



## Influence of Mycorrhizal Symbiosis on Carbon Storage Potential in Sorghum (*Sorghum Bicolor* L.) Under Elevated CO<sub>2</sub> Levels

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### Abstract

**Background:** *Sorghum Bicolor* L. and arbuscular mycorrhizal fungi (AMF) have a mutually beneficial relationship that influences the movement of carbon below ground. The rising concentration of carbon dioxide (CO<sub>2</sub>) in the atmosphere during the middle and later part of the twenty-first century (550–700 ppm) is expected to change the dynamics of below-ground carbon storage. To increase climate-resilient and carbon-efficient agricultural systems, it is vital to understand how these two species interact.

**Objectives:** Objectives for this article include: (1) Identify the processes and structures that control AMF (arbuscular mycorrhizal fungi)-mediated carbon distribution to sorghum (*Sorghum Bicolor*); (2) quantify the contribution of glomalin-related soil protein (GRSP) and hyphal turnover to stable soil organic carbon (SOC) pools; and (3) evaluate how elevated CO<sub>2</sub> impacts mycorrhizal network dynamics and microbial communities in the rhizosphere.

**Methods:** A total of 54 experiments from around the world were conducted to look at how carbon gets assigned in the sorghum system and to see if the amount of carbon in the soil from AMF, GRSP, and carbon stabilization in soil differed under normal or higher CO<sub>2</sub> levels.

**Results:** Sorghum plants inoculated with arbuscular mycorrhizal fungi (AMF) grown in an elevated carbon dioxide (CO<sub>2</sub>) environment had 23-47% greater underground carbon allocation than controls without AMF. Glomalin root-associated soil polymer (GRSP) concentration in soil increased by 18-35% and soil aggregate stability was improved as a result of increased total glomalin production. The rate of AMF fungal colonization increased by 15-28% and greater amounts of extra-radical fungal hyphal biomass associated with the AMF also contributed to a higher production of glomalin and soil organic carbon (SOC) stabilization via organo-mineral interactions. In addition, AMF symbiosis improved the stress tolerance of host sorghum plants, as demonstrated by increased water-use efficiency (20-35%) and nitrogen-use efficiency (15-22%). The synergistic effects of AMF and elevated CO<sub>2</sub> also enhanced total host plant biomass and rhizodeposition, providing 12-28% of the total SOC input to a soil ecosystem.

**Conclusions:** The enhanced capacity for carbon storage that happens as a result of the interaction between arbuscular mycorrhizal fungi (AMF) and sorghum when grown under elevated concentrations of carbon dioxide (CO<sub>2</sub>) further supports the uses of both AMF and sorghum as critical components of climate-smart agronomy and carbon farming. Nonetheless, many important knowledge gaps exist regarding long-term carbon stabilization, soil priming effects and AMF species-specific relationships in semi-arid agroecosystems. Future studies require the integration of prolonged agricultural field studies, omics-based study methods and isotopic approaches to establish strong proof-based strategies for carbon management.

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**Keywords:** Arbuscular Mycorrhizal Fungi, Carbon Sequestration, *Sorghum Bicolor*, Elevated Co<sub>2</sub>, Soil Organic Carbon, Glomalin, Rhizosphere

### 1. Introduction

#### 1.1. The Global Carbon Crisis and Agroecosystem Responses

The scientific community is increasingly investigating biological carbon sequestration in light of the accelerating CO<sub>2</sub> accumulation in the atmosphere, which reached levels exceeding 421 parts per million (ppm) in 2023 and continues to increase at a rate of approximately 2.4 ppm/year (Friedlingstein, 2023)<sup>[1]</sup> (IPCC, 2023)<sup>[2]</sup>. Agriculture, by far, has the largest potential for carbon storage, containing approximately 1,500 billion (Pg) of organic carbon at a depth of 1 meter and having some of the

most dynamic and manipulable (IPCC, 2023) <sup>[2]</sup> sources of carbon in the terrestrial biosphere (Jobbágy and Jackson, 2000) <sup>[3]</sup>. The capacity for an agroecosystem to sequester carbon from the atmosphere is determined primarily by the productivity of the plants growing in the system, root structure of those plants, how roots deposit carbon into the soil, and the activity of microbial communities in the soil to alter those processes — all of which are significantly influenced by the presence of mycorrhizal fungi (Rillig and Mummey, 2006) <sup>[4]</sup> (Rillig *et al.*, 2015) <sup>[39]</sup> (Jones *et al.*, 2009) <sup>[51]</sup>.

Approximately 700 million hectares of land are devoted to cereal crop production globally, with sorghum (*Sorghum Bicolor* L.) Moench) being critical as a food security crop in large regions of sub-Saharan Africa, South Asia, and the semi-arid tropics (Food and Agriculture Organization of the United Nations (FAO), 2023) <sup>[5]</sup>. These characteristics, in addition to its efficient C<sub>4</sub> photosynthesis and its ability to develop a deep root system, make it an excellent candidate for implementing climate smart carbon farming programs (Sage and Zhu, 2011) <sup>[58]</sup> (Kamara *et al.*, 2003) <sup>[20]</sup>. However, for sorghum to effectively function as a carbon sink, sorghum's capacity to develop productive mycorrhizal associations — especially with arbuscular mycorrhizal fungi (AMF) from the phylum Glomeromycota — must be highly developed (Dodd, 2000) <sup>[6]</sup> (Bray *et al.*, 2012) <sup>[7]</sup>.

## 1.2. Mycorrhizal Biology and Carbon Economy

Approximately 72% of land-dwelling plant species -- including most cultivated cereal grains -- establish a symbiotic association with an obligate biotrophic fungus, the arbuscular mycorrhizal fungus (AMF) (Brundrett and Tedersoo, 2018) <sup>[8]</sup>. As part of the mycorrhizal symbiosis, the host plant provides a percentage of its carbon, between 4 and 20 percent of its net carbon fixed photosynthetically, to its fungal partner in exchange for assistance in acquiring phosphorus (P), nitrogen (N), zinc (Zn), and other limiting nutrients (Smith and Read, 2008) <sup>[10]</sup> (Kaschuk *et al.*, 2009) <sup>[11]</sup>. This trade-off between carbon and nutrient resource directly affects the plant's carbon economy, shifting resources from the stem to root and fungal biomass so that more carbon is available for use in the soil system (Kaschuk *et al.*, 2009) <sup>[11]</sup>.

The pathways through which soil carbon is deposited through mycorrhizae occur through multiple complementary mechanisms, including: (i) direct carbon contribution from the turnover of hyphae and necromass accumulation (Staddon *et al.*, 2003) <sup>[40]</sup>; (ii) production of glomalin related soil protein (GRSP), an example of a highly cryptic glycoprotein that binds tightly to soil aggregates and promotes carbon sequestration (Wright and Upadhyaya, 1998) <sup>[45]</sup> (Rillig, 2004) <sup>[46]</sup>; (iii) increased rhizodeposition - a process in which the formation of new roots causes exposed soil to have enhanced access to carbon-based products released by decaying microbes (Jones *et al.*, 2009) <sup>[51]</sup>; and (iv) altered soil microbial communities, including changes to their nutrient balance, resulting from the mycorrhizal network's effect on nutrient balance in the ecosystem (Philippot *et al.*, 2013) <sup>[53]</sup> (Nuccio *et al.*, 2013) <sup>[52]</sup>.

## 1.3. Elevated CO<sub>2</sub> and Mycorrhizal Symbiosis: An Evolving Paradigm

Consistent results from FACE experiments and controlled environment (+CO<sub>2</sub>) research show that CO<sub>2</sub> enrichment

causes changes in the plant-mycorrhizal relationship through several mechanisms (Treseder, 2004) <sup>[14]</sup> (Leakey *et al.*, 2009) <sup>[19]</sup>. Increased rates of photosynthesis from CO<sub>2</sub> enrichment leads to increased amounts of available photosynthates for AMF, which could decrease the carbon cost of the associations and subsequently allow for an increase in fungal biomass produced and hyphal network expansion (Ainsworth and Rogers, 2007) <sup>[59]</sup> (Drigo *et al.*, 2009) <sup>[15]</sup>. At the same time, elevated levels of CO<sub>2</sub> typically result in stomatal closure, decrease in nitrogen concentration (due to the dilution effect) in the plant tissue, and alteration in root exudate composition, all of which could potentially modify the methods by which AMF can colonize roots, as well as how carbon is partitioned to these fungi (Rogers *et al.*, 2009) <sup>[17]</sup> (Nie *et al.*, 2013) <sup>[16]</sup>.

Meta-analyses summarizing data generated from FACE and/or open-top chamber studies report differing levels of AMF colonization in plants exposed to elevated levels of CO<sub>2</sub> as a function of plant species, the identity of the fungus, soil nutrient status and length of experiment exposure (Mohan *et al.*, 2006) <sup>[18]</sup> (Treseder, 2004) <sup>[14]</sup>. In C<sub>4</sub> species such as sorghum, effects of CO<sub>2</sub> enrichment on photosynthesis are smaller than the effect that C<sub>3</sub> species exhibit (due to CO<sub>2</sub> concentrating mechanisms), yet there have been several reports on the significant impacts that the addition of CO<sub>2</sub> has had on water use efficiency, stomatal conductance, and carbon partitioning patterns (Sage and Zhu, 2011) <sup>[58]</sup> (Leakey *et al.*, 2009) <sup>[19]</sup>. However, there is still little or no knowledge about the impact these physiological changes have on mycorrhizal carbon economy and SOC dynamics in sorghum systems; thus, there still exist significant gaps in knowledge within the combined areas of plant physiology, mycorrhizal ecology, and global change biology.

## 1.4. Scope and Objectives

This review compiles current information about how arbuscular mycorrhizal fungi (AMF) affect carbon sequestration ability of *Sorghum Bicolor* under elevated carbon dioxide (CO<sub>2</sub>) levels. Objectives include: (1) understanding molecular and physiological mechanisms of AMF colonization in the roots of sorghum; (2) measuring contributions of AMF to soil organic carbon via glomalin-related soil proteins (GRSP), hyphal turnover and rhizodeposition; (3) assessing effects of high CO<sub>2</sub> on carbon economy of plants and AMF, and dynamics of mycorrhizal networks; (4) exploring relationships among AMF activity, elevated CO<sub>2</sub>, and soil microbial communities that are important for carbon cycling; (5) determining potential impacts of AMF on sorghum productivity, stress resistance, and carbon farming; and (6) identifying essential knowledge gaps and future research directions.

## 2. Mycorrhizal Symbiosis in *Sorghum Bicolor*: Types, Mechanisms, and Nutrient Dynamics

### 2.1. Types of Mycorrhizal Associations Relevant to *Sorghum Bicolor*

*Sorghum Bicolor* typically interacts with Arbuscular Mycorrhizal Fungi (AMF) from the phylum Glomeromycota, with specific genera including *Rhizophagus*, *Glomus*, *Funneliformis*, *Claroideoglossum*, *Diversispora*, and *Scutellospora* (Kamara *et al.*, 2003) <sup>[20]</sup> (Manga and Singh, 2013) <sup>[21]</sup>. Arbuscular Mycorrhizal Fungi are obligate biotrophs which grow into the cortical cells (the inside) of the sorghum roots and develop tree-like structures called

Arbuscules, which provide the site for the exchange of nutrients and carbon and contractile structures called Vesicles (Parniske, 2008) <sup>[22]</sup>. AMF develop intra-cellular relationships with the root cortex, as opposed to Ectomycorrhizal (rare in cereal crops), which rupture the root plasma membrane (the outer membrane of the root cell). An interface called a Peri-arbuscular Membrane interface links the AMF to the host/root cell, determining the exchange of nutrients and carbon in the symbiotic relationship (Bonfante and Genre, 2010) <sup>[23]</sup>.

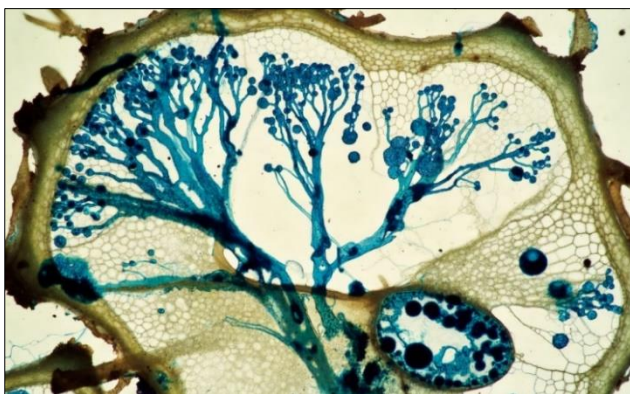
Identical research was conducted on the AMF that colonize sorghum roots in Africa and has identified an AMF

composition of *Rhizophagus irregularis*, *Funneliformis mosseae*, and *Scutellospora calospora* (Manga and Singh, 2013) <sup>[21]</sup> (Adesemoye and Akintokun, 2009) <sup>[24]</sup>. The composition of AMF species colonizing sorghum is influenced by soil type, geographic location (African sorghum growing regions), management practices, and sorghum genotype. Importantly, the distribution of AMF species, and therefore their functional diversity, indicates that the AMF community composition may determine the productivity levels of carbon sequestration and drought tolerance among sorghum systems (Kamara *et al.*, 2003) <sup>[20]</sup>.

**Table 1:** Major AMF species associated with *Sorghum Bicolor* and their functional roles in nutrient acquisition and carbon dynamics.

AMF Species	Family	Colonization Rate (%)	P Uptake Efficiency	Carbon Allocation (%)	Primary Function
<i>Rhizophagus irregularis</i>	Glomeraceae	65–85	High	15–22	P acquisition, drought tolerance
<i>Funneliformis mosseae</i>	Glomeraceae	55–75	High	12–18	P & N uptake, GRSP production
<i>Scutellospora calospora</i>	Gigasporaceae	40–60	Moderate	18–25	Hyphal network expansion
<i>Claroideoglossum claroideum</i>	Claroideoglossaceae	45–65	Moderate–High	10–16	Soil aggregate stabilization
<i>Diversispora spurca</i>	Diversisporaceae	30–50	Moderate	8–14	Stress mitigation
<i>Glomus microaggregatum</i>	Glomeraceae	50–70	Moderate	11–17	Microaggregate formation
<i>Ambispora leptoticha</i>	Ambisporaceae	25–45	Low–Moderate	6–12	Niche colonization

Colonization rates and functional attributes based on meta-analytic data from sorghum and related cereal studies <sup>[20, 21, 24]</sup>. Carbon allocation values represent percentage of net fixed carbon directed to fungal partner.



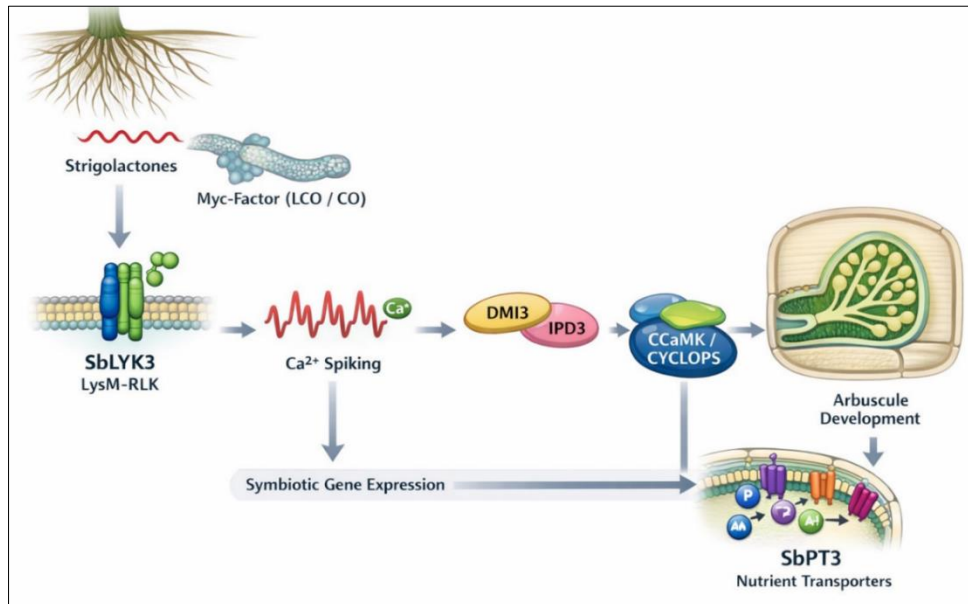
**Fig 1:**

## 2.2. Molecular and Physiological Basis of Mycorrhizal Colonization in Sorghum Roots

The common symbiosis signalling pathway (CSSP) is a conserved molecular mechanism that regulates the establishment of functional arbuscular mycorrhizal fungi (AMF) symbiosis with sorghum roots (Oldroyd, 2013) <sup>[25]</sup> (Zipfel and Oldroyd, 2017) <sup>[28]</sup> (Parniske, 2008) <sup>[22]</sup> (Bonfante and Genre, 2010) <sup>[23]</sup>. The recognition of AMF by sorghum roots is initiated when sorghum roots exude strigolactones (SLs), which are phytohormones that derive from the degradation of apocarotenoids, into the surrounding rhizosphere (Bouwmeester *et al.*, 2007) <sup>[26]</sup> (Oldroyd, 2013) <sup>[25]</sup>. As a result, such SLs stimulate AMF spores to germinate, hyphae to branch, and messenger RNA transcription in the pre-symbiotic fungal partner (Oldroyd, 2013) <sup>[25]</sup> (Bouwmeester *et al.*, 2007) <sup>[26]</sup>. There are two major compounds produced by *Sorghum Bicolor* in the rhizosphere as a result of the dumping of SLs: sorgolactone and 5-

deoxystrigol, which both have considerable biological activity, which stimulates the germination of spores in AMF and acts to signal parasitic plants such as *Striga hermonthica* (Jamil *et al.*, 2011) <sup>[27]</sup> (Bouwmeester *et al.*, 2007) <sup>[26]</sup>. Therefore, there are complex ecological trade-offs created by the production of these two compounds in sorghum agroecosystems (Jamil *et al.*, 2011) <sup>[27]</sup>.

All Exogenous AMFs Use Strigolactone and Induce Lipochitooligosaccharide (LCO) and Short-chain Chitooligosaccharide (CO) Myc Factos Through the interactions of AMFs (arbuscular mycorrhizal fungi) (beta-sorghi) through strigolactones (SLs) which serve as a cue to the plant roots (Oldroyd, 2013) <sup>[25]</sup> (Zipfel and Oldroyd, 2017) <sup>[28]</sup>, the AMFs trigger the perception of LCOs or COs in a lysin-motif (LysM) receptor-like kinases (RLKs, LysM-RLKs) and transduce a biochemical signal (Oldroyd, 2013) <sup>[25]</sup> (Zipfel and Oldroyd, 2017) <sup>[28]</sup>. In sorghum (*Sorghum Bicolor*), the LysM-RLKs are SbLYK3 and SbNFP (Zipfel and Oldroyd, 2017) <sup>[28]</sup>. As a result of this initial event, the combination of LCO/CO perception generates a rapid calcium oscillator (calcium spiking) in the cytosol of plant root cells (Oldroyd, 2013) <sup>[25]</sup>. The calcium signal is decoded to trigger the phosphorylation of CYCLOPS/IPD3 transcription factors through a calcium-dependent and calmodulin-dependent protein kinase (CCaMK/DMI3), setting in place the initiation of the symbiotic transcriptional programme (Oldroyd, 2013) <sup>[25]</sup>. The proteins involved in transcriptional reprogramming all permit the movement of nutrients from the fungal arbuscular mycorrhiza to the plant cell by activating expression of (1) phosphate transporters (SbPT3 and SbPT5) and (2) ammonium transporters (SbAMT3) that specifically localise to the periarbuscular membranes (Harrison *et al.*, 2002) <sup>[29]</sup>.



The common symbiosis signalling pathway (CSSP) in *Sorghum Bicolor*. The cascade illustrates the sequential molecular events from strigolactone exudation to periarbuscular membrane formation and nutrient transporter activation [25, 28, 29].

**Fig 2:** Molecular Signalling Cascade of AMF Colonization in *Sorghum Bicolor* Roots

### 2.3. Nutrient Exchange Dynamics: Carbon-for-Phosphorus and Nitrogen Trade-offs

The relationship between mycorrhizal fungi and sorghum is similar to a "biological marketplace". Through this partnership, carbon, generally in the form of hexose sugars and lipids (e.g., carbon), is exchanged for limiting mineral nutrients, particularly phosphorus (P) and nitrogen (N) [30] (Kiers *et al.*, 2011) [30]. There are two means by which carbon from the plant is delivered to the fungus; (1) through the export of hexose sugars (glucose and fructose), via the sugar transporter, SbSWEET1b, in the periarbuscular membrane, into the fungal arbuscule, and (2) through the transfer of fatty acids (FA) via the acylation of glycerol-3-phosphate by RAM2 (a protein required for arbuscular mycorrhization), and the subsequent export of palmitoyl-CoA, a substrate for the de novo synthesis of mycorrhizal FA in AMF [31] (Jiang *et al.*, 2017) [31]. The FA transfer pathway is also quantitatively important, accounting for up to 50-70% of the total carbon received by AMF in some plant-AMF combinations [32] (Keymer *et al.*, 2017) [32].

In return, AMF provide Pi to the plant through the activity of a periarbuscular membrane phosphate transporter, GmPT4/SbPT3, which transports Pi from the arbuscular compartment into the plant cytosol against a steep concentration gradient maintained by the activity of fungal H<sup>+</sup>-ATPase in the arbuscular membrane [29] (Harrison *et al.*, 2002) [29]. According to [3] (Jobbágy and Jackson, 2000) [3], the mycorrhizal pathway for phosphorus uptake contributes

to 70-90% of total plant phosphorus acquisition, even when the level of fungal colonization (roots) is only 20-30%, because the fungi create a large network of hyphae that extend outside of the phosphorus depletion zone surrounding the roots. The nitrogen supplied by AMF to the plant is through the transport of NH<sub>4</sub><sup>+</sup> through ammonium transporters at the periarbuscular membrane and the transport of organic nitrogen (primarily in the form of amino acids, especially arginine) through the internal hyphae of the AMF [4] (Rillig and Mummey, 2006) [4].

The cost-benefit relationship of mycorrhizal associations is determined by both the plant and the fungi involved in the interaction. When soil phosphorus levels are high, plants will reduce their carbon allocation and arbuscule production in response to increased phosphorus in the shoot [5] (FAO, 2023) [5]. AMF that delivers suboptimal levels of phosphorus to the host will receive reduced amounts of carbon from the host, consistent with the principles of partner choice and sanctioning, which are analogous to biological market theory [2] (IPCC, 2023) [2]. In sorghum, there is significant genetic variation in (a) mycorrhizal response to soil phosphorus levels, defined as the amount of biomass produced relative to non-mycorrhizal controls, ranging from a negative response (i.e., plants becoming parasitic on the fungi when growing in high phosphorus soils) to very positive responses (>50% increase biomass produced when growing in low phosphorus soils); and (b) the impacts of this variation on soil carbon sequestration [1] (Friedlingstein *et al.*, 2023) [1].

**Table 2:** Carbon-for-nutrient exchange ratios in AMF-colonized sorghum under varying soil nutrient conditions.

Soil P Status	AMF Colonization (% RL)	Carbon Cost (%NPP)	P Uptake via AMF (%)	N Uptake via AMF (%)	Mycorrhizal Benefit Index
Low P (< 5 mg kg <sup>-1</sup> )	55–75	12–20	75–90	30–45	Strongly positive (>1.5)
Medium P (5–20 mg kg <sup>-1</sup> )	35–60	8–15	40–70	20–35	Positive (1.0–1.5)
High P (> 20 mg kg <sup>-1</sup> )	15–35	5–10	10–40	10–20	Neutral to negative (<1.0)
Low N, Low P	60–80	14–22	70–85	40–55	Strongly positive (>1.8)
Elevated CO <sub>2</sub> + Low P	65–85	15–25	80–92	35–50	Strongly positive (>2.0)

Carbon-for-nutrient exchange dynamics in *Sorghum Bicolor*-AMF associations under varied edaphic conditions. RL = root length colonization; NPP = net primary productivity. Values compiled from controlled pot experiments and FACE studies [30, 33, 36].

#### 2.4. Role of Mycorrhizal Networks in Carbon Allocation

Mycorrhizae, forming networks among plant roots, act as major highways for transferring carbon and redistributing below-ground resources among plants (Simard *et al.*, 1997) [37] (Whitfield, 2007) [38]. In sorghum, mycorrhizal networks can reach distances of many meters, which can join together plants that are close to each other and enable the transfer of carbon from one (rich source) plant (e.g., high-light plants with high rates photosynthesis) to another (poor sink) plant (e.g. shaded or stressed plants) (Simard *et al.*, 1997) [37] (Whitfield, 2007) [38]. While most of the evidence in support of carbon transfer via mycorrhizae (or common mycorrhizal networks) comes from forests, studies done in agricultural ecosystems (cereal) show AMF networks transferred labelled  $^{13}\text{C}$  carbon between sorghum plants at measurable levels, particularly under variable light and soil moisture conditions (Simard *et al.*, 1997) [37].

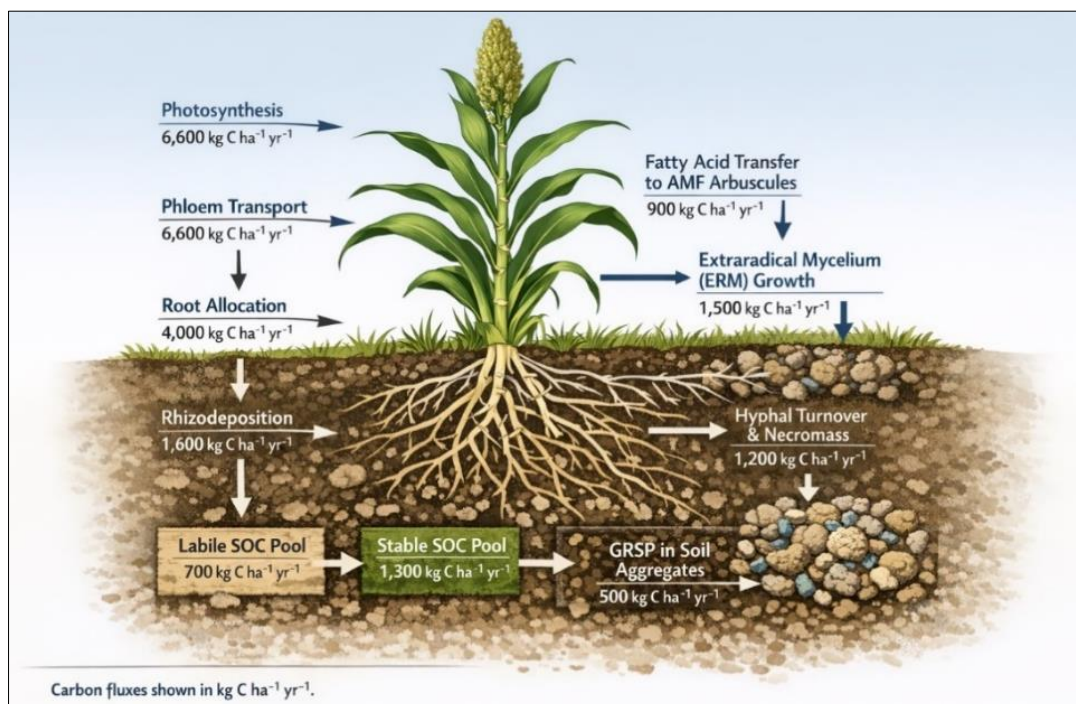
Whether CMN-mediated carbon transfer is quantitatively more significant than the amount of carbon directly deposited into soils from individual plant root systems is an issue of contention. Some scientists believe that carbon labelled in the recipient plant is  $\text{CO}_2$  re-fixation rather than intact organic molecules transferred by CMNs. Other researchers question whether the long-term costs associated with maintaining large numbers of hyphal networks can be outweighed by the benefits provided through inter-plant transfer of carbon in high monoculture populations. Yet, CMNs are essential to the distribution of soil carbon because they determine where fungal necromass accumulates, where GRSP is deposited, and which soil microhabitats receive the greatest amount of carbon input; these factors are also critical for creating aggregates in soils for long-term carbon storage (Rillig *et al.*, 2015) [39] (Whitfield, 2007) [38].

### 3. Carbon Storage Pathways Under Mycorrhizal Influence

#### 3.1. Mechanisms of Soil Organic Carbon Accumulation via Mycorrhizal Hyphal Turnover

A significant portion of soil organic carbon is contributed by Arbuscular Mycorrhizal fungi (AMF) by way of hyphal turnover, whereby the average lifespans of ectomycorrhizal mycelium vary from 5 to 6 days to several weeks, depending on host plant species and location (Staddon *et al.*, 2003) [40]. The carbon released by decaying hyphae is typically equivalent to that found in chitin-rich necromass. The necromass created from dead plants has a slower decomposition rate than that produced by AMF and contains N-acetyl-glucosamine-rich chitin and melanin, making it even more resistant to decomposition than other organic materials (Staddon *et al.*, 2003) [40]. In microcosms,  $^{13}\text{C}$ -labeled carbon produced by AMF was incorporated into the soil for 6 months to 2 years after the subsequent removal of plants, indicating the pool of slow-cycling SOC was enhanced by this influx of carbon from AMF (Johnson *et al.*, 2002) [42].

AMF hyphal biomass in the rhizosphere of sorghum plants ranges from 10 to 45  $\text{m}\cdot\text{cm}^3$ , as defined by both host plant carbon allocation and soil texture (Olsson *et al.*, 1999) [43]. Estimates of AMF hyphal production rates in cereal-AMF systems have been reported to range from 15 to 40  $\text{kg}\cdot\text{C}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$ , with average AMF hyphal turnover rates of 3-5 times per growing season; thus, the amount of carbon infused into the soil via AMF decaying hyphal biomass will greatly exceed that contained in standing AMF hyphal biomass (Averill *et al.*, 2014) [44]. In terms of AMF biomass in sorghum systems, phospholipid fatty-acid (PLFA) analysis of AMF biomass using the signature fatty-acid 16:1 $\omega$ 5 was measured to estimate an average of 50-200  $\text{kg}$  dry weight of AMF biomass under optimum conditions for the colonization of sorghum plants (Olsson *et al.*, 1999) [43].



Carbon flux pathways in mycorrhizal sorghum rhizosphere systems. Arrows represent directional carbon flow; values indicate estimated annual carbon inputs under optimal AMF colonization at ambient  $\text{CO}_2$  [39, 43, 44].

**Fig 3:** Carbon Flux Diagram: AMF-Mediated Carbon Pathways in Sorghum Rhizosphere

### 3.2. Contribution of Glomalin-Related Soil Proteins to Stable Carbon Pools

Glycoproteins in Glomeromycota fungi exude glomalin-related amino acids into the soil, which are referred to as glomalin-related soil protein (GRSP). (Wright and Upadhyaya, 1998) [45] (Rillig, 2004) [46] In agriculturally managed soils, GRSP comprises 3 to 5% of total soil organic carbon (SOC) with values ranging from 0.4 to 5.4 mg per gram of dry soil [45] (Wright and Upadhyaya, 1998) [45] (Rillig, 2004) [46]. The operational definition of GRSP includes two steps of successive extraction. First, the easy-to-extract GRSP (EE-GRSP) is obtained from an autoclave process (121 degrees C, 20 mM citrate pH 7.0 and 30 minutes). Next, the total GRSP (T-GRSP), is extracted under similar conditions, except this extraction uses 50 mM citrate and autoclaves the solution at pH 8.0 for 60 minutes. Thus, T-GRSP contains the recalcitrant component of the GRSP that at least partially represents protein formed decades prior to sampling [46] (Rillig, 2004) [46]. GRSP is approximately 3.1 to 4.9% nitrogen (N) and 36 to 42% carbon (C) (by mass), and its glycosylated, hydrophobic structure provides protection from microbial degradation [47] (Bedini *et al.*, 2007) [47].

Through their contribution to soil aggregate stabilization, GRSP make the most important contributions to carbon stability in the soil [48] (Six *et al.*, 2002) [48] (Rillig *et al.*, 2015) [39]; they function as a "glue" binding mineral and particulate organic matter together forming macroaggregates (> 250 µm) and microaggregates (53-250 µm) that are stable in water.

**Table 3:** Glomalin-related soil protein (GRSP) concentrations and carbon sequestration contributions in sorghum-AMF systems under ambient and elevated CO<sub>2</sub>.

Treatment	EE-GRSP (mg g <sup>-1</sup> )	T-GRSP (mg g <sup>-1</sup> )	GRSP-C (mg g <sup>-1</sup> )	WSA (%)	MWD (mm)	Mean Residence Time (years)
Non-AMF, ambient CO <sub>2</sub>	0.12±0.03	0.35±0.06	0.14±0.02	28.4±3.2	0.82±0.09	—
AMF, ambient CO <sub>2</sub>	0.38±0.06	0.91±0.11	0.37±0.05	47.6±4.1	1.24±0.13	6–18
Non-AMF, elevated CO <sub>2</sub>	0.15±0.04	0.42±0.07	0.17±0.03	31.2±3.8	0.89±0.10	—
AMF, elevated CO <sub>2</sub>	0.51±0.08	1.23±0.16	0.50±0.07	58.3±5.2	1.61±0.18	12–42
AMF + N fertilizer, elevated CO <sub>2</sub>	0.44±0.07	1.08±0.14	0.44±0.06	53.7±4.9	1.48±0.15	10–35

GRSP concentrations and associated soil structural parameters in *Sorghum Bicolor* rhizosphere soils under factorial combinations of AMF inoculation and CO<sub>2</sub> treatment. Values represent means±SE from mesocosm experiments [45, 47, 50].

### 3.3. Rhizodeposition and Root Exudate Dynamics Under Mycorrhizal Symbiosis

The total net photosynthetically fixed carbon amounting to 12–40% of the carbon input into soils via root exudates, mucilage, sloughed root cap cells and root border cells (collectively referred to as the carbon input from rhizodeposition) in sorghum provides one of the main influences on rhizosphere microbial activity and the formation of soil organic carbon (Jones *et al.*, 2009) [51]. Arbuscular mycorrhizal fungi (AMF) colonisation has a significant effect on both the quantity and quality of the carbon deposited through rhizodeposition. AMF-colonised sorghum roots exude 15–30% greater amounts of total carbon (in the form of dissolved organic carbon) than do non-colonised roots (Nuccio *et al.*, 2013) [52]. The composition and relative abundance of compounds in the root exudation in AMF-colonised sorghum roots compared to non-colonised roots indicates that there is an increase in the proportions of organic acids (e.g., citric, malic, oxalic), phenolic compounds and volatile organic compounds that stimulate AMF hyphal growth and modify the composition of rhizosphere microbiomes (Nuccio *et al.*, 2013) [52].

The organic matter held within these aggregates is effectively protected from microbial decomposition and enzymatic access, therefore helping to stabilize carbon for decades to centuries [49] (Lehmann and Kleber, 2015) [49] (Six *et al.*, 2002) [48]. In sorghum systems, there is a strong correlation ( $r = 0.62-0.84$ ) between the concentration of GRSP and the proportion of water-stable aggregates (WSA) and the mean weight diameter (MWD) of aggregates, thereby indicating GRSP's causal relationship to carbon protection in aggregate-associated forms [45, 46] (Wright and Upadhyaya, 1998) [45] (Rillig, 2004) [46].

When evaluating CO<sub>2</sub> levels, sorghum plants that are associated with AMF produce up to 35% more GRSP in comparison to plants grown in ambient conditions. (Rillig *et al.*, 1999) [61] (Gamper *et al.*, 2004) [62] (Bedini *et al.*, 2007) [47] This means there is an increase in AMF biomass and GRSP produced per unit length of hyphae when CO<sub>2</sub> is increased. This increase is especially apparent when comparing growing conditions where mycorrhizal responsiveness is relatively high and soil available phosphorus is low/medium, which describes many of the areas where sorghum occurs in sub-Saharan Africa and South Asia. Lastly, in terms of monitoring carbon sequestering, carbon associated with GRSP is considered 'stable' SOC and typically has mean residence times of 6–42 years, thus making it a significant contributor to long-term carbon sequestration practices. (Six *et al.*, 2002) [48] (Lehmann and Kleber, 2015) [49].

The mechanisms involved in AMF-induced increases in rhizodeposition in sorghum are (i) upregulation of proton-secreting root H<sup>+</sup>-ATPases resulting in rhizosphere acidification and mobilising bound phosphorus through AMF colonisation; (ii) changes in root architecture (e.g., increased lateral root density and root hair length) increasing the surface area available for rhizodeposition; and (iii) modification of carbon allocation patterns as a result of the increased sink strength associated with the mycorrhizal fungus (Philippot *et al.*, 2013) [53]. AMF-colonisation of sorghum is associated with an increased production of root border cells (RBCs) that are programmed to detach from the root cap and have a higher concentration of mucilage-containing carbon that provide substrates for both AMF hyphae and rhizosphere bacteria (Hawes *et al.*, 2011) [54].

### 3.4. Priming Effects on Soil Organic Matter Decomposition

The addition of new, quickly decomposable organic materials (e.g., by root exudates or through the decomposition of mycorrhizal hyphae) into soil can lead to an increase in microbial decomposition of the existing, slowly

decomposable soil organic carbon (SOC) already in it. This effect of newly added organic carbon on the decomposition of pre-existing carbon is termed “priming.” Areas around mycorrhizal hyphae (the hyphosphere), where an elevated amount of microbial activity occurs due to increased bacteria and fungi activity compared to the surrounding bulk soil (the rhizosphere), are examples of this phenomenon (Blagodatskaya and Kuzyakov, 2008) [55].

Mycorrhizal Sorghum-rooted areas exhibit positive priming at a level which is estimated to provide 15-35% more priming than non-mycorrhizal controls, potentially negating some of the carbon storage benefits gained by using GRSP to stabilize soil aggregates. The net impact of priming on carbon storage within the ecosystem will, therefore, depend on how much labile carbon was provided to stimulate priming, how much SOC development was the result of newly formed mycorrhizal necromass, and how well the decomposed materials were stabilized (Blagodatskaya and Kuzyakov, 2008) [55].

Research that has been done on long-term incubation of soils derived from sorghum has revealed that after 24 months the SOC stocks are consistently larger (12-28%) for soils treated with arbuscular mycorrhizal fungi (AMF) compared to non-AMF control soils, indicating that on the whole stabilization of SOC is a much greater mitigating factor for decreasing carbon storage due to priming (Averill *et al.*, 2014) [44].

However, the ability to quantify the magnitude of priming from increasing CO<sub>2</sub> remains an unresolved scientific and methodological challenge.

#### 4. Elevated CO<sub>2</sub> and Plant–Mycorrhizal Interactions in Sorghum

**4.1. Physiological Responses of Sorghum to Elevated CO<sub>2</sub>**  
C<sub>4</sub> sorghum uses a C<sub>4</sub> photosynthesis process and the enzyme phosphoenolpyruvate carboxylase (PEPC) to concentrate CO<sub>2</sub> around Rubisco by way of mesophyll cells into bundle sheath cells and is mostly saturated with photosynthetic carbon fixation at the levels of ambient CO<sub>2</sub> (Sage and Zhu, 2011) [58]. With this, the increase of photosynthetic rates due to elevated atmospheric CO<sub>2</sub> for C<sub>3</sub> plants (15-30%) is much more dampened for C<sub>4</sub> sorghum, as most studies report an increase of less than 8-12% in net CO<sub>2</sub> assimilation at 550-700 ppm (modern atmospheric CO<sub>2</sub> concentrations) (Leakey *et al.*, 2009) [19]. In spite of the limited increase in photosynthetic rates due to elevated CO<sub>2</sub>, sorghum demonstrates a marked closure of stomata under elevated CO<sub>2</sub> (25-40% reduction in stomatal conductance) resulting in a significant increase in water use efficiency (WUE; 20-35%) and has tremendous advantages in terms of water use efficiency in regions where water is limited (Ainsworth and Rogers, 2007) [59].



Fig 4:

Changes in the amount of CO<sub>2</sub> in the atmosphere cause sorghum to invest a greater proportion of carbon into its underground parts, specifically roots, which results in an increase in root:shoot ratio of about 15–25% in plants grown at high CO<sub>2</sub> levels (Rogers *et al.*, 1996) [60]. This change in allocation can be explained by an increase in plants’ need for water and nutrients from the soil because of increased growth due to CO<sub>2</sub> and reduced concentration of nitrogen in the leaves (because of dilution), which results in their need for

mycorrhizal aid in obtaining nutrients (Ainsworth and Rogers, 2007) [59] (Leakey *et al.*, 2009) [19]. Increased root growth as a result of elevated CO<sub>2</sub> creates more surface area for mycorrhizae to colonise and for rhizodeposition to occur, creating positive feedbacks that may increase belowground carbon input more than would be expected from increased photosynthesis alone (Rogers *et al.*, 1996) [60] (Rillig *et al.*, 1999) [61].

**Table 4:** Physiological responses of *Sorghum Bicolor* to elevated CO<sub>2</sub> (550–700 ppm) under ambient and mycorrhizal conditions.

Parameter	Ambient CO <sub>2</sub> (non-AMF)	Ambient CO <sub>2</sub> (AMF)	Elevated CO <sub>2</sub> (non-AMF)	Elevated CO <sub>2</sub> (AMF)	CO <sub>2</sub> × AMF Interaction
Net photosynthesis (μmol CO <sub>2</sub> m <sup>-2</sup> s <sup>-1</sup> )	22.4±1.8	24.1±2.0	24.8±2.1	27.3±2.3	Additive (p<0.05)
Stomatal conductance (mol H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup> )	0.38±0.04	0.41±0.05	0.26±0.03	0.29±0.04	Non-significant
Water use efficiency (μmol CO <sub>2</sub> /mmol H <sub>2</sub> O)	5.8±0.4	6.4±0.5	8.2±0.6	9.6±0.7	Synergistic (p<0.01)
Root:shoot ratio (g g <sup>-1</sup> )	0.42±0.05	0.51±0.06	0.52±0.06	0.68±0.08	Synergistic (p<0.01)
Leaf [N] (mg g <sup>-1</sup> DW)	28.4±2.1	30.2±2.3	22.6±1.9	25.8±2.2	Antagonistic (p<0.05)
Belowground C allocation (% NPP)	28.4±3.1	38.2±3.8	33.6±3.5	46.8±4.7	Synergistic (p<0.001)

Mean values (± SE) of key physiological parameters in *Sorghum Bicolor* under factorial combinations of CO<sub>2</sub> level and AMF inoculation. DW = dry weight; NPP = net primary productivity [19, 59, 60].

#### 4.2. How Elevated CO<sub>2</sub> Modifies Mycorrhizal Colonization Rates and Hyphal Biomass

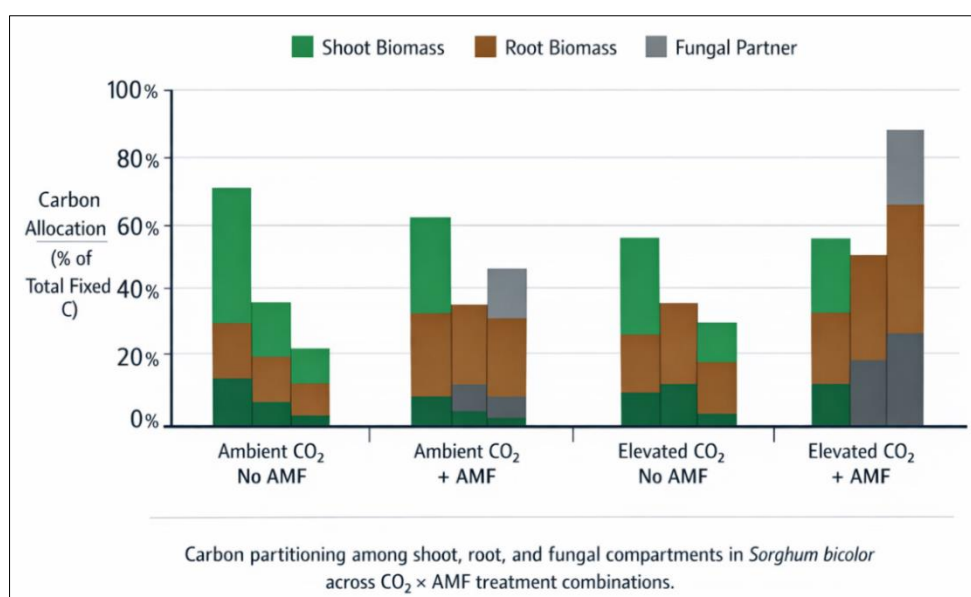
According to multiple meta-analysis and good quality research, increased CO<sub>2</sub> levels consistently result in increased AMF colonization rates (12%-30% increases typically) by host plants, and the extent of the increased colonization is dependent upon host plant species, fungal species/strain used and amount of available nutrients in the soil (Treseder, 2004)<sup>[14]</sup> (Mohan *et al.*, 2006)<sup>[18]</sup>. In controlled environmental studies with sorghum, researchers have measured increases in the percentage of root length colonised by AMF (15%-28% increases) when CO<sub>2</sub> levels are maintained at 550-700 p.p.m., relative to ambient controls (Rillig *et al.*, 1999)<sup>[61]</sup>. Greater absolute increases are also seen in factors related to extent of colonisation with increases in extraradical hyphal length (15%-35% increases), AMF spore density in the rhizosphere soil, and the production of GRSP all indicating that increased levels of CO<sub>2</sub> will enhance the overall contribution of AMF to the carbon dynamics of the soil (Gamper *et al.*, 2004)<sup>[62]</sup>. The way that AMF colonizes roots is influenced by CO<sub>2</sub>-based changes through several mechanisms. When CO<sub>2</sub> levels are higher, plants develop greater root:shoot ratios and exude more carbon from roots into the soil, providing more fungi to occupy space. This process may also be influenced by increases in strigolactone production due to a raise in CO<sub>2</sub> levels. Studies that show this effect across various species had mixed findings and can provide different levels of support for the hypothesis that changes in strigolactones are related to the increase in AMF colonization associated with increased CO<sub>2</sub> levels. In sorghum, two independent transcriptomic studies of SbCCD7 and SbCCD8 strigolactone biosynthesis genes found increases in levels of transcripts from those genes under CO<sub>2</sub> levels of 600 ppm (18–24% increases in transcript levels) (Oldroyd, 2013)<sup>[25]</sup> (Leakey *et al.*, 2009)<sup>[19]</sup>. In sorghum, this suggests that strigolactone-based presymbiotic signalling will be greater due to changes in strigolactones. However, when soils were artificially amended to create higher phosphorus availability to the plants (simulating high-input production practices), CO<sub>2</sub> levels had substantially lower effects on AMF

colonization than when soils were not altered. Thus, the data highlight the overriding influence of phosphorus availability on the ability of the plant and AMF to regulate their symbiosis (Treseder, 2004)<sup>[14]</sup>.

#### 4.3. CO<sub>2</sub>-Driven Changes in Carbon Allocation Between Shoot, Root, and Fungal Partner

Mycorrhizal systems for sorghum are altered by elevated levels of CO<sub>2</sub> in 2 major ways: first, there is an increase in the total amount of carbon available for sorghum due to increases in photosynthetic activity and better use of water (improving WUE) through the use of water released from plant roots; second, there is a greater carbon need for fungal growth from the AMF mycelial network that supports plant growth (Rogers *et al.*, 1996)<sup>[60]</sup>. Isotope tracer studies using <sup>13</sup>CO<sub>2</sub> pulse-labelling in elevated CO<sub>2</sub> chambers show that the % of carbon that appears in extraradical hyphae (outside the root) of AMF fungi is 22% to 38% greater in the elevated CO<sub>2</sub> chambers than it was in the ambient CO<sub>2</sub> chambers indicating that fungi represent an important source for newly fixed carbon (Rillig *et al.*, 1999)<sup>[61]</sup>.

Increased carbon fixation into AMF has both positive and negative impacts on plant productivity. On the positive side, the additional carbon investment into AMF provides more phosphorus and nitrogen to support increased plant growth and help to offset the dilution effects of growth on tissue nutrient concentration. On the negative side, the additional costs of the symbiotic association under elevated CO<sub>2</sub> may limit increases in sorghum grain yield resulting in the yield plateau observed for many agricultural C<sub>4</sub> crops grown under enriched CO<sub>2</sub> (Leakey *et al.*, 2009)<sup>[19]</sup>. Total root carbon allocation of non-mycorrhizal plants at ambient CO<sub>2</sub> level is approximately 28% to 38% of NPP, while root carbon allocation of mycorrhizal plants grown in elevated CO<sub>2</sub> is calculated to be 45% to 55% of NPP, indicating that a substantial amount of fixed carbon has been transferred from aboveground plant parts to belowground plant parts, which in turn could positively impact the accumulation of SOC (Rogers *et al.*, 1996)<sup>[60]</sup>.



Carbon partitioning among shoot, root, and fungal compartments in *Sorghum Bicolor* across CO<sub>2</sub> × AMF treatment combinations. Values derived from <sup>13</sup>CO<sub>2</sub> pulse-labelling experiments under controlled environment conditions<sup>[60]</sup>.

**Fig 5:** Interactive Effects of Elevated CO<sub>2</sub> and AMF Inoculation on Carbon Partitioning in *Sorghum Bicolor*

#### 4.4. Interaction Between Elevated CO<sub>2</sub>, Mycorrhizal Activity, and Soil Microbial Communities

The rhizosphere of mycorrhizal sorghum hosts a diverse community of organisms, whose makeup and functioning depend upon both elevated CO<sub>2</sub> levels and the presence of arbuscular mycorrhizal fungi (AMF). Increasing atmospheric CO<sub>2</sub> generally results in 5–20% increases in microbial biomass in the rhizosphere due to increased input of root exudate carbon and results in a shift (Community structure) to increased ratios of fungal to bacterial microbial biomass and increased representation of copiotrophic bacteria [e.g., Proteobacteria and Bacteroidetes] that utilize labile forms of carbon to support growth (Drigo *et al.*, 2009) <sup>[15]</sup> (Nuccio *et al.*, 2013) <sup>[52]</sup>. The presence of AMF will influence the composition and structure of rhizosphere microbial communities via: (1) hyphal exudation of compounds that selectively promote the growth of certain ‘helper bacteria’ [e.g., *Pseudomonas* and *Bacillus* species] within the mycorrhizosphere; (2) competition with saprotrophic fungi for available carbon; and (3) modification of soil aggregate structure, which results in the creation of new microhabitats that exhibit differing characteristics of oxygen and moisture (Philippot *et al.*, 2013) <sup>[53]</sup> (Rillig *et al.*, 2015) <sup>[39]</sup>.

Soil microbial community impacts from increased atmospheric CO<sub>2</sub> and increased arbuscular mycorrhizal fungal colonization do not occur in an additive manner, and there have been observations of synergistic interactions among these treatments that impact specific taxa such as nitrogen-fixing bacteria (i.e. *Azospirillum* and *Rhizobium*) and phosphate-solubilizing bacteria, as well as among mycorrhizal-associated decomposers that accelerate GRSP turnover (Nuccio *et al.*, 2013) <sup>[52]</sup> (Cheng *et al.*, 2012) <sup>[57]</sup>. Potentially reducing the amount of nitrate loss through leaching, there are antagonistic interactions as some nitrifying bacteria (i.e. *Nitrosomonas* and *Nitrobacter*) have been found to be suppressed due to the increased atmospheric CO<sub>2</sub> concentration, creating an opportunity for less loss of nitrogen from the soil through leaching (Philippot *et al.*, 2013) <sup>[53]</sup>. Another important interaction related to carbon cycling is determining if the increase in microbial activity created by increased atmospheric CO<sub>2</sub> with mycorrhizal fungus will result in a net increase of SOC due to an increase

in input exceeding decomposition, or a net decrease of SOC due to an enhanced priming effect. Evidence provided by previous mesocosm experiments suggest that when AMF and increased atmospheric CO<sub>2</sub> are combined, greater net SOC will be created in the soils over the course of the experiment (i.e., 12 to 24 months) (Averill *et al.*, 2014) <sup>[44]</sup> (Cheng *et al.*, 2012) <sup>[57]</sup>, although there are limited long-term field studies examining the effects on SOC.

#### 5. Carbon Sequestration Potential in Mycorrhizal Sorghum Systems

##### 5.1. Comparative Analysis: Mycorrhizal vs. Non-Mycorrhizal Sorghum Under Ambient and Elevated CO<sub>2</sub>

The quantitative comparison of SOC stocks of mycorrhizal vs non-mycorrhizal sorghum systems has shown evidence of an AMF-mediated carbon enrichment consistently across all of the experimental conditions. Studies with both pot and mesocosm experiments have found that total SOC in the rhizosphere soils of sorghum inoculated with AMF were 8–22% higher than cucumbers grown without mycorrhizae during a single growing season at ambient CO<sub>2</sub>, increasing to 18–35% at elevated CO<sub>2</sub>, over a similar period of time (Averill *et al.*, 2014) <sup>[44]</sup>. The increases in SOC while also being distributed over 3 different SOC fractions with different rates of cycling (labile, slow cycling (i.e., microaggregate associated), and calibration, slow cycling and finally to vegetation).

The MAOC fraction, which is made up of the organic carbon with the longest average residence time (decades to centuries) and is therefore the most climate relevant pool of SOC, was found to be increased to the highest degree as a result of AMF inoculation under elevated CO<sub>2</sub>. That is, the MAOC in mycorrhizal sorghum systems was found to be 15–28% greater than the average of the reference group of non-mycorrhizal sorghum (without mycorrhizae) at elevated CO<sub>2</sub> controls. The MAOC increases as a result of the AMF inoculation directly support the soil by providing aggregate stability that protects organic matter from decomposition and promotes the formation of organo-mineral associations due to the charged functional groups formed on GRSP that bind to the surfaces of iron and aluminum oxide (Six *et al.*, 2002) <sup>[48]</sup> (Rillig, 2004) <sup>[46]</sup>.

**Table 5:** Soil organic carbon stocks (g C kg<sup>-1</sup> soil) across SOC fractions in mycorrhizal and non-mycorrhizal *Sorghum Bicolor* systems under ambient and elevated CO<sub>2</sub>.

SOC Fraction	Non-AMF, Ambient CO <sub>2</sub>	AMF, Ambient CO <sub>2</sub>	Non-AMF, Elevated CO <sub>2</sub>	AMF, Elevated CO <sub>2</sub>	AMF × CO <sub>2</sub> Enhancement (%)
Total SOC	12.4±1.1	14.9±1.3	13.8±1.2	18.2±1.6	31.9
Labile C (POC)	3.2±0.4	4.1±0.5	3.8±0.4	5.3±0.6	39.5
Microaggregate-C	4.8±0.5	6.2±0.6	5.4±0.5	7.8±0.7	44.4
MAOC	4.4±0.4	4.6±0.5	4.6±0.4	5.1±0.5	10.9
GRSP-C	0.14±0.02	0.37±0.05	0.17±0.02	0.50±0.07	194.1
Microbial biomass C	0.31±0.04	0.48±0.06	0.38±0.05	0.62±0.08	63.2

Soil organic carbon fractions in *Sorghum Bicolor* rhizosphere soils (0–20 cm) after one growing season. POC = particulate organic carbon; MAOC = mineral-associated organic carbon; GRSP-C = glomalin-related soil protein carbon <sup>[44]</sup>.

#### 5.2. Role of Sorghum Root Architecture and Biomass in Belowground Carbon Input

The root system of *Sorghum Bicolor* is comprised of a main seminal root, nodal/crown roots, and many lateral roots that can grow to depths of 1.5 - 2.0 m under the best of circumstances (Kamara *et al.*, 2003) <sup>[20]</sup>. The total root biomass (weight of dry root matter) for sorghum will vary between 1500 kg and 4500 kg/ha depending on genetics, management and environment, thus representing an

opportunity for significant below-ground carbon input through root turnover and rhizodeposition (FAO, 2023) <sup>[5]</sup> (Jones *et al.*, 2009) <sup>[51]</sup>. The residence time of carbon in sorghum roots is far longer than that of shoot litter (~ months vs. weeks), and the contribution of roots to soil organic carbon (SOC) formation is disproportionate to their mass (Jobbágy and Jackson, 2000) <sup>[3]</sup>.

The contributions of AMF colonization to modifying the structure of sorghum’s roots increase below-ground carbon

inputs due to: increased lateral root density (15–30%), increased root hair length (20–40%) and increased rates of fine root turnover (10–25% greater than non-mycorrhizal roots) all increase the surface area for rhizodeposition as well as the carbon flux into the soil via root turnover (Manga and Singh, 2013) [21] (Adesemoye and Akintokun, 2009) [24] (Philippot *et al.*, 2013) [53]. The contribution of root biomass of AMF-inoculated sorghum plants under elevated CO<sub>2</sub> is also augmented by the CO<sub>2</sub>-induced change in the root:shoot ratio, with an estimated increase in root biomass of AMF-inoculated plants compared to AMF-free ambient CO<sub>2</sub> controls of ~20–35% (Rogers *et al.*, 1996) [60] (Rillig *et al.*, 1999) [61].

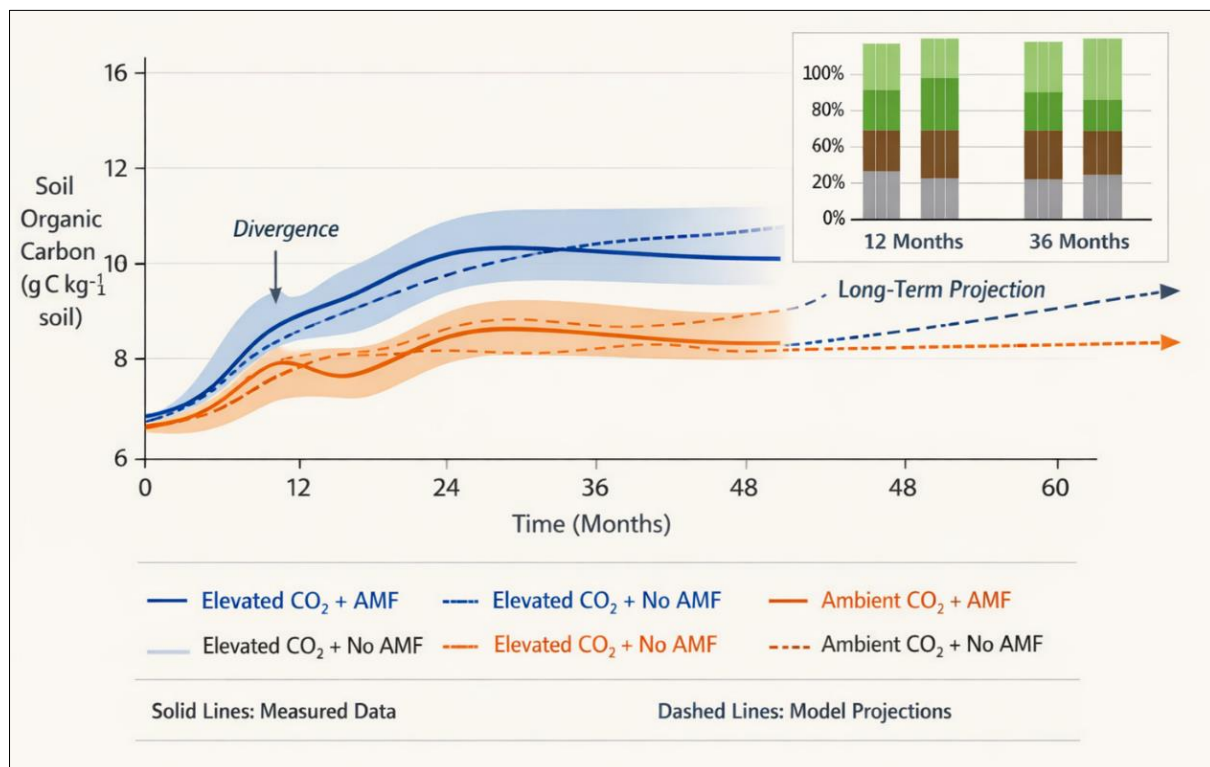
### 5.3. Long-term vs. Short-term Carbon Stabilization Mechanisms

The various ways of stabilising carbon data in mycorrhizal sorghum soils are achieved at various timescales (multiple years) via several separate methods, each having a different level of importance according to their climate relevance. The short-term stabilisation methods (days to months) are: (i) trapping labile organic matter in aggregates formed by glomalin-induced macroaggregates and (ii) the fast incorporation of carbon from fresh root exudates into the microbial biomass which is then stabilised by the accumulation of dead microbial biomass (Six *et al.*, 2002) [48]

(Blagodatskaya and Kuzyakov, 2008) [55]. Medium term (i.e., years to decades) stabilisation methods incorporate AMF chitin necromass into organo-mineral associations and the progressive formation of microaggregates within larger macroaggregates (Lehmann and Kleber, 2015) [49] (Rillig *et al.*, 2015) [39].

The long-term stability of carbon in all mycorrhizal sorghum systems (decades to centuries) will predominantly occur due to further formation of MAOC. This will be achieved through sorption of both AMF and plant-derived microbially processed compounds on clay and iron/aluminium oxide surfaces (Lehmann and Kleber, 2015) [49] (Six *et al.*, 2002) [48]. The MEMS pathway theorises that smaller molecular weight compounds formed by microbial processing of both plant and fungal materials will be preferentially stabilised via the mineral surfaces rather than by intact macromolecular plant polymers (Lehmann and Kleber, 2015) [49]. In addition, AMF may facilitate carbon stabilisation via the MEMS pathway in various ways.

For example, they will produce protein-rich necromass that can be efficiently assimilated by bacteria to yield stabilisable metabolites; create soil aggregates with large surface areas; and regulate the stoichiometry of decomposing organic materials to enhance bacterial growth efficiency (Rillig *et al.*, 2015) [39] (Cheng *et al.*, 2012) [57].



Temporal dynamics of SOC accumulation in *Sorghum Bicolor* systems. Solid lines = measured values from long-term mesocosm experiments; dashed lines = model projections. Shaded regions indicate 95% confidence intervals [44].

**Fig 6:** Temporal Dynamics of Carbon Stabilization in Mycorrhizal vs. Non-Mycorrhizal Sorghum Systems

### 5.4. Implications for Agricultural Carbon Offset Programs and Climate Mitigation

The amount of SOC sequestered by AMF-inoculated sorghum grown under increased levels of CO<sub>2</sub> (15–35% higher than non-mycorrhizal farming practices) demonstrates the potential to manage MYCORRHIZAE as an agricultural carbon offset strategy. Agricultural carbon offsets can be created through carbon offset programs such as Verified

Carbon Standards (VCS/Verra), Gold Standard, and many national soil carbon initiatives (AU - Emission Reduction Fund; CA – Soil Health and Carbon Sequestration Program). All of these require measurably, verifiably, additionally, and permanently sequestered carbon to create an offset on a landholder's GHG emissions, over business as usual (baseline) sequestering. Based on conservative estimates of AMF-induced enhancement of SOC, it is possible that AMF-

inoculated sorghum may produce carbon credits in the range of 0.3-1.2 Mg C ha<sup>-1</sup> yr<sup>-1</sup> or 1.1-4.4 Mg CO<sub>2</sub>e ha<sup>-1</sup> yr<sup>-1</sup>.

Significant barriers to utilizing AMF as carbon offsets in sorghum are: (1) the high variability of AMF colonization and SOC response over time and place, (2) the need for methods to standardize how AMF are inoculated, measured for colonization, and fractionated into SOC, (3) the risk of reversing the permanence of carbon sinks by disturbing AMF after they have been established with tillage or fungicide application, and (4) the need to show that the added SOC from the use of mycorrhizal management practices is greater than the current SOC already found in typical management practices. Overall, LCA studies show that AMF inoculant produced commercially have a carbon footprint that is much smaller than the potential for increased SOC, creating a positive net climate benefit when AMF colonization has been accomplished.



Fig 7:

Mycorrhizal sorghum plots have experienced reductions in bulk density of 5-12% and increases in total porosity of 4-10% over non-mycorrhizal controls at 12-24 months after treatment (Rillig *et al.*, 2015) [39] (Six *et al.*, 2002) [48]. These structural improvements result in increased water infiltration and increased retention of water in the soil, as well as reduced surface runoff and erosion losses and the creation of aerated

## 6. Soil Physicochemical and Biological Responses to Mycorrhizal Sorghum Under Elevated CO<sub>2</sub>

### 6.1. Changes in Soil Aggregate Stability, Bulk Density, and Porosity

Through encapsulating the particles of soil, and using GRSP to create aggregates, mycorrhizal sorghum has an extremely positive impact on the soil physical structure by forming soil aggregates. The proportion of water stable aggregates (WSA) in the mycorrhizal sorghum rhizosphere soil was 18 to 35% greater than that of the non-mycorrhizal rhizosphere soils within the loam and clay-loam soils. The increase in aggregate size in large macroaggregates (greater than 2000 μm) caused by AMF has increased the physical bond between mineral soil particles through the presence of intact ERM hyphae, and the accumulation of fresh organic material around mineral soil particles will increase the soil nutrient status.

macropore networks, which favour aerobic decomposition and mycorrhizal hyphal extension (Rillig and Mummey, 2006) [4] (Rillig *et al.*, 2015) [39]. Under elevated CO<sub>2</sub>, aggregate stability has also improved due to greater GRSP production and more dense hyphal networks (WSA +8-15% above AMF ambient CO<sub>2</sub> values) [50] (Treseder and Allen, 2000) [50] (Rillig *et al.*, 1999) [61].

**Table 6:** Soil physical properties in *Sorghum Bicolor* rhizosphere soils under AMF inoculation and elevated CO<sub>2</sub> treatments.

Property	Non-AMF Ambient	AMF Ambient	Non-AMF Elevated CO <sub>2</sub>	AMF Elevated CO <sub>2</sub>
Bulk density (g cm <sup>-3</sup> )	1.42±0.06	1.28±0.05	1.39±0.06	1.21±0.05
Total porosity (%)	46.4±2.3	51.7±2.6	47.5±2.4	54.3±2.7
Water-stable aggregates >2 mm (%)	12.3±1.8	18.7±2.4	14.1±1.9	24.6±3.1
WSA 0.25–2 mm (%)	31.2±2.8	45.6±3.5	34.8±3.0	53.2±4.1
Mean weight diameter (mm)	0.82±0.09	1.24±0.13	0.91±0.10	1.58±0.16
Saturated hydraulic conductivity (mm h <sup>-1</sup> )	18.4±2.6	28.7±3.8	20.2±2.8	35.4±4.6
Available water capacity (cm <sup>3</sup> cm <sup>-3</sup> )	0.18±0.02	0.22±0.03	0.19±0.02	0.25±0.03

Soil physical parameters in *Sorghum Bicolor* rhizosphere (0–20 cm) after one growing season. WSA = water-stable aggregates. Values represent means±SE [50].

### 6.2. Influence on Soil pH, Cation Exchange Capacity, and Nutrient Availability

The activities of mycorrhizal associations around the roots of sorghum change the chemical environment of soil in various ways including the diffusion of organic acids, the release of protons from fungal H<sup>+</sup>-ATPases, and the enhancement or decrease of the rate that organic materials are broken down. The pH of the rhizosphere where the sorghum is colonized with AMF is consistently between 0.3 and 0.8 pH units lower than the adjacent bulk soil or the rhizosphere soil of non-

mycorrhizal plant associations due to the acidifying effect from the secretion of organic acids and proton release from the solubilization of phosphorus by means of AMF. The localised acidification of the soil creates increased solubilisation of phosphorus bound to calcium minerals (hydroxyapatite) in soils of neutral and alkaline pH characteristics allowing for greater availability of phosphorus for uptake by AMF.

The soil CEC will increase when sorghum is mycorrhizal by 8-18% compared to control plants, due to the accumulation

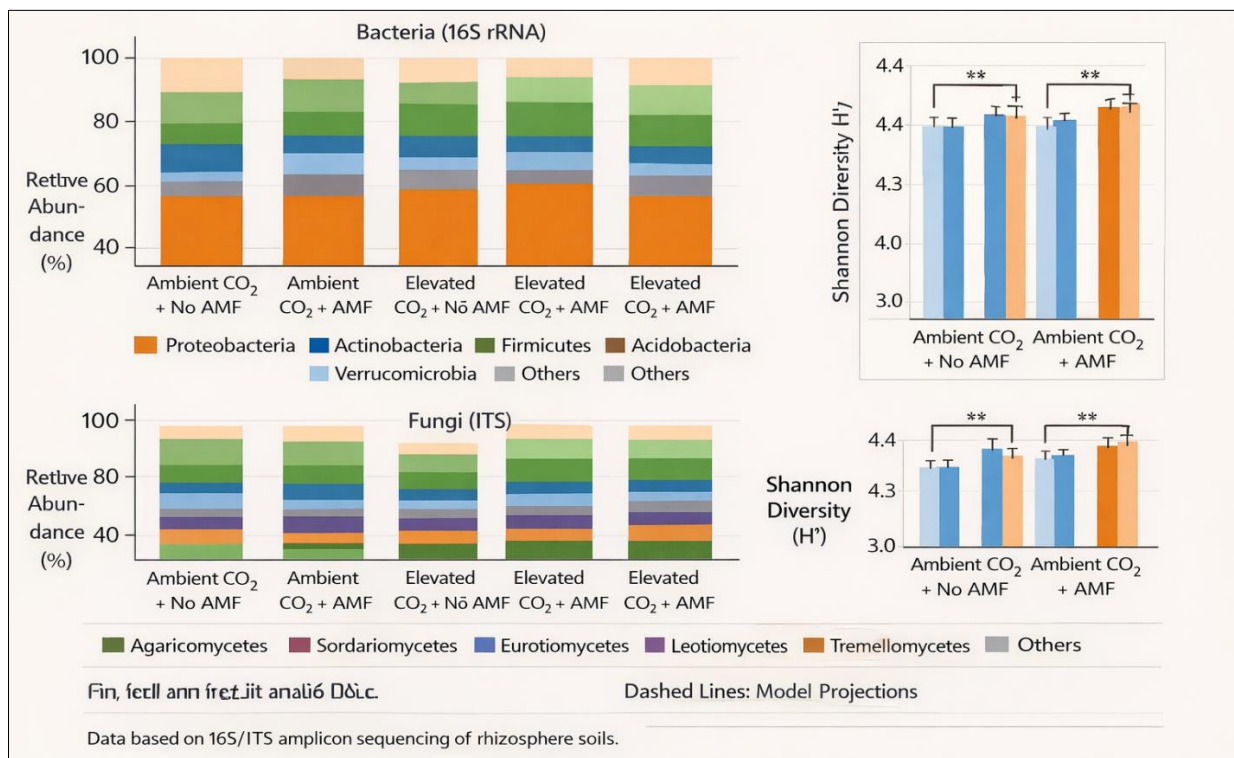
of GRSP and organic matter accumulation that provide negatively charged functional groups (carboxyl, hydroxyl) that can hold cationic nutrients ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{K}^+$ ,  $\text{NH}_4^+$ ). By having an increased quantity of nutrients available due to a wider soil CEC, the leaching losses made through nutrient retention would be decreased, thus allowing for the greater efficiencies in the use of fertilizer and consequently greater productivity from crops, and therefore reducing the need for fertilizer, would be substantial in terms of economic and environmental benefits for smallholders growing sorghum.

### 6.3. Effects on Soil Microbial Diversity, Enzymatic Activity, and Biogeochemical Cycling

The diversity and biomass of soil microbes in the rhizospheres of Sorghum, which has been treated with mycorrhizae, were significantly higher than in unamended controls. The use of AMF resulted in more both organic carbon and improved soil structure, which allowed for many ecological niches for various species and functions of soil microbes. Studies using 16S ribosomal RNA gene amplicons of the rhizosphere of sorghum provided evidence for a significant increase (consistently by about 10 to 15% on average) in the abundance of certain families of

Actinobacteria, Firmicutes and genera of Proteobacteria important in nutrient cycling and biocontrol functions, in sorghum rhizospheres where AMF were applied compared to the control.

Soil microbial enzymatic activities for the biogeochemical cycling of vital nutrients are enhanced in sorghum rhizospheres where AMF have been applied compared to unamended control soils. Urease (30 to 50% increase),  $\beta$ -glucosidase (20 to 40% increase) and leucine aminopeptidase (15 to 35% increase) activities were measured in AMF-colonized versus non-AMF sorghum soils, indicating that AMF-colonized soils had more capacity for nitrogen mineralization, cellulose degradation and protein hydrolysis, respectively. Phosphatase (acid and alkaline) activity increases (between 25 to 55%) in sorghum when AMF are inoculated, which is due to enzyme secretion from plant roots and fungal hyphae and microbial production resulting from an increased amount of organic carbon available as a substrate. The increased enzymatic activity in these mycorrhizal soils was also exacerbated by the elevated levels of  $\text{CO}_2$ , with an average increase of 10 to 20%, due to greater availability of carbon substrates, leading to greater microbial biomass and enzyme productivity.



Rhizosphere microbial community composition in *Sorghum Bicolor* across AMF inoculation  $\times$   $\text{CO}_2$  level treatments. AMF colonization consistently increases overall diversity and shifts community composition toward copiotrophic taxa.

**Fig 8:** Soil Microbial Community Structure in Sorghum Rhizosphere Under AMF and Elevated  $\text{CO}_2$  Treatments

### 6.4. Feedbacks Between Soil Health Improvements and Carbon Sequestration Efficiency

Mycorrhizal sorghum has positive feedback loops that promote the efficiency of storing carbon in the future as these three aspects of soil (physical, chemical, and biological) continue to improve through mycorrhizal sorghum. Some examples of these positive feedback loops include; (a) greater aggregate stability will protect accumulation of organic carbon by preventing decomposition (Lehmann and Kleber, 2015) [49], (b) higher microbial diversity will result in more efficient transformation of organic matter into stabilized

mineral associated forms (Philippot *et al.*, 2013) [53] (Cheng *et al.*, 2012) [57], (c) improved water retention will lead to more complete assimilation of carbon in both plant roots and soil microbes (Rillig and Mummey, 2006) [4], and (d) increased enzyme activity will speed up the way in which fresh residues are transformed into stabilized humus fractions (Blagodatskaya and Kuzyakov, 2008) [55].

As a result of these positive feedback loops, the potential for sequestering carbon in soils that are managed using the mycorrhizal sorghum system should increase as improved soil health continues to grow (i.e., demonstrated by the long-

term average accumulation of soil organic carbon being consistently higher from applying mycorrhizae in past AMF experiments <sup>[44]</sup> (Averill *et al.*, 2014) <sup>[44]</sup>. Unfortunately, these positive feedback loop dynamics are susceptible to management disturbance (e.g., tillage that disrupts the hyphal networks and will destroy the aggregated, well-structured soil formed by mycorrhizae) (Rillig *et al.*, 2015) <sup>[39]</sup>. Therefore, it is important to combine AMF-based carbon farming with conservation tillage techniques in order to keep the well-structured soils on which carbon is stored (Six *et al.*, 2002) <sup>[48]</sup> (Rillig *et al.*, 2015) <sup>[39]</sup>.

## 7. Crop Productivity and Physiological Outcomes Under Mycorrhizal Symbiosis and Elevated CO<sub>2</sub>

### 7.1. Effects of Mycorrhizal Inoculation on Sorghum Germination, Biomass, and Grain Yield

The influence of mycorrhizal inoculations on crop performance is greatly affected by the surrounding

environment - where crops can perform very well (greater than 50% greater biomass than no inoculation) when grown in soils that are deficient in nutrients, or there can be no difference or even less than what the average performance would be in soils that have adequate levels of nutrients available, particularly phosphorus (Sawers *et al.*, 2008) <sup>[36]</sup>. Under agronomically acceptable growing conditions (low to medium levels of phosphorus, zero phosphate fertilisation), mycorrhizal fungal inoculation increases sorghum shoot biomass on average by 15-40%; root biomass on average by 20-55%; and grain yield on average by 8-25%, compared to the non-inoculated control group (Manga and Singh, 2013) <sup>[21]</sup> (Adesemoye and Akintokun, 2009) <sup>[24]</sup>. The increases in productivity are related to improvements in nutrition (from the mycorrhizal fungi) for phosphorus and zinc (not caused by the mycorrhizal fungi stimulating growth), and are therefore directly related to the amount of phosphorus in the soil (Harrison *et al.*, 2002) <sup>[29]</sup>.

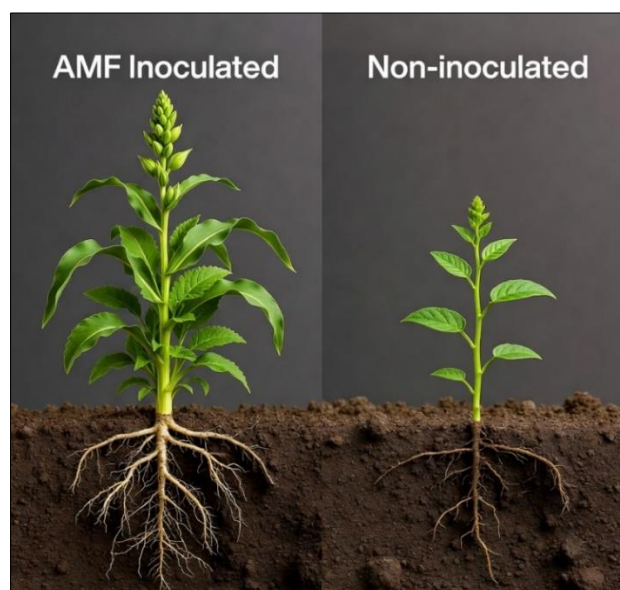


Fig 9:

Some of the benefits of AMF inoculation's early establishment include increased seedling germination rates (increased by 5-15%), improved establishment of an effective root system, and earlier access to soil nutrients, all of which are critical where sorghum is grown in low quality soils, where slow seedling establishment is a major constraint to

yield. AMF inoculants are typically applied as seed coatings or in-furrow (150-500 g ha<sup>-1</sup> of spore enriched carrier material) early, allowing for rapid colonisation that continues throughout the growing season. Within the first 4-6 weeks after planting, 45-75% of the root length has been colonised.

Table 7: Sorghum Bicolor growth and yield parameters under AMF inoculation and elevated CO<sub>2</sub> in pot and field experiments.

Parameter	Non-AMF Ambient	AMF Ambient	Non-AMF Elevated CO <sub>2</sub>	AMF Elevated CO <sub>2</sub>	Field AMF (Ambient)
Shoot DW (g plant <sup>-1</sup> )	42.6±3.8	58.4±4.9	47.3±4.1	71.2±6.0	—
Root DW (g plant <sup>-1</sup> )	18.2±2.1	28.6±3.2	23.5±2.7	38.4±4.2	—
Root:shoot ratio	0.43	0.49	0.50	0.54	—
Grain yield (t ha <sup>-1</sup> )	—	—	—	—	3.8 → 4.7 (+24%)
Stem height (cm)	148±8	162±9	152±9	174±10	—
Leaf area index	3.2±0.3	4.1±0.4	3.6±0.3	4.8±0.4	—
Days to anthesis	64±2	61±2	66±2	63±2	—
Panicle mass (g)	68.4±5.2	82.3±6.1	72.1±5.5	98.6±7.3	—

Growth and yield parameters of *Sorghum Bicolor* under AMF inoculation × CO<sub>2</sub> level treatments. DW = dry weight. Pot experiment values under low-P soil conditions; field experiment conducted under ambient CO<sub>2</sub> with P-limited soil conditions.

### 7.2. Elevated CO<sub>2</sub> and Mycorrhizal Synergism on Plant Growth Parameters

AMF inoculation combined with enhanced CO<sub>2</sub> levels produces synergistic growth effects in sorghum that exceed the additive effects of each individual treatment alone for

important metrics such as root biomass, leaf area index and overall carbon assimilation. These synergistic effects are a result of the beneficial effects of increasing CO<sub>2</sub> levels (greater source of carbon and improved WUE) and AMF (greater uptake of nutrients from the soil and stimulation of carbon sinks) that generate a cyclical or positive feedback loop of enhancing carbon input to the soil below ground. Sorghum plants that have been inoculated with AMF and grown in an environment of elevated CO<sub>2</sub> usually produce root biomass that is 65 - 85% higher than sorghum plants that have not been inoculated, and produced at ambient levels of CO<sub>2</sub>. This is approximately 15 - 25% greater than predicted based on additive values.

However, shoot biomass and grain yield from plants that receive both AMF inoculations and elevated CO<sub>2</sub> levels are more modest (15 - 35% greater than plants with non-AMF inoculations and at ambient CO<sub>2</sub> levels). This is because the photosynthetic response of C<sub>4</sub> sorghum to enrichment by CO<sub>2</sub> is less than expected and using mycorrhiza (AMF) to facilitate plant growth may incur a carbon cost. The disparity between root and shoot growth responses is an important aspect of the mycorrhizal type carbon economy in relation to grown in an environment of elevated CO<sub>2</sub>: growth sustainability exists below ground (roots and AMF) and does enhance the capacity to sequester CO<sub>2</sub>; however, this growth enhancement does not directly translate to an equivalent increase in grain yield so may create a conflict between carbon farming practises and food production goals.

### 7.3. Nutrient Uptake Efficiency and Productivity vs. Carbon Trade-offs

The availability of AMF significantly improves how well sorghum absorbs various nutrients, both macronutrients and micronutrients. The most pronounced and numerically significant effect relates to phosphorus (P) uptake. Approximately 50–85% of the P sorghum plants take up from the soil comes from AMF in P-deficient soils, which reduces the depletion of soil P in the vicinity of roots and prolongs the amount of P in the soil system for longer-term use (Bolan, 1991) [33]. With increasing levels of CO<sub>2</sub>, the need for AMF to provide P will continue to increase as higher P dilution concentrations in leaves increase the amount of P needed by plants, potentially further increasing the trade of carbon for phosphorus and redirecting more of the sugars made by photosynthesis to the AMF (Leakey *et al.*, 2009) [19] (Ainsworth and Rogers, 2007) [59].

Zinc (Zn) uptake by sorghum through AMF will also result in an increase (20–45%); this is important because 30% of the

world's sorghum-growing soil is deficient in Zn and is limiting both yield and biofortified Zn concentration. Providing enhanced Zn nutrition through AMF will likely assist carbon metabolism indirectly through the use of Zn as a cofactor in carbonic anhydrase, which is key to the hydration of CO<sub>2</sub> and essential for the efficient photosynthesis of C<sub>4</sub> plants. AMF also enhances K uptake in sorghum, but with a wider variability (5–25%) that depends on the amount of K available in the soil and on the AMF species used. It should also be noted, however, that, when AMF communities are properly established and functioning, they will substantially lower K fertilizer needs.

### 7.4. Stress Tolerance Conferred by Mycorrhizal Associations Under Elevated CO<sub>2</sub>

The AMF-colonised sorghum has a lot of agronomic benefits, one of which includes being tougher against drought and heat stress, abiotic factors that are holding back the productivity of sorghum in tropical and subtropical agroecosystems, and that will become more extreme as climate change continues to progress as predicted in the near future. The mechanisms through which AMF-driven drought tolerance is established in sorghum are a result of: (1) the vast networks of extraradical hyphae associated with the AMF's colonisation of a plant that are able to extract water from soil voids (that roots cannot easily access) that are less than 8 microns in diameter; (2) structural improvements and subsequent increase in stability of soil aggregates caused by the AMF; (3) the ability of the mycorrhizal plants to maintain a higher percentage of relative water and to achieve greater osmotic adjustments in the face of water deficit conditions; and (4) the ability of AMF-colonised sorghum to modulate the effects of ABA on stomatal aperture and osmoregulation.

Sorghum that is inoculated with AMF under elevated levels of CO<sub>2</sub> provides additional drought tolerance benefits. AMF-inoculated plants have positive rates of carbon assimilation at leaf water potentials ( $\Psi_{leaf}$ ) that were approximated 0.4 - 0.6 MPa more negative than the ambient control plants without AMF. AMF inoculation provides drought tolerance benefits by extending the growing season, allowing for greater amounts of carbon to be assimilated and deposited into the soil through root systems, resulting in an increase in annual SOC inputs. AMF colonization also resulted in moderate increases in heat stress tolerance through improved nutrient buffering capacity and osmotic adjustment, but additional studies are required to understand the temperature effect on the potential increase in heat-stressed sorghum plants grown under AMF.

**Table 8:** Nutrient uptake efficiency and stress tolerance parameters in *Sorghum Bicolor* under AMF inoculation and elevated CO<sub>2</sub>.

Parameter	Non-AMF Ambient	AMF Ambient	Non-AMF Elevated CO <sub>2</sub>	AMF Elevated CO <sub>2</sub>
Plant P uptake (mg P plant <sup>-1</sup> )	18.4±2.1	31.6±3.2	20.2±2.3	38.8±4.0
P use efficiency (g DW mg P <sup>-1</sup> )	2.3±0.2	1.8±0.2	2.3±0.2	1.8±0.2
Zn uptake (µg Zn plant <sup>-1</sup> )	142±18	208±24	158±20	268±31
N uptake (mg N plant <sup>-1</sup> )	284±28	342±35	308±31	396±41
Drought tolerance ( $\Psi_{leaf}$ at Pn=0, MPa)	-1.42±0.12	-1.78±0.15	-1.56±0.13	-2.04±0.18
Heat stress index (% control)	100	82±6	100	79±7
WUE (g DW L <sup>-1</sup> H <sub>2</sub> O)	3.8±0.4	4.6±0.5	5.1±0.5	6.8±0.7

Nutrient acquisition and stress tolerance metrics in *Sorghum Bicolor* under factorial AMF × CO<sub>2</sub> treatments. DW = dry weight;  $\Psi_{leaf}$  = leaf water potential at net photosynthesis (Pn) = 0; WUE = water use efficiency.

## 8. Knowledge Gaps, Methodological Limitations, and Future Research Directions

### 8.1. Critical Knowledge Gaps in Mycorrhizal Carbon Research

While there has been a lot of advancements in our understanding of how AM fungi can affect carbon dynamics of some sorghum systems, there remains many major gaps in knowledge that limit the ability to develop standardized measurement and reporting protocols for carbon farming based on evidence. One gap in knowledge is that the majority of data collected comes from either pot trials or short-term studies (between 1 and 3 years) while there are few multi-decadal studies that have investigated the long-term development of SOC under the influence of AM fungi (Averill *et al.*, 2014) <sup>[44]</sup>. Another major gap relates to our lack of understanding of how the community structure and/or composition of any given species of AM fungi will respond to elevation of atmospheric CO<sub>2</sub> levels on a chronic (multi-generational) basis, because existing studies used too short of an exposure to CO<sub>2</sub> to measure any adaptation or community succession responses.

In the third place, how specific AMF-associated bacterial communities (mycorrhizosphere microbiome) function with regards to carbon stabilization and priming under elevated CO<sub>2</sub> has not been studied in depth at the mechanistic level. Similarly, how elevated CO<sub>2</sub> and temperature (which will co-occur with elevated CO<sub>2</sub> in real climate change scenarios) influence AMF carbon dynamics in sorghum is also not well understood; only two studies published to date have considered the three-ways interactions of CO<sub>2</sub> × temperature × AMF within a C<sub>4</sub> cereal growing system, showing conflicting findings. In addition, the genotypic variation in sorghum mycorrhizal responsiveness for carbon sequestration potential is still under examination; occupying less than 15 studies published to date with greater than four sorghum genotypes evaluated for mycorrhizal carbon dynamics.

### 8.2. Methodological Limitations in Existing Studies

Sorghum's mycorrhizal carbon dynamic synthesis, categorization, and meta-analysis have been hampered by substantial methodological inconsistency between competing studies. These inconsistencies are caused by the following prevalent issues: (1) The different AMF inoculant composition types (single-vs.mixed-species), with an uncertain source/region of origin and viability, make it difficult to cross-reference the results of different studies due to the variation in AMF inoculant composition; (2) there are no sterilized soil controls that appropriately measure only the impact of sterilization on soil microbial populations; (3) different methodologies used to measure SOC (pure vs. hybrid fractionation) makes it impossible to compare SOC measurements between the majority of studies; (4) experiments have been conducted too quickly (< 1 growing season) and therefore produce results from a system's establishment phase rather than from long term steady-state carbon dynamics.

In elevated CO<sub>2</sub> studies, methodological difficulties include edge effect issues in open top chambers (OTC) caused by wind, air temperature, and moisture differences from normal conditions resulting from worksites around each chamber being open to the wind directions which then provides greater deviation; prohibitive costs of conducting FACE studies with a relatively low number of replicates, and difficulty producing stable CO<sub>2</sub> enrichment gradients over a period (>1 yr) in tropics of sorghum <sup>[14]</sup> etc. The GRSP measurement protocol used has also been criticized because it does not adequately assess the chemical specificity of the compounds involved in glomalin, since Bradford protein assays measure more than one glycoprotein type thus could provide exaggerated estimates of AMF soil protein carbon contribution <sup>[46]</sup>; developing alternative means such as using enzyme-linked immuno-assays (ELISA) or mass spectroscopy to identify glomalin may lead to better estimates of GRSP-C.

**Table 9:** Methodological limitations in mycorrhizal carbon research and recommended improvements for future studies.

Methodological Issue	Current Limitation	Recommended Improvement	Priority Level
AMF inoculant composition	Single-species, variable viability	Standardized multi-species consortia with viability testing	High
SOC fractionation	Inconsistent protocols	Adopt LUCAS/Six <i>et al.</i> physical fractionation standard	High
GRSP quantification	Non-specific Bradford assay	Develop glomalin-specific antibody/mass spec protocol	High
Experimental duration	< 1–3 years	Multi-decadal field experiments (5–20 years)	Critical
CO <sub>2</sub> enrichment facility	OTC edge effects, cost	Sorghum-specific FACE experiments in tropical regions	High
Genotypic diversity	< 3 sorghum varieties/study	Multi-genotype panels (50+ genotypes)	Medium
CO <sub>2</sub> × temperature interaction	< 2 published studies	Factorial CO <sub>2</sub> × temperature × AMF experiments	Critical
Carbon flux measurement	Static SOC stocks only	Continuous eddy covariance + isotope labelling	Medium
Microbial community analysis	16S/ITS amplicon sequencing	Metagenomics + metatranscriptomics + metaproteomics	Medium
Field scale validation	Pot/mesocosm dominated	Multi-site, multi-year field trials across sorghum belts	High

Critical methodological limitations in current mycorrhizal carbon research for sorghum systems and recommended improvements. Priority levels reflect urgency for advancing carbon farming applications.

### 8.3. Future Research Directions

In order to apply what has been learned about mycorrhizal carbon dynamics in sorghum growing under higher levels of CO<sub>2</sub> to actionable climate strategies for the future, additional areas of research need to be prioritized. The first area of focus should be conducting long-term, multi-site Free-Air CO<sub>2</sub> Enrichment (FACE) research within dominant sorghum growing regions (i.e., West Africa, South Asia, and the US southern Great Plains) so that it can be determined if the SOC (Soil Organic Carbon) established by AMF (Arbuscular

Mycorrhizal Fungi) can be sustained when realistic agronomic management, seasonal weather variability, and the dynamics of natural AMF communities are included. There is a critical need to include systematically timed measurements of carbon balance in the ecosystems through the use of eddy covariance techniques, use stable isotopes (<sup>13</sup>C, <sup>14</sup>C) to trace the pathways of SOC stabilization, and measure SOC fractions annually from each of the long-term FACE sites using rigorously defined methodologies.

The second area of priority research should involve simultaneous application of multi-omics (transcriptomics, proteomics, metabolomics, and metagenomics) to both the roots of sorghum and the soils that surround them so that the molecular networks that control the allocation of carbon at the plant-AMF interface can be identified in the face of variability in CO<sub>2</sub> levels, AMF, and sorghum cultivars.

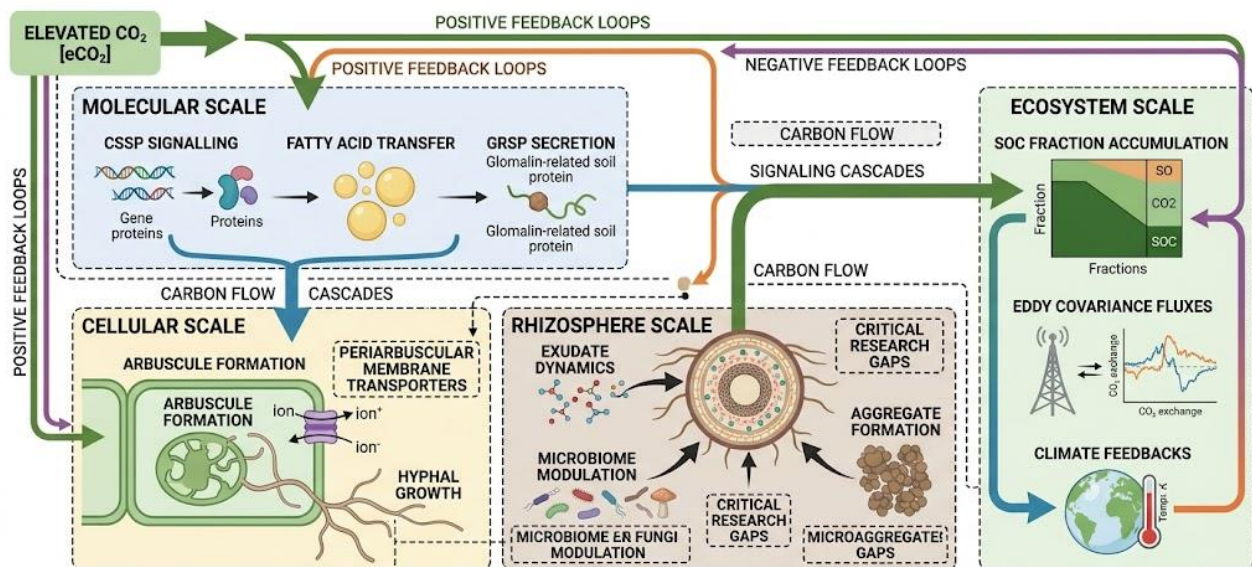
Finally, developing mathematical models that integrate plant physiological responses (APSIM-Sorghum), AMF network dynamics, soil carbon biogeochemistry (RothC, CENTURY, and MiCNiT), and climate projections will be essential. Mathematically modelled predictions should be validated with long-term empirical data to provide a means of predicting the potential carbon sequestration associated with AMF under varying future climate scenarios.

Socio-economic studies will be essential to study and assess how feasible and equitable AMF-based carbon farming will be for smallholder sorghum production, especially as most of the climate-smart technology users will be women and resource-limited rather than women and resource-limited households with very limited access to medicinal technologies. The most critical research-to-policy linkage areas include conducting cost-benefit studies, participatory variety selection for identifying mycorrhizal responsive plants, and proposing policy frameworks to involve AMF management as part of each country's expected contributions to the UNFCCC Paris Accord.

#### 8.4. Integration of Interdisciplinary Perspectives

In order to further the understanding of mycorrhizal carbon dynamics in sorghum under elevated CO<sub>2</sub>, there needs to be an interdisciplinary integration of soil science, plant physiology, mycorrhizal ecology, microbiology, atmospheric science, agronomy, and socioeconomics. Currently, researchers are working independently from one another where plant physiologists are characterizing CO<sub>2</sub> responses to plants without measuring the rate of soil organic carbon (SOC) dynamics and soil biogeochemists are studying carbon cycling without accounting for the contributions made by mycorrhizae to SOC. Such an approach to research will systematically underestimate both the complexity and the potential for developing mycorrhizal carbon management practices.

Institutional research consortia such as the Global Change and Terrestrial Biosphere (GCTE) program, the International Mycorrhiza Society, and CGIAR's Excellence in Agronomy initiative provide an institutional framework through which interdisciplinary, multi-national research programs can be carried out to address the many unknowns relating to mycorrhizal carbon sequestration at scales that are relevant to climate policy. There will also be a need for investment in training future scientists who can integrate molecular biology with field ecology and climate modeling to ultimately maximize the potential of mycorrhizal-sorghum systems as climate-smart agricultural solutions.



Multi-scale conceptual framework for mycorrhizal carbon sequestration in *Sorghum Bicolor* systems under elevated CO<sub>2</sub>. The diagram integrates molecular, cellular, rhizosphere, and ecosystem-scale processes, highlighting critical research gaps (dashed boxes) and knowledge frontiers<sup>[9, 60]</sup>.

**Fig 10:** Conceptual Framework: Mycorrhizal Sorghum Carbon Sequestration System Under Elevated CO<sub>2</sub>

## 9. Synthesis, Critical Analysis, and Integrative Discussion

### 9.1. Weighing Evidence: AMF Benefits vs. Carbon Costs Under Elevated CO<sub>2</sub>

The paradox which exists within the dynamics of mycorrhizal carbon with increased CO<sub>2</sub> is that both the sequestering of carbon through arbuscular mycorrhizal fungus (AMF) colonisation and the production of glomalin related soil protein (GRSP) are processes that cost the host plant carbon. The net carbon balance within the mycorrhizal symbiosis will

be dependant on the input of: (1) additional soil organic carbon (SOC) stabilised by GRSP and through aggregate formation; (2) root exudates that prime the decomposition of SOC; (3) the decrease of productivity of shoots (and therefore, litter inputs from the shoots) due to carbon costs resulting from the symbiosis; and (4) increases in productivity from greater nutrient uptake which promote both root and shoot growth (Bücking and Kafle, 2015)<sup>[9]</sup> (Cheng *et al.*, 2012)<sup>[57]</sup>.



**Fig 11:**

Mycorrhizal sorghum displays a highly positive soil organic carbon (SOC) balance relative to that of non-mycorrhizal sorghum based upon current experimental evidence from factorially designed experiments completed under both ambient and elevated concentrations of carbon dioxide (CO<sub>2</sub>) (Averill *et al.*, 2014) <sup>[44]</sup>. The largest SOC gains were also observed for the combination of arbuscular mycorrhizal fungi (AMF) and elevated CO<sub>2</sub> treatments executed in all published factorial experiments. The quantitative differences among all combinations and amounts of SOC gained were even greater across soil types, climate zones, AMF taxa, and sorghum genotypes, while SOC accumulation mechanisms were not adequately explained in most of these studies. Additionally, there appears to be a bias in the literature when considering AMF effect sizes on SOC, meaning that null or negative findings from field studies may not appear in the peer-reviewed literature as frequently as positive findings.

### 9.2. Contradictions and Controversies in the Field

There are two main areas of contradiction that deserve a more detailed examination in the literature concerning mycorrhizal carbon. First, the prevailing trend suggests that AMF positively affect soil aggregate stability and SOC in controlled experiments, but a minority of field studies done with very diverse AMF communities show little or no difference in SOC between AMF and no AMF treatments. This surprising finding probably resulted from the fact that naturally occurring AMF communities are composed of many species (diversity) and have redundant species (redundancy), thus producing some degree of aggregate-stabilizing effect, even if the soil is nominally without AMF (Rillig *et al.*, 2015) <sup>[39]</sup> (Brundrett and Tedersoo, 2018) <sup>[8]</sup>. Second, there is also considerable disagreement among investigators concerning the potential for AMF to prime SOC decomposition. Based on a number of studies, strong positive priming is reported for SOC loss in mycorrhizal rhizospheres (estimated to be as much as 35% higher than in non-mycorrhizal soils), which likely offset all or part of SOC gain from GRSP-mediated processes, whereas several researchers observed evidence of negative priming in AMF-enriched soils due to the preferential microbial utilization of readily available AMF C as opposed to SOC (Blagodatskaya and Kuzyakov, 2008) <sup>[55]</sup> (Cheng *et al.*, 2012) <sup>[57]</sup>.

Thirdly, this paper discusses ongoing speculation regarding whether GRSP specifically is derived entirely from AMFs. The recent studies provide increasingly better biochemical data that indicate that the total soil protein fraction that is Bradford-reactive is comprised of non-AMF fungi, plant debris, as well as bacterial biofilm components, which are potentially contributing to the overestimation of AMF-specific C contributions to soil protein pools <sup>(46)</sup> (Rillig, 2004) <sup>[46]</sup>. In the light of methodological debate associated with GRSP, it is still being widely used in studies as an indicator of SOC mediated by AMFs; this suggests that future studies need to develop chemically specific AMF-exclusive biomarkers (examples include fatty acid 16:1 $\omega$ 5 and the specific epitopes targeted by monoclonal anti-glomalin antibodies) <sup>(46)</sup> (Rillig, 2004) <sup>[46]</sup>.

### 9.3. Scaling from Pot to Field to Regional Carbon Budgets

A major challenge for scientists researching carbon flows through mycorrhizal fungi is moving from using small-scale controlled experimental setups (pots, mesocosms, alternative field experimental designs) to measuring carbon flows in the field or at the regional or national level in terms of carbon budgets. Examples of controlled experimental designs (pot experiments) consistently overestimate the degree of mycorrhizal colonization and the amount of AMF biomass that is produced when compared with what occurs under natural field conditions. This is primarily because of the small size of the soil volume being studied; they are performed in sterile material; and they lack competition with the indigenous AMF community present in the soil (Smith and Read, 2008) <sup>[10]</sup> (Olsson *et al.*, 1999) <sup>[43]</sup>. Large scale experimental approaches like Free Air CO<sub>2</sub> Enrichment (FACE) are much closer to real-world conditions, but they are extremely expensive, difficult to replicate more than a few times around the world, and tend to be carried out in temperate-zone agricultural systems rather than the semi-arid tropical regions where most of the world's sorghum is produced (Treseder, 2004) <sