$) contributed significantly beyond the stage effect. In contrast, genotype identity significantly explained composition within the LGS1 panel at both V5 ($R^2 = 0.77$, $p = 0.02$) and the reproductive stage ($R^2 = 0.75$, $p = 0.01$).

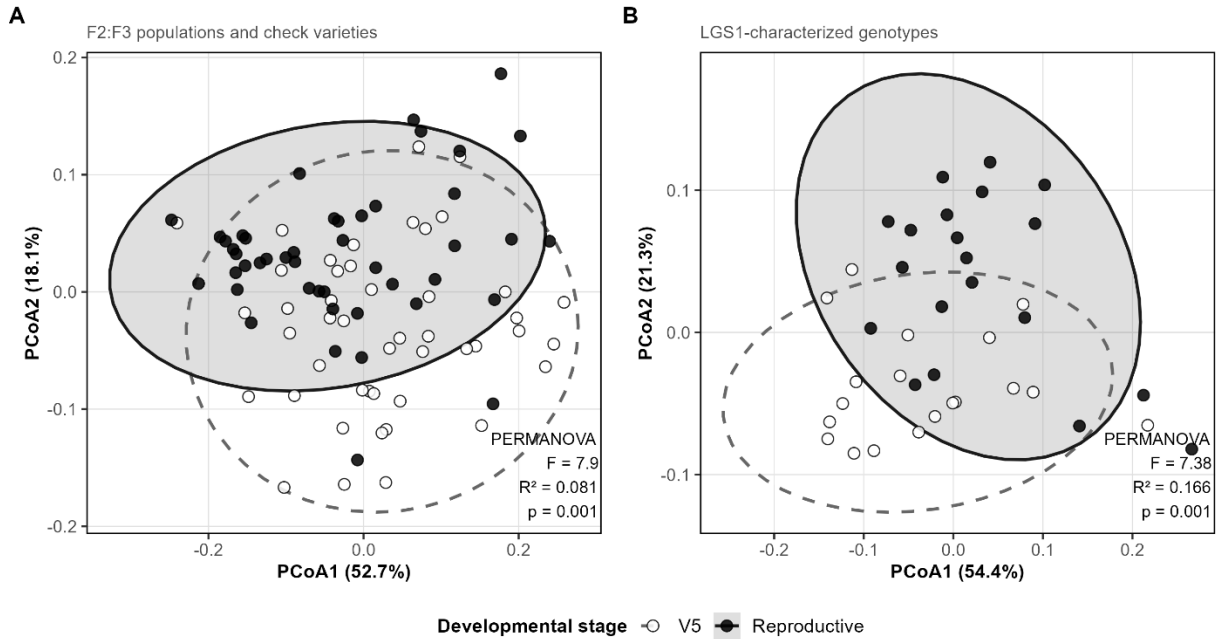


Figure 4.3 Principal coordinates analysis (PCoA) of rhizosphere microbial community composition based on Bray-Curtis dissimilarity of PLFA/NLFA biomarker profiles across four functional groups. (A) F_{2:3} breeding populations (P1-P8) and check varieties (C1 and C2). (B) LGS1-characterized genotypes. Samples were collected at V5 (vegetative) and Reproductive developmental stages (n = 131; 65 V5, 66 reproductive). Points represent individual samples; ellipses depict 95% confidence regions.

AMF colonization

AMF root colonization percentage and AMF NLFA abundance were not significantly correlated within the LGS1-characterized genotype panel when pooled across both developmental stages ($R = -0.23$, $p = 0.16$; Fig. 4.4A). When genotypes were split by LGS1 functional status, this relationship remained non-significant among *lgs1* loss-of-function genotypes ($R = -0.26$, $p = 0.41$) but was significantly negative among LGS1 functional genotypes ($R = -0.57$, $p = 0.0021$; Fig. 4.4B). Given the modest sample size within each LGS1

status group, this apparent difference in the strength of coupling between root colonization and storage lipid accumulation should be interpreted cautiously and warrants confirmation in the ongoing greenhouse study. The absence of a strong positive relationship between root colonization and AMF NLFA abundance overall indicates that these two measures capture largely decoupled aspects of AMF activity under the sufficient-nutrient conditions of this conventionally managed field: root colonization reflects the extent of intraradical fungal structures (hyphae and arbuscules), and NLFA reflects storage lipid accumulated in vesicles and spores within the soil. Also during reproductive root sampling, the dry soil conditions contributed to many root samples being stripped, making it difficult to accurately quantify AMF colonization for the second developmental stage. This likely contributed to the negative correlation seen below.

This decoupling is consistent with the underlying AMF life cycle: fungal carbon demand is low during the initial phase of root colonization but rises substantially once spore and vesicle formation begins later in fungal development (Kokkoris, 2025). Root colonization percentage would be expected to establish relatively early in the growing season, while AMF NLFA, as a biomarker of storage structures, would be expected to continue accumulating later into plant development. This is consistent with the significant V5-to-reproductive increase in AMF NLFA observed across both the F_{2:3} and LGS1 panels in this study ($p < 0.001$, F_{2:3} panel) and offers another plausible explanation for why NLFA and root colonization were decoupled when measured concurrently in the same rhizosphere samples.

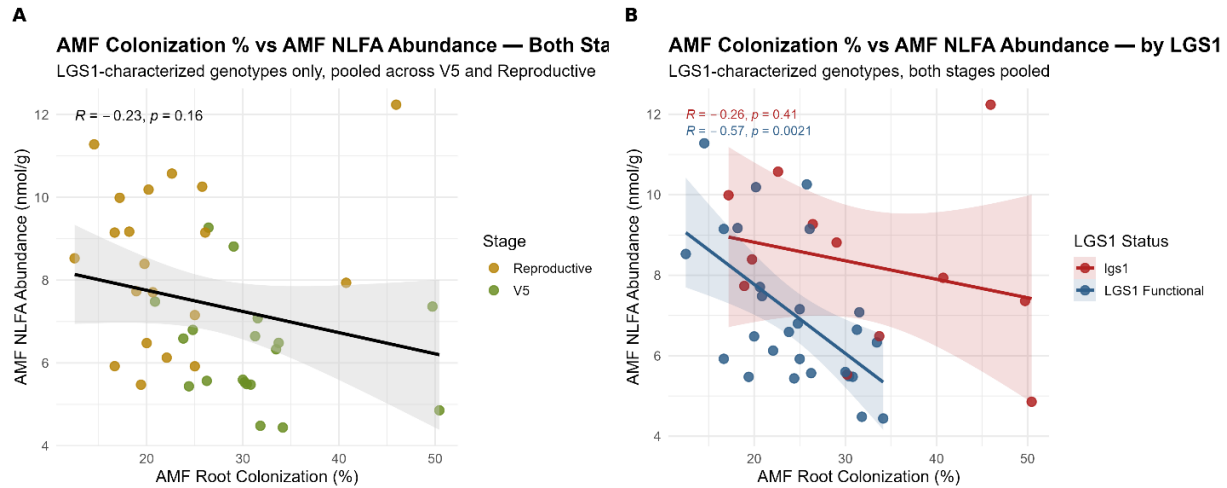


Figure 4.4 Relationship between AMF root colonization (%) and AMF NLFA abundance (nmol NLFA g⁻¹ dry soil) within the LGS1-characterized genotype panel, pooled across V5 and reproductive stages. (A) All genotypes combined. (B) Genotypes grouped by LGS1 functional status. Lines represent ordinary least squares regression with 95% confidence intervals.

Relationships between AMF colonization and plant performance

At the reproductive stage, relationships between AMF root colonization percentage and plant-performance traits were weak and non-significant within the LGS1-characterized genotype panel, both when analyzed separately by LGS1 functional status and when tested for a status-dependent interaction. For aboveground biomass, colonization percentage explained limited variation in both the LGS1 Functional ($R^2 = 0.05$, $p = 0.46$) and lgs1 ($R^2 = 0.03$, $p = 0.75$) groups, with no significant colonization $\times$ Group interaction ($p = 0.67$). Yield showed a similar pattern, with colonization percentage again explaining limited variation in the LGS1 Functional ($R^2 = 0.08$, $p = 0.35$) and lgs1 ($R^2 = 0.54$, $p = 0.09$) groups; despite the comparatively higher R^2 observed in the lgs1 group, this relationship did not reach significance, and the colonization $\times$ Group interaction was also non-significant ($p = 0.21$). Total nitrogen uptake showed essentially no relationship with colonization percentage in either group (LGS1 Functional: $R^2 = 0.01$, $p =$

0.72; lgs1: $R^2 = 0.00$, $p = 0.97$), with no significant interaction ($p = 0.79$). Phosphorus uptake followed the same pattern, with weak, non-significant relationships in both the LGS1 Functional ($R^2 = 0.05$, $p = 0.44$) and lgs1 ($R^2 = 0.03$, $p = 0.73$) groups and no significant colonization $\times$ Group interaction ($p = 0.56$).

Across all four traits, the absence of significant colonization $\times$ Group interactions indicates that the relationship between AMF root colonization and plant performance did not differ meaningfully between LGS1 Functional and lgs1 genotypes. Although the lgs1 group showed a numerically higher R^2 for yield than any other trait-by-group combination in the panel, this relationship remained non-significant given the available sample size, and should not be interpreted as evidence of a colonization-yield association specific to LGS1 status.

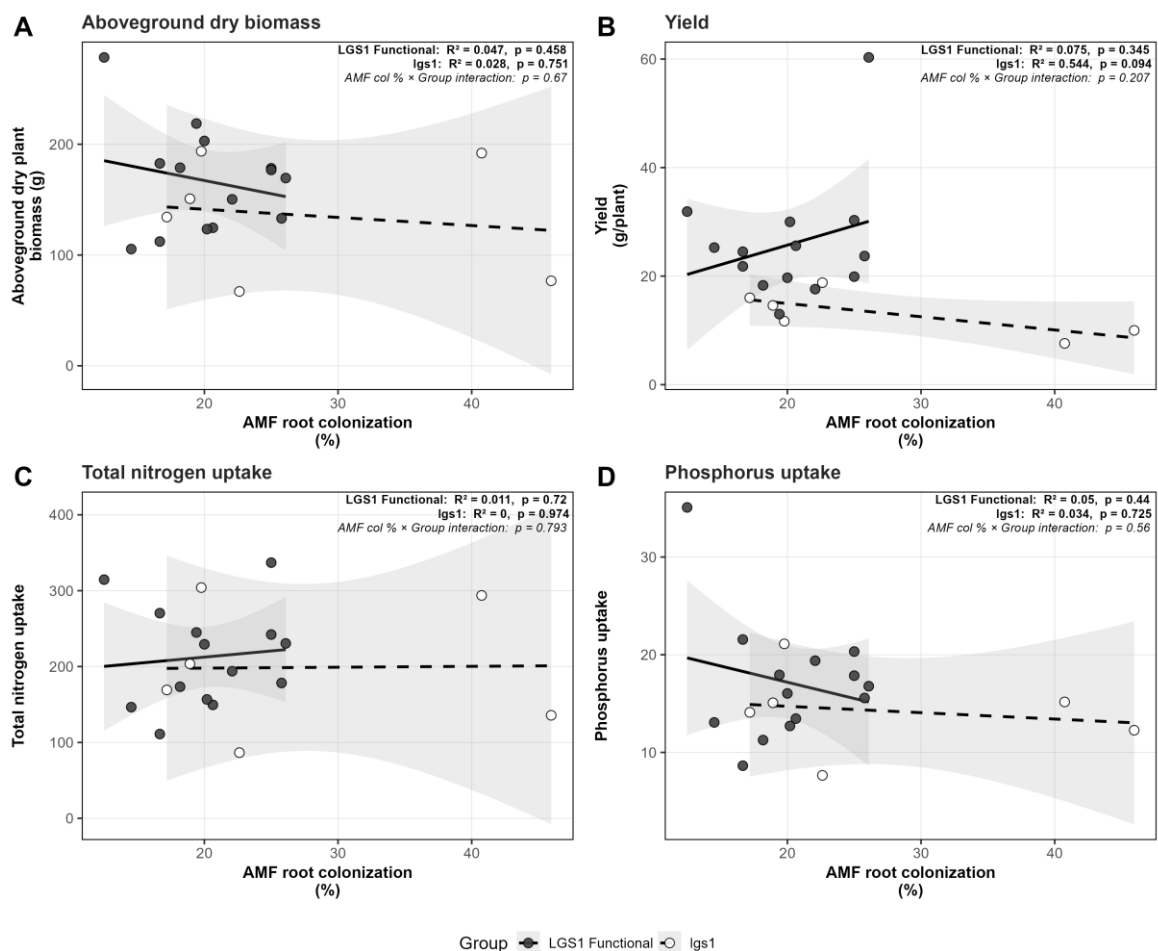


Figure 4.5 Relationships between rhizosphere AMF % colonization and aboveground plant characteristics at the reproductive stage (LGS1-characterized genotypes, $n = 22$). (A) Biomass; (B) Grain yield; (C) N uptake; (D) P uptake.

Aboveground Plant Characteristics and Agronomic Performance

Among the eight $F_{2:3}$ populations and two check varieties, aboveground biomass ranged from 106 ± 12.6 g (P3) to 256 ± 46 g (P7), with P3 significantly lower in biomass than all other entries; grain yield (17.7 – 33.7 g plant⁻¹) and plant height did not differ significantly among populations or checks (Table 4.4). Days to flowering was similar across the $F_{2:3}$ populations and Check 1 (Macia; 76.1 – 80.6 days) but Check 2 (85P58) flowered significantly earlier (69.9 ± 1.8 days), consistent with its identity as an early-maturing hybrid. Tissue nutrient concentrations showed more population-level structure than the agronomic traits: nitrogen and phosphorus were significantly lower in P3 and the check varieties than in most $F_{2:3}$ populations, tissue carbon was highest in P4 through 8, and tissue potassium was significantly higher in P1 and both checks (up to $2.40 \pm 0.37\%$ in Check 2) than in the remaining $F_{2:3}$ populations.

Within the LGS1-characterized genotype panel, plant height varied more widely among genotypes than among the $F_{2:3}$ populations, ranging from 120.5 – 130.5 cm in the shorter genotype group to 172.5 – 200.5 cm in the taller group (Table 4.4). The most notable contrast was between the RTx430 near-isogenic pair: despite comparable vegetative biomass (114.4 – 134.3 g) and plant height (120.5 – 121.0 cm), the LGS1-functional NIL produced roughly three-fold greater grain yield (27.6 ± 2.4 vs. 8.8 ± 1.2 g plant⁻¹) and higher tissue potassium ($2.5 \pm 0.1\%$ vs. $1.4 \pm 0.1\%$) than the *lgs1-2* loss-of-function line. Because this comparison included only two plants per genotype, it was evaluated using a priori contrast rather than the Tukey-adjusted comparisons

used for the remaining genotypes, and is reported here as an exploratory finding rather than a definitive genotype effect.

Table 4.4 Aboveground plant characteristics of F_{2:3} sorghum populations and check varieties at the reproductive stage. Within each column, entries sharing a superscript letter are not significantly different (Tukey-adjusted, $\alpha = 0.05$). Entries with no letter did not differ significantly from any other entry in the overall ANOVA ($p \geq 0.05$).

Population / Check	Biomass (g)	Height (cm)	Yield (g plant ⁻¹)	N (%)	P (%)	K (%)
P1	123 ± 15 a	125 ± 3.2	17.7 ± 3.5	1.38 ± 0.06 a	0.13 ± 0.01 a	1.77 ± 0.08 a
P2	136 ± 16 a	116.5 ± 4.4	19.3 ± 3.1	1.47 ± 0.10 a	0.15 ± 0.01 a	1.54 ± 0.11 b
P3	106 ± 12.6 b	124.3 ± 6.8	24.9 ± 3.4	1.23 ± 0.04 b	0.10 ± 0.01 b	1.55 ± 0.13 b
P4	215 ± 39 a	131.9 ± 7.1	23.2 ± 5.4	1.55 ± 0.12 a	0.16 ± 0.02 a	1.64 ± 0.14 b
P5	205 ± 33 a	120.0 ± 7.9	23.4 ± 2.9	1.59 ± 0.06 a	0.17 ± 0.00 a	1.54 ± 0.12 b
P6	164 ± 19.6 a	122.7 ± 5.3	21.0 ± 1.3	1.27 ± 0.07 a	0.13 ± 0.01 a	1.30 ± 0.06 b
P7	255.9 ± 46.1 a	122.2 ± 4.3	27.8 ± 5.6	1.48 ± 0.07 a	0.14 ± 0.01 a	1.34 ± 0.06 b
P8	150.5 ± 25.2 a	135.4 ± 6.0	19.1 ± 1.8	1.21 ± 0.07 b	0.13 ± 0.01 a	1.18 ± 0.04 b
Check 1 (Macia)	227.1 ± 51.1 a	127.7 ± 4.7	33.7 ± 7.6	1.24 ± 0.01 a	0.08 ± 0.00 b	1.86 ± 0.05 a
Check 2 (85P58)	200.3 ± 60.0 a	114.3 ± 4.8	22.9 ± 0.8	1.14 ± 0.09 b	0.10 ± 0.01 b	2.40 ± 0.37 a

Table 4.5 Aboveground plant characteristics of LGS1-characterized sorghum genotypes at the reproductive stage. Within each column, entries sharing a superscript letter are not significantly different ($\alpha = 0.05$). Letters for most traits derive from Tukey-adjusted pairwise comparisons following one-way ANOVA. Letters for RTx430 and RTx430 NIL in Yield and K (%) derive from a planned priori contrast (Welch t-test) between the near-isogenic pair, because the one-way ANOVA across all 10 genotypes has insufficient power at $n = 2$ per genotype to detect pairwise differences.

Genotype	LGS1 Status	Biomass (g)	Height (cm)	Yield (g plant ⁻¹)	N (%)	P (%)	K (%)
RTx430	lgs1-2	134.3 ± 57.6	121.0 ± 4.0 b	8.8 ± 1.2 b	1.65 ± 0.12 a	0.12 ± 0.04	1.36 ± 0.08
RTx430 NIL	LGS1	114.4 ± 9.0	120.5 ± 1.5 b	27.6 ± 2.4 a	1.33 ± 0.06 a	0.11 ± 0.01	2.46 ± 0.0
Tx7000	LGS1	151.3 ± 18.2	130.0 ± 16.0 b	42.0 ± 18.3	1.35 ± 0.01 a	0.11 ± 0.01	2.00 ± 0.10
BTx3042-2-4	LGS1	137.4 ± 12.9	130.5 ± 2.5 b	21.6 ± 4.0	1.25 ± 0.05 a	0.12 ± 0.01	1.99 ± 0.70
BTx3042-2-10	LGS1	180.5 ± 2.2	172.5 ± 3.5 a	27.4 ± 2.9	1.69 ± 0.20 a	0.12 ± 0.00	1.62 ± 0.14
BTx3042-2-13	LGS1	240.7 ± 37.7	200.5 ± 3.5 a	25.8 ± 6.1	1.13 ± 0.00 a	0.10 ± 0.02	1.72 ± 0.02

BTx398-1-1	LGS1	145.5 ± 33.3	176.0 ± 23.0 a	20.1 ± 1.7	0.98 ± 0.01 b	0.07 ± 0.01	2.06 ± 0.10
BTx398-1-2	lgs1-1	130.4 ± 63.3	184.5 ± 16.5 a	15.2 ± 3.6	1.43 ± 0.14 a	0.11 ± 0.00	1.75 ± 0.27
BTx398-1-3	LGS1	197.8 ± 20.9	177.0 ± 4.0 a	16.4 ± 3.4	1.25 ± 0.12 a	0.09 ± 0.01	2.00 ± 0.22
BTx398-1-5	lgs1-7	142.6 ± 8.3	186.5 ± 4.0 b	15.3 ± 0.7	1.31 ± 0.05 a	0.10 ± 0.01	1.94 ± 0.06

Discussion

Developmental stage as the dominant driver of rhizosphere microbial dynamics

Developmental stage was the strongest and most consistent factor structuring rhizosphere microbial dynamics across the two experimental panels. Community-level analyses identified a significant effect of plant developmental stage, indicating that microbial composition changed between the V5 and reproductive stages even when population or genotype effects were limited. However, the microbial responses did not reflect a uniform increase in total microbial biomass. Total PLFA biomass was not significantly different between stages in the F_{2:3} populations ($p = 0.141$), however, a significant stage effect was detected in the LGS1 panel ($p < 0.001$), together with a significant genotype-by-stage interaction ($p = 0.001$).

The LGS1 lines, by contrast, are advanced, nearly homozygous lines with minimal within-line genetic variation, allowing developmental changes in root physiology to be expressed reproducibly across replicates of the same genotype. This pattern is consistent with findings in

maize, where Wagner et al. (2020) compared rhizosphere and leaf microbiomes of inbred lines against their F₁ hybrids and found that inbred lines and hybrids differed consistently in bacterial and fungal community composition, with broad-sense heritability of microbiome features substantially exceeding narrow-sense heritability. This gap between broad- and narrow-sense heritability indicates that non-additive and stochastic sources of variance are more effectively averaged out, and genotype-associated microbial signals are more reliably detected, in fixed, homozygous genotypes than in segregating or heterozygous material. This same logic extends to the F_{2:3} vs. LGS1 comparison here: the LGS1 panel's genetic fixation improves the signal-to-noise ratio for detecting both stage main effects and genotype-by-stage interactions, while the F_{2:3} panel's residual heterozygosity partially masks stage-driven shifts in total microbial biomass, even though compositional (community-level) differences between stages remain detectable. Developmental progression affected overall microbial biomass only in the LGS1 comparison, while the F_{2:3} response was expressed primarily through changes in microbial composition and functional groups rather than through a significant shift in total PLFA.

The most consistent stage-associated response was the increase in NLFA abundance from V5 to the reproductive stage in both panels ($p < 0.001$). This pattern shows greater accumulation of neutral lipid storage compounds, including the AMF-associated marker measured in this study, as plants matured. The fungal-to-bacterial ratio also increased significantly in both the F_{2:3} ($p = 0.0007$) and LGS1 ($p < 0.001$) panels, suggesting that fungal-associated biomass became more prominent relative to bacterial biomass during reproductive development. One plausible explanation is a shift in root tissue chemistry as roots mature and lignify, since fungi possess a broader range of extracellular enzymes capable of degrading recalcitrant, lignin-associated compounds, whereas bacteria are comparatively specialized for rapid assimilation of

labile carbon (de Boer et al., 2005). If root-derived carbon inputs become progressively more recalcitrant as plants advance toward reproductive maturity, this would favor fungal over bacterial dominance in the rhizosphere. The consistency of this pattern across both experimental panels, despite their differing genetic backgrounds and levels of fixation, suggests that the fungal-to-bacterial shift reflects a broadly conserved developmental process rather than a genotype-specific response. In the F_{2:3} populations, this broader shift was accompanied by significant increases in actinomycetes ($p = 0.0007$) and saprophytic fungi ($p = 0.033$), while total PLFA remained statistically unchanged.

The comparison between panels also demonstrates that developmental effects were context dependent rather than identical across all genotypes. Actinomycetes and saprophytic fungi responded significantly only in the F_{2:3} panel. These groups did not differ significantly across stages in the LGS1 panel. Conversely, the LGS1 panel showed a significant stage effect for Gram-positive bacteria ($p = 0.011$), despite no significant F_{2:3} stage effect for this group in the F_{2:3} panel ($p = 0.303$). The GP:GN ratio declined in the F_{2:3} populations ($p = 0.019$) but did not change significantly in the LGS1 panel ($p = 0.449$). Plant development was the dominant temporal driver of rhizosphere assembly, while genotype and genetic background modified which microbial groups expressed that response.

Undetectable population-level genetic effects

The absence of significant population-level differences in total PLFA biomass or microbial functional group composition at either developmental stage was expected at the F_{2:3} generation and is interpretable through two factors. First, residual heterozygosity at the F_{2:3} stage is approximately 25%, meaning within-population genetic variance likely exceeds between-population variance for quantitative traits governing root exudate chemistry and rhizosphere

microbial recruitment. The eight populations evaluated occupy unreplicated plots within the augmented design, preventing statistically defensible between-population comparisons and further reducing the power to detect genetic signals against the background of developmental and environmental variation. Second, PLFA profiling resolves microbial functional groups rather than individual taxa and therefore cannot detect the family- or genus-level compositional differences that genotype-linked exudate variation might produce. Sorghum genotypes with high-organic-acid or high-sugar exudates foster different soil microbial memberships and metabolic functions (Aira et al., 2010), but these differences are evident at taxonomic resolutions inaccessible to PLFA. This interpretation is supported by the contrasting result within the LGS1 genotype panel, where genotype identity did significantly explain total PLFA biomass despite non-significant pairwise differences among individual functional groups, suggesting that LGS1-linked effects operate through subtle shifts across multiple functional groups simultaneously rather than through large changes in any single group. Advancing to later generations with reduced residual heterozygosity, and pairing PLFA with 16S rRNA and ITS amplicon sequencing, would substantially improve the ability to detect genotype-linked rhizosphere signals.

LGS1 pleiotropy and the RTx430 NIL comparison

Within the LGS1-characterized genotype panel, genotype identity significantly explained variation in total PLFA biomass ($p = 0.002$), while total PLFA also showed a significant developmental-stage effect ($p < 0.001$) and a significant genotype-by-stage interaction ($p = 0.001$). These results indicate that both genotype and developmental stage contributed to overall microbial biomass, but that the direction and magnitude of the stage-associated biomass shift depended on genotype. At the level of individual microbial groups, Gram-positive bacteria

showed a significant stage effect ($p = 0.011$), and the fungal-to-bacterial ratio increased from V5 to the reproductive stage ($p < 0.001$). In contrast, gram-negative bacteria, actinomycetes, and saprophytic fungi did not show significant stage effects, and the gram-positive-to-gram-negative ratio remained unchanged ($p = 0.449$).

AMF storage-lipid dynamics showed a different pattern. AMF NLFA abundance increased significantly from V5 to the reproductive stage within the LGS1-characterized panel, and genotypes carrying the loss-of-function *lgs1* allele exhibited higher AMF NLFA than LGS1-functional genotypes at both stages. At the reproductive stage, RTx430 had the highest AMF NLFA value in the panel, whereas BTx398-1-1 had the lowest, with this specific pairwise difference reaching significance ($p < 0.05$). However, the pooled LGS1-status comparison combines genotypes from different genetic backgrounds, including BTx3042, BTx398, and RTx430. Consequently, the higher AMF NLFA associated with *lgs1* genotypes cannot be attributed to LGS1 status alone.

These findings are consistent with the broader pleiotropic effects of LGS1 loss-of-function, which extend beyond changes in strigolactone stereochemistry. In CRISPR-Cas9-edited sorghum, LGS1 loss-of-function was associated with systemic changes in photosystem-related gene expression, including significant down-regulation of 11 of 36 photosystem II genes in shoots (Bellis et al., 2020). Such changes could reduce photosynthetic carbon supply to roots and alter the quantity or composition of root-derived resources available to rhizosphere microorganisms. At the same time, *lgs1* genotypes have been reported to exhibit slower growth, developmental delay, altered gibberellin precursor accumulation, and enhanced lateral root growth (Kawa et al., 2021). Increased lateral root development could expand rhizosphere volume and partially offset the effects of reduced carbon allocation, making the net microbial response

difficult to predict from strigolactone chemistry alone. The significant genotype effects on total PLFA and the contrasting AMF NLFA patterns should be interpreted as potential signatures of broader LGS1-associated physiology rather than as direct evidence of a single strigolactone-mediated recruitment mechanism.

The RTx430 near-isogenic comparison should therefore be discussed separately and treated as exploratory. The functional RTx430 NIL and the *lgs1-2* RTx430 line had comparable vegetative biomass and plant height, but the functional NIL showed approximately three-fold greater grain yield and higher tissue potassium concentration (Table 3.5). These differences were evaluated using a priori contrast between the near-isogenic pair because the sample size was only two plants per genotype per stage. The NIL comparison does not support a significant Gram-positive bacterial difference, nor does it demonstrate a significant effect of LGS1 status on AMF NLFA dynamics. The yield and potassium differences may instead reflect the combined consequences of altered photosynthetic capacity, carbon allocation, gibberellin signaling, root architecture, and reproductive partitioning. The RTx430 results provide evidence that the *lgs1-2* and LGS1 haplotypes can differ in agronomic performance under field conditions, but they do not isolate strigolactone chemistry as the causal mechanism.

AMF colonization and plant performance

At the reproductive stage, AMF root colonization showed no significant relationship with any of the four agronomic traits examined. Within the LGS1 Functional genotypes, colonization was not significantly associated with aboveground dry biomass ($R^2 = 0.05$, $p = 0.46$), yield ($R^2 = 0.08$, $p = 0.35$), total nitrogen uptake ($R^2 = 0.01$, $p = 0.72$), or phosphorus uptake ($R^2 = 0.05$, $p = 0.44$). The same pattern held within the *lgs1* genotypes, where none of the corresponding relationships reached significance (biomass: $R^2 = 0.03$, $p = 0.75$; yield: $R^2 = 0.54$, $p = 0.09$;

nitrogen uptake: $R^2 = 0.00$, $p = 0.97$; phosphorus uptake: $R^2 = 0.03$, $p = 0.73$), despite the comparatively higher R^2 observed for yield in this panel. AMF colonization $\times$ Group interaction terms were also non-significant across all four traits ($p = 0.67$, 0.21 , 0.79 , and 0.56 , respectively), indicating that the relationship between colonization percentage and plant performance did not differ meaningfully between the two genetic backgrounds.

This absence of a detectable relationship between percent root colonization and plant performance is not unexpected given the broader mycorrhizal literature. Percent root length colonization is the most widely used metric of AMF abundance, but its functional significance is inconsistent: a meta-analysis of manipulated colonization across laboratory and field trials found that changes in colonization explained only a small fraction of the variation in plant biomass and phosphorus response, with substantial variability among individual trials (Treseder, 2013). Field-scale evidence reinforces this disconnect. In a large on-farm inoculation study across 54 fields, there was no correlation between root colonization and plant growth response, with colonization success instead better explained by soil microbiome and pathogen-suppression indicators than by colonization intensity itself (Lutz et al., 2023). Percent colonization is a structural measure, capturing the proportion of root length occupied by fungal structures, but it does not necessarily reflect the metabolic or nutrient-transfer activity of the symbiosis. These results suggest that root colonization percentage alone may be an incomplete indicator of symbiotic functional activity in this system, and that lipid-based markers such as NLFA may better capture the physiologically relevant dimension of the AMF-host interaction, especially in conventional management systems.

Limitations and future directions

Several limitations should be considered when interpreting these results. PLFA/NLFA profiling resolves broad microbial functional groups and AMF storage-lipid abundance, but it does not identify individual taxa or distinguish taxonomic changes within a functional group. Consequently, genotype-associated shifts in bacterial or fungal membership, including family- or genus-level changes, may have occurred without producing detectable differences in the measured PLFA categories. The experiment was also conducted at a single field site during one growing season under conventional management, so the magnitude and direction of LGS1- and AMF-associated responses may differ under contrasting nitrogen and phosphorus availability, soil pH, moisture conditions, temperature, or low-input environments. Root sample quality at the reproductive stage was also affected by soil moisture conditions at the time of excavation. Because the soil profile was notably dry during reproductive-stage sampling, a substantial number of fine roots were stripped from the root system during excavation, reducing the amount of intact root length available for colonization scoring. This constraint may partly explain the lower AMF root colonization values observed at the reproductive stage relative to what would otherwise be expected given the concurrent increase in AMF NLFA abundance, and it should be considered when interpreting the root colonization results in Figure 3.4.

Interpretation of the $F_{2:3}$ population results is further limited by residual heterozygosity and the unreplicated placement of breeding populations within the augmented field design. These factors reduce the ability to distinguish between-population genetic effects from within-population variation and environmental heterogeneity. Advancing the materials to $F_{3:4}$ or later generations may reduce heterozygosity, but later-generation experiments should also use replicated plots and larger numbers of plants per genotype. Pairing PLFA/NLFA profiling with

16S rRNA and ITS amplicon sequencing would provide taxonomic resolution needed to determine whether population or genotype effects occur at a level not captured by lipid biomarkers.

The RTx430 NIL comparison should be treated as exploratory because only two plants per genotype were evaluated at each developmental stage. Although the near-isogenic contrast provided evidence of differences in grain yield and tissue potassium, it did not establish a significant NIL-level difference in Gram-positive bacterial biomass or AMF NLFA dynamics. A controlled experiment using replicated near-isogenic lines is currently underway to isolate the effect of LGS1 from variation in genetic background, field heterogeneity, and developmental timing. The greenhouse study will evaluate root architecture, PLFA/NLFA profiles, AMF root colonization, and plant nutrient acquisition under controlled nutrient and moisture conditions. Direct measurement of root-exuded strigolactones, including the relevant LGS1 and neighboring-locus haplotypes, would further test whether changes in strigolactone composition are associated with rhizosphere recruitment or whether the observed effects primarily reflect broader pleiotropic changes in photosynthesis, gibberellin signaling, root development, and carbon allocation.

Future studies should also include more frequent sampling across the transition to flowering and through post-anthesis development. Repeated measurements of AMF NLFA, AMF root colonization, root biomass, plant nutrient uptake, and flowering progression would determine whether higher NLFA in earlier-flowering plants reflects a longer interval for storage-lipid accumulation, a distinct developmental allocation strategy, or a causal relationship between phenology and AMF dynamics. AMF inoculation or exclusion treatments would be particularly

important for testing whether higher NLFA represents functional nutrient acquisition, fungal storage investment, or both.

Conclusions

This study used PLFA/NLFA profiling to examine how plant developmental stage, $F_{2:3}$ population identity, and *LGS1* status shape rhizosphere microbial biomass in 131 field-grown sorghum plants. Developmental stage was the dominant driver of community composition in both experimental panels, with increases in the fungal-to-bacterial ratio and AMF storage-lipid abundance from V5 to the reproductive stage. In the $F_{2:3}$ populations, stage-associated changes also included increased actinomycete abundance, while population identity did not significantly explain community composition. These findings extend previous sequencing-based work showing that sorghum rhizosphere communities shift across development and that genotype effects may become more detectable later in the growing season (Schlemper et al., 2017). The *LGS1* panel showed additional, context-dependent differences, including significant developmental effects on total PLFA biomass, gram-positive bacteria, the fungal-to-bacterial ratio, and AMF NLFA, but did not establish a consistent direct effect of *LGS1* status on individual microbial groups. The RTx430 NIL comparison provided exploratory evidence of differences in grain yield and tissue potassium, but not a significant NIL-level microbiome effect. Relationships between AMF NLFA abundance and plant-performance traits were weak and generally non-significant within datasets. The association with days to flowering differed between the $F_{2:3}$ population and check dataset and the *LGS1*-characterized panel, with a significant AMF NLFA-by-dataset interaction. Overall, this study demonstrates that developmental stage must be accounted for when evaluating sorghum rhizosphere microbiomes and that PLFA/NLFA profiling can detect stage-dependent functional shifts while offering

limited resolution for genetic effects in early-generation materials. Replicated later-generation field trials, taxonomic sequencing, and controlled NIL experiments will be needed to determine how LGS1-associated physiology and strigolactone variation influence AMF recruitment and broader rhizosphere assembly.

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