Utilizing mycorrhizal responses to guide selective breeding for agricultural sustainability

Adam B. Cobb¹ | Eric B. Duell¹ | Kaitlin B. Haase² | R. Michael Miller³ | Yanqi Q. Wu⁴ | Gail W. T. Wilson¹

¹Department of Natural Resource Ecology and Management, Oklahoma State University, Stillwater, OK, USA
²School of Earth and Sustainability, Northern Arizona University, Flagstaff, AZ, USA
³Environmental Science Division, Argonne National Laboratory, Lemont, IL, USA
⁴Department of Plant and Soil Sciences, Oklahoma State University, Stillwater, OK, USA

Correspondence
Adam B. Cobb, Department of Natural Resource Ecology and Management, Oklahoma State University, Stillwater, OK 008C AGH, USA.
Email: abcobb@okstate.edu

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Societal Impact Statement
Agriculture touches all aspects of society and global environmental health. Dwindling phosphorous reserves are a looming crisis for civilization, and soil erosion typically far outpaces pedogenesis. Improving plant–mycorrhizal symbioses can enhance sustainable agriculture because mycorrhizas typically improve host-plant nutrition and stabilize soils. Selective breeding of plants that gain greater benefits from mycorrhizas can provide considerable economic and environmental benefits. Our assessments demonstrate switchgrass genetic improvement increased or maintained production of two populations, and low-input breeding increased mycorrhizal responsiveness, compared to parent lines. Selecting for increased mycorrhizal reliance may be an effective strategy for more sustainable and economical agricultural production.

Summary
• Plant–mycorrhizal interactions are not typically assessed in crop breeding programs. Our experiment addresses this by determining host-plant outcomes of newly developed synthetic (agronomic) populations compared with parent lines, following low-input selective breeding. Assessing the potential of low-input breeding to enhance crop mycorrhizal benefits is a critical step toward more sustainable agricultural production.
• We compared four synthetic populations of Panicum virgatum, from a low-input biofuel breeding program at Oklahoma State University, to corresponding parent lines. Plants were grown in a greenhouse in native prairie soils that were either steam-pasteurized (nonmycorrhizal) or non-steamed (mycorrhizal).
• We assessed shoot and root biomass, shoot P concentration and P content, mycorrhizal growth response (MGR), and mycorrhizal phosphorous response (MPR). Importantly, we provide novel evidence that low-input selective breeding increased mycorrhizal reliance of switchgrass synthetics compared to parent lines, with implications for global agricultural systems.
• There are substantial opportunities for plant traits associated with increased MGR and MPR to be transferred to a wide array of crops. Our findings indicate...
1 | INTRODUCTION

Humanity faces looming environmental challenges that are linked with current agricultural production strategies and addressing the negative impacts of food systems on planetary health is vital (Duhamel & Vanderkoornhuyse, 2013; Garcia et al., 2020). For example, one of the most pressing concerns for global food security is loss of productive soils, with mounting evidence that agricultural mismanagement leads to vicious cycles of environmental degradation (Wuepper et al., 2020). To meet these challenges, we need sustainable agricultural systems that optimize fertilizer use while regenerating soil quality and maintaining or increasing agroecosystem productivity (Edwards, 2020). Indeed, healthy carbon-rich soils are foundational to most food production systems and to achieving the United Nation’s Sustainable Development Goals (Lal, 2020).

North American prairie grasses represent a strategy to help reduce agricultural inputs and improve soil health, particularly as a source of a low-input biofuel feedstock with potential under proper management to increase soil C stocks (de Graaff et al., 2014; McGowan et al., 2019; Revillini et al., 2019). Switchgrass (Panicum virgatum) is a North American native perennial species that shows great promise as a low-input biofuel feedstock source and as a model crop for improving agroecosystem efficiency due to its general adaptability, yield potential, and soil benefits (Emery et al., 2018; Parrish & Fike, 2005). Perennial crop cultivation and mutualistic microbial interactions are broadly transferable to multiple agricultural settings, with potential to decrease fertilizer inputs while enhancing soil quality. Previous research indicates enhancing host-plant reliance on mycorrhizal symbioses can facilitate efforts to improve soil stability and agricultural sustainability (Cobb & Wilson, 2018; Duhamel & Vanderkoornhuyse, 2013; Galván et al., 2011; Hetrick et al., 1995).

Researchers are examining strategies for integrating mycorrhizal symbioses across varied agricultural landscapes, to enhance efficient food, fiber, and biofuel feedstock production (Mitter et al., 2019; Rillig et al., 2019). Mycorrhizas represent a critical mutualism for the vast majority of global plant species, including North American warm-season grasses (Miller et al., 2012; Wilson & Hartnett, 1998). Therefore, our current research focuses on assessing switchgrass populations, following low-input breeding, as a potential strategy to improve sustainability of biofuel production systems. The emerging holobiont conceptual framework, identifying host-plants as both primary drivers and scaffolding for complexes of diverse microbial communities that function as an evolutionary unit, can be used by breeders to enhance plant production (Gopal & Gupta, 2016; Ravanbakhsh et al., 2021; Wei & Jousset, 2017; Ye & Siemann, 2020). A holobiont describes multipartite relationships where a host-plant genome is tightly linked with an associated microbiome, forming a nascent and complex hologenome (Theis et al., 2016). In crop breeding, it is critical to directly assess binary interactions between host-plants and key mutualists, such as AM fungi, that likely drive production outcomes. Such assessments can establish breeding benchmarks toward further elucidation and utilization of hologenome dynamics. We propose the holobiont framework can inform alternative selection criteria and strategies for the development of genetic resources that can enhance agricultural sustainability (see Figure 1). As arbuscular mycorrhizal (AM) fungi often drive multiple host-plant benefits, mycorrhizal relationships represent a flagship mutualism within root-associated microbial communities, suggesting the effects of plant breeding on AM fungi provide a solid baseline for effective hologenome breeding.

Selective breeding for improved host-plant partnership with mycorrhizal fungi is a necessary step, setting the stage for additional efforts to use soil amendments and agroecosystem management to support effective microbial symbioses (Cobb, Wilson, & Goad, 2018; Cobb, Wilson, Goad, & Grusak, 2018; Duhamel & Vanderkoornhuyse, 2013; Smith et al., 1992). Mycorrhizal symbioses are widespread and typically beneficial, suggesting that evaluation of mycorrhizal relationships is critical groundwork for disentangling additional plant-associated microbial dynamics of the total holobiont. For example, switchgrass breeders are also assessing the influence of nitrogen-fixing bacteria on switchgrass growth in low-N soils (Rodrigues et al., 2017; Roley et al., 2020). As mycorrhizas enhance nutrient uptake, water-use efficiency, and soil quality in native ecosystems, plant breeding programs that select for increased reliance on mycorrhizas in agroecosystems can potentially deliver synthetic (agronomic) populations that reduce fertilizer requirements (Galván et al., 2011; Porter & Sachs, 2020).

AM fungi contribute to resource-use optimization, regeneration of degraded soils, and enhanced sustainability in food, fiber, and bioenergy production systems (Cobb et al., 2017; Rillig et al., 2019; Zhang et al., 2019). Plant breeders are making clear progress by selecting traits that improve aboveground biomass yields, yet plant-mycorrhizal interactions were not historically incorporated into breeding programs and breeding under high-input conditions can profoundly reduce reliance on resources supplied by mycorrhizal symbioses.
relationships (Cobb et al., 2016; Hetrick et al., 1995). Increasing multiple ecosystem services, such as soil carbon storage and downstream water quality, is an emerging paradigm in agriculture, and AM fungi represent a strategic component of improved agroecosystem management (Rillig et al., 2019; Rillig & Lehmann, 2019). Hence, incorporating host-plant reliance on resources supplied by AM fungi should be an important component for crop breeding programs concerned with improving agricultural sustainability, including mycorrhizal contributions to soil regeneration and fertilizer use optimization.

It is critical to assess breeding strategies, such as hologenome breeding, that potentially enhance mycorrhizal growth responses (MGRs) and can be scaled up to improved agroecosystem efficiency. Switchgrass is known to associate with AM fungi, and advanced molecular tools have provided valuable insights into the genetic diversity and evolution of switchgrass across geographic regions (Evans et al., 2018; Zhang et al., 2011). However, linking switchgrass breeding practices with mycorrhizal responsiveness is underexplored. These knowledge gaps constrain our capacity to advise crop breeders on incorporating key rhizosphere traits, such as mycorrhizal symbioses into selective breeding programs. Our current experiment addresses these gaps by comparing host-plant outcomes of newly developed switchgrass synthetics with parent lines, following low-input selective breeding, to examine potential alterations in mycorrhizal reliance. The Oklahoma State University (OSU) switchgrass breeding program is focused on the development of high-yielding cultivars targeted for bioenergy feedstock production. Genotypic and phenotypic recurrent selection has been used to advance populations, whereas selection and evaluation are conducted in low-input environments. Assessing the potential of low-input breeding, as part of a hologenome breeding framework, to enhance mycorrhizal benefits is a step toward more sustainable agricultural production, and ecological insights gained are transferable to a wide array of agroecosystems.

2 | MATERIALS AND METHODS

2.1 | Soil and inoculum preparation

Prairie soil, a Chase silty clay loam, fine mont-morillonitic, mesic Aquic Argiudoll (pH = 7.5; plant-available N = 9.6 mg/kg; plant-available P = 10 mg/kg [Bray test I]), was freshly collected from Konza Prairie Biological Station (KPBS), Manhattan, Kansas, USA, and transported to OSU. To eliminate soil microorganisms, including AM fungi, soil was steam-pasteurized at 80°C for 2 hr and allowed...
to cool for 72 hr with no measurable changes in soil chemistry (pH, NH₄, NO₃, plant-available P). Communities of indigenous AM fungi and other soil organisms were added back in a controlled manner by inoculating one-half of the pots (mycorrhizal) with 20 g of living soil from KPBS. The living soil inoculum was added directly below seedling roots during transplantation. The remaining half of the pots (nonmycorrhizal) filled with only sterile soil as a control for each switchgrass population. All pots were also amended with 60 ml non-sterile soil sievate. Sievates were prepared by blending soil:water in a 1:2 ratio and passing the slurry through a 38-µm sieve. The relatively large AM fungal spores were trapped on the sieve, whereas smaller organisms pass through, allowing for the addition of the majority of soil microbes while excluding AM fungi (Koide & Li, 1989).

2.2 | Plant preparation

*Panicum virgatum*, including four synthetics from the OSU switchgrass breeding program, SL93 C2-2, NL94 C2-3, NL94 C2-4, and NLSL 2009-1, and two parental cultivars, Alamo and Kanlow, were selected. These populations originate in the southern great plains and are commonly planted for biofuel feedstock and selected for increased production. Lowland switchgrass germplasm was used to initiate two populations, South Lowland population in 1993 (SL93) and North Lowland population in 1994 (NL94; Bartley et al., 2013; Wu, 2014). All four OSU experimental synthetics were developed at Cimarron Valley Research Station, Perkins, OK, by crossing elite individuals from cycle two evaluation nurseries that were given low-input fertilization: 112 kg/ha (18-46-0, N-P-K) at establishment and 56 kg/ha plant-available N in subsequent years. Seeds were pregerminated in vermiculite and 14-day-old seedlings (second-leaf stage) were transplanted into pots (6 cm × 25 cm) containing 600 g of soil (one seedling per pot).

2.3 | Experimental design and maintenance

Plants were grown in a glasshouse at OSU, Stillwater, Oklahoma, USA. Pots were blocked by switchgrass population, and plants were arranged in a completely randomized design. Each population (mycorrhizal and nonmycorrhizal controls) was replicated six times for a total of 72 pots. Plants were watered daily and fertilized every other week with 35 µg/g N and 35 µg/g K, applied as Peter's No-Phos Special Fertilizer solution (25:0:25, Robert B. Peter's Co., Inc., Allentown, Pennsylvania). Plants were grown during the summer with full sunlight (918–1027 mmol m⁻² s⁻¹) and maintained at 21–28 (daily mean 24.5) °C for 14 weeks. At the end of 14 weeks, plants were harvested, roots washed free of soil, and biomass was oven-dried at 60°C for 48 hr. Subsamples of dried roots were stained in trypan blue (Phillips & Hayman, 1970) and examined microscopically to assess percentage root colonization (McConigle et al., 1990). Shoot tissue P concentrations were determined using a Spectro Blue ICP following acid digestion (NFTA, 1993).

2.4 | Statistical analyses

Prior to analyses, all data were tested for normality and homogeneity of variances using Shapiro-Wilk and Levene's tests, respectively. To assess effects of population on biomass production, shoot P concentration and content, and AM fungal root colonization, one-way analyses of variance (ANOVA) were conducted, with population as the sole factor. Within populations, mycorrhizal and nonmycorrhizal replications were also compared using one-way ANOVA. Tukey's honest significant differences (HSDs) tests were performed post hoc to assess differences among populations, and mycorrhizal status, with α = 0.05. Due to normal distribution of proportional data, shoot phosphorus and root colonization were not transformed. We determined MGR and mycorrhizal phosphorus response (MPR) using equations from Cavagnaro et al. (2003). All MGR calculations are expressed as %MGR = (dry wt. mycorrhizal plant) – (mean dry wt. nonmycorrhizal plant) / (mean dry wt. nonmycorrhizal plant) × 100, such that all values indicate a percentage change in biomass of mycorrhizal plants, compared with mean biomass of nonmycorrhizal plants. All MPR calculations are expressed as %MPR = (shoot P content mycorrhizal plant) – (mean shoot P content nonmycorrhizal plant) / (mean shoot P content nonmycorrhizal plant) × 100, such that all values indicate a percentage change in shoot P content of mycorrhizal plants, compared with mean shoot P content of nonmycorrhizal plants. Values of MGR and MPR calculations were compared using one-way ANOVAs. All statistical analyses were performed using base R 3.6.1 (R Core Team, 2019).

3 | RESULTS

3.1 | Mycorrhizal growth response and mycorrhizal phosphorus response following low-input selective breeding

Synthetics developed by OSU’s switchgrass biofuel breeding program originated from lowland populations in the Southern Great Plains; however, these modified populations express substantially greater MGR than parents (Figure 2). We also found evidence selective breeding under low-input nutrient conditions influenced MPR, with synthetics expressing substantially greater MPR than parents (Figure 3).

3.2 | Plant biomass, arbuscular mycorrhizal fungal root colonization, and shoot P

There was at least one synthetic within each mycorrhizal comparison that produced equivalent or greater shoot, root, and total biomass, than the corresponding parent line; however, nonmycorrhizal synthetics generally produced less biomass compared with corresponding parent lines (Table 1). In particular, SL93 C2-2 and NLSL 2009-1 produced significantly greater total biomass when grown with AM
fungi, compared with corresponding parent lines, and significantly reduced total biomass when grown in the absence of AM fungi, compared with corresponding parent lines (Table 1). For mycorrhizal plants, AM fungal root colonization and shoot P concentration or P content did not differ between synthetic and parent lines (Table 2). However, nonmycorrhizal synthetic plants derived from Kanlow were characterized by lower shoot P content, compared with the parent line (Table 2).
**Table 1** Shoot biomass, root biomass, and total biomass (dry wt. g) for OSU switchgrass and corresponding parent lines

| Switchgrass population | Shoot biomass (dry wt. g) | Root biomass (dry wt. g) | Total Biomass (dry wt. g) |
|------------------------|---------------------------|--------------------------|---------------------------|
|                        | M (± SE)                  | NM (± SE)                | M (± SE)                  | NM (± SE)                |
| **Comparison A**       |                           |                          |                           |                           |
| Alamo                  | 2.65 ± 0.20a              | 0.13 ± 0.01a             | 1.32 ± 0.11b              | 0.14 ± 0.02a             |
| SL 93 C2-2             | 3.20 ± 0.11a              | 0.10 ± 0.01a             | 2.31 ± 0.13a              | 0.07 ± 0.02b             |
| **Comparison B**       |                           |                          |                           |                           |
| Kanlow                 | 3.02 ± 0.11xy             | 0.23 ± 0.01x             | 1.88 ± 0.13y              | 0.24 ± 0.02x             |
| NL94 C2-3              | 2.80 ± 0.10x              | 0.15 ± 0.02x             | 1.38 ± 0.16xy             | 0.10 ± 0.02y             |
| NL94 C2-4              | 1.81 ± 0.23y              | 0.05 ± 0.01x             | 1.11 ± 0.10yz             | 0.04 ± 0.01y             |
| NLSL 2009-1            | 3.52 ± 0.13x              | 0.13 ± 0.01y             | 3.28 ± 0.31y              | 0.07 ± 0.01y             |

Note: Oklahoma State University (OSU) switchgrass synthetic populations compared with corresponding parents. Comparison A = SL93 C2-2 with Alamo (Parent); Comparison B = NL94 C2-3, NL94 C2-4, and NLSL 2009-1 with Kanlow (Parent). Grown with (M) or without (NM) arbuscular mycorrhizal fungi; results expressed as means ± standard error (n = 6), based on one-way ANOVA and Tukey’s honest significant difference. All mycorrhizal replications produced significantly greater biomass than corresponding nonmycorrhizal replications. Within a column, for each parent line comparison, values that do not share a letter are significantly different (p < .05).

**Table 2** Shoot P concentration (µg/g), shoot P content (mg), and arbuscular mycorrhizal (AM) fungal root colonization (%) for OSU switchgrass and corresponding parent lines

| Switchgrass population | Shoot P concentration (µg/g) | Shoot P content (mg) | Colonization (%) |
|------------------------|------------------------------|----------------------|------------------|
|                        | M (± SE)                     | NM (± SE)            | M (± SE)         |
| **Comparison A**       |                              |                      |                  |
| Alamo                  | 1.428 ± 79a                  | 610 ± 69a            | 4.61 ± 0.30a     | 0.12 ± 0.03a            | 47.9 ± 1.5a            |
| SL 93 C2-2             | 1.175 ± 70a                  | 593 ± 62a            | 3.99 ± 0.38a     | 0.06 ± 0.03a            | 50.2 ± 3.0a            |
| **Comparison B**       |                              |                      |                  |
| Kanlow                 | 1.265 ± 184x                 | 630 ± 47x            | 4.23 ± 0.64xy    | 0.21 ± 0.04x            | 37.3 ± 2.7x            |
| NL94 C2-3              | 1.340 ± 67x                  | 503 ± 35xy           | 4.12 ± 0.21xy    | 0.10 ± 0.02y            | 40.9 ± 2.2x            |
| NL94 C2-4              | 1.638 ± 135x                 | 555 ± 27xy           | 2.56 ± 0.45xy    | 0.02 ± 0.01y            | 41.6 ± 1.1x            |
| NLSL 2009-1            | 1.155 ± 44x                  | 455 ± 27x            | 4.34 ± 0.20x     | 0.04 ± 0.01y            | 43.3 ± 2.2x            |

Note: Oklahoma State University (OSU) switchgrass synthetic populations compared with corresponding parents. Comparison A = SL93 C2-2 with Alamo (Parent); Comparison B = NL93 C2-3, NL94 C2-4, and NLSL 2009-1 with Kanlow (Parent). Grown with (M) or without (NM) arbuscular mycorrhizal fungi; results expressed as means ± standard error (n = 6), based on one-way ANOVA and Tukey’s honest significant difference. All mycorrhizal replications produced significantly greater biomass than corresponding nonmycorrhizal replications. Within a column, for each parent line comparison, values that do not share a letter are significantly different (p < .05).

1AM fungal structures were not present in nonmycorrhizal roots.

4 | DISCUSSION

The most important and novel findings of our study are low-input selective breeding generally increased host-plant reliance on AM symbioses, and, when grown with AM fungi, two lines from OSU’s breeding program produced ~39% greater total biomass, compared with corresponding parent lines. Our results suggest that breeders can develop agronomic populations that outperform less reliant parent lines. If low-input breeding improves MGR and MPR of synthetic populations compared with parent lines, the high nutrient and water-use efficiency associated with effective mycorrhizal symbioses can facilitate economical switchgrass biomass production as a cellulosic bioenergy crop. Additionally, our results indicate low-input selective breeding has substantial potential to improve mycorrhizal reliance, with applications across a diverse array of agricultural crops.

With increasing recognition that plants and associated microbes should be assessed together as a holobiont, and that crop breeding strategies must account for complex symbiotic amalgams (Gopal & Gupta, 2016; Ravanbakhsh et al., 2021; Wei & Jousset, 2017; Ye & Siemann, 2020), our findings add an important dimension to switchgrass research that can be extrapolated to multiple agricultural production systems. We assessed plant responses to AM fungi in conditions where additional holobiont components (i.e., local soil microbes) were consistent across the experiment. We used this approach because AM fungi represent a flagship mutualism within...
root-associated microbial communities, making them a convenient target for hologenome breeding. Future research and breeding efforts will likely discover multiple selection criteria within holobiont consortia, as diverse microbiota have both complementary and competitive influences on the hologenome (Theis et al., 2016). By considering the influence of AM fungi, among the most widespread symbiotic partners in both natural and agroecosystems, our work sets a baseline for further study involving additional mutualistic microorganisms such as nitrogen-fixing bacteria (Roley et al., 2020).

Agricultural breeding generally focuses on aboveground performance traits and qualities, whereas belowground traits and microbial partnerships receive less attention, impacting the potential utility of crops for use in agricultural systems designed around microbial-sensitive practices. Selective breeding in highly fertilized and irrigated systems reduced MGR of modern Sorghum bicolor genotypes compared with less modified varieties, presumably due to inadvertent decoupling of host-plant dialogue with AM fungi (Cobb et al., 2016). Similarly, ancestral land races of wheat often respond very positively to mycorrhizas, whereas modern cultivars respond negatively—even in low-P soils (Hetrick et al., 1993). The authors concluded wheat cultivars are selectively bred under high P input, profoundly reducing reliance on mycorrhizas. In fact, genetic inheritance studies identified alleles on several chromosomes in wheat linked with responsiveness to mycorrhizas (Hetrick et al., 1995). Comparison of synthetic populations with parent lines suggest altered traits may link to critical rhizosphere signaling pathways. If commercial crop breeding has unintentionally selected against these signaling pathways, we propose the process of can be reversed through refined breeding protocols. Indeed, there are considerable opportunities for hologenome breeding following assessments of MGR and MPR. Our results demonstrate alternations in plant genomes are not solely responsible for crop biomass production, as a consortium of microorganisms, especially AM fungi, interact with the biological scaffolding provided by plants to determine the productivity of the holobiont.

Switchgrass breeding must avoid negative unintended outcomes associated with high-input breeding. Understanding the influence of breeding protocols on mycorrhizal dynamics may enable optimization of agricultural production in low-input conditions. Indeed, we previously proposed targeted breeding for increased MGR, especially for C4 grass species (Cobb et al., 2017; Wilson et al., 2015). It is important to note that reduced nonmycorrhizal biomass in synthetic populations had an influence on MGR and MPR calculations. This type of selective alteration is not always considered an effective breeding target (Galván et al., 2011); however, in our study, two synthetic populations also produced greater total biomass than parent lines, when grown with AM fungi. Therefore, improvement of MGR and MPR was likely a function of increased mycorrhizal reliance following low-input breeding, with both negative consequences for nonmycorrhizal synthetic populations and growth benefits for mycorrhizal plants. By identifying and assessing modified populations of switchgrass, as a model mycorrhizal plant species, our findings represent a step toward breeding for greater beneficial belowground interactions. Because of emerging technologies, new genotype/population development is increasing rapidly. Cutting-edge tools are improving our capacity to design crop varieties that are locally adapted (Cortinovis et al., 2020), and development of CRISPR gene-editing provides a reason for optimism regarding the future of appropriate crop development (Zhang et al., 2020). Gene-editing enables additional options to stack multiple beneficial traits (Khan et al., 2021), for example genes associated with greater mycorrhizal reliance, into highly productive lines. Considering the findings of our current study, we advocate for eventual application of gene-editing technology to use traits associated with locally adapted plant germplasm in agronomic varieties that meet the economic requirements of producers while simultaneously enhancing the nutrient-use efficiency benefits of mycorrhizal associations in agroecosystems.

A meta-analysis by Zhang et al. (2019) describes mycorrhizal contributions to commodity crop production and grain quality but highlights persistent variation in mycorrhizal-mediated outcomes across agricultural systems and management practices. We propose that inconsistencies in management are potentially driven by study selection of host plants that have insufficient reliance on mycorrhizas. For example, experimental comparisons of different nutrient amendments or cropping diversity that compare crop genotypes that are marginally responsive to AM symbioses will potentially lead to biased conclusions. We propose that prior to assessing influences of farm management decision or soil amendments on crop-mycorrhizal outcomes at farm-scale, researchers need a foundational understanding of the influence of host-plant traits on AM fungal associations, as the flagship mutualism of plant holobionts. Moving forward, we suggest tighter collaborations between mycorrhizologists and plant breeders that focus on developing germplasm that are guided by MGR outcomes. This initial step should be instituted prior to assessing complex influences of soil amendments and agroecosystem management that potentially contribute to AM fungal benefits and sustainable plant production. Additionally, increasing reliance of agricultural crops on AM symbioses can improve global soil health, as mycorrhizal fungal hyphae are tightly linked to key measurements of soil quality, such as soil aggregation and soil C storage (Wilson et al., 2009). These dynamics are vital for operationalizing sustainable production systems.

By understanding relationships between switchgrass and mycorrhizal symbioses, we can better achieve host-plant breeding that simultaneously maximizes biomass production and soil quality. OSU’s switchgrass breeding program focuses on improving drought resilience and nutrient-use efficiency through identification of root morphological characteristics such as coarse and elongated roots, capable of tapping into deep soil horizons. There is a positive correlation between root coarseness and plant MGR (Bergmann et al., 2020; Miller et al., 2012), suggesting root morphology is a critical target for future breeding efforts. Indeed, root morphological mechanisms may help explain improved MGR and MPR observed in our switchgrass populations, following breeding under low-input conditions. For the most productive and mycorrhizal reliant synthetics, we measured similar shoot biomass and
significantly greater root biomass than parent lines, suggesting plants acquired additional resources and allocated those to root production. Although total root colonization by AM fungi was similar, greater allocation to root biomass likely had an overall positive influence on nutrient uptake. Following low-input breeding, increased reliance on AM fungi and greater root production both have implications for soil quality and agricultural efficiency. A critical next step is combining transcriptome measurements with well-quantified plant gene expression. Cutting-edge transcriptomic work is being conducted with other warm-season grasses to associate host-plant gene expression with functional AM symbioses (Watts-Williams et al., 2019). Future research of this type may disentangle the influence of plant morphological traits from mycorrhizal signaling pathways and provide multiple strategic breeding targets, especially in the context of gene-editing advances that continue to expand our capacity to stack multiple plant traits into modification events (Khan et al., 2021). As an attractive model and production crop, switchgrass represents another exciting opportunity to enhance mycorrhizal contributions across a wide array of agroecosystems.

5 | CONCLUSIONS

Our strategy of identifying more mycorrhizal reliant populations of *P. virgatum* elucidates a path forward, as *P. virgatum* serves as a model plant for sustainable biofuel production (Emery et al., 2018), with implications for numerous additional crops. Developing in situ management of mycorrhizas using MGR and MPR crop genotypes can potentially reduce cost to farmers (Benami et al., 2020). Many natural ecosystems display stability and resilience, in large part due to interactions such as AM symbioses (Jia et al., 2020; Yang et al., 2018). Discovery and utilization of mycorrhizal mechanisms and modification of plant genetics underlying holobiont interactions have potential to help us meet key agricultural sustainability goals, such as enhancing soil health and improving fertilizer efficiency. We propose low-input breeding creates selective pressure for switchgrass and other crops to invest more resources in chemical signaling with AM fungi, potentially enhancing both production efficiency and soil quality. If loss of symbiotic potential follows high-input plant breeding, our current study indicates an opportunity to harness the benefits of AM fungi under low-input breeding and other conditions that are based on a hologenome breeding framework.

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CONFLICT OF INTEREST

The authors declare research was conducted without commercial or financial relationships that can be construed as a conflict of interest.

AUTHOR’S CONTRIBUTIONS

R.M.M., Y.Q.W., and G.W.T.W. designed the research. K.B.H. and G.W.T.W. conducted the research. A.B.C., E.B.D., K.B.H., R.M.M., and G.W.T.W. carried out data collection and analyses. A.B.C., E.B.D., R.M.M., and G.W.T.W. interpreted data. A.B.C. and G.W.T.W. wrote the manuscript; all authors contributed to editing the final paper.

DATA AVAILABILITY STATEMENT

Authors agree to make data and materials available to readers on reasonable request.

ORCID

Adam B. Cobb https://orcid.org/0000-0002-3305-1360
Eric B. Duell https://orcid.org/0000-0001-8692-5271
R. Michael Miller https://orcid.org/0000-0003-2114-7856
Yanqi Q. Wu https://orcid.org/0000-0003-0802-6881
Gail W. T. Wilson https://orcid.org/0000-0003-1396-6480

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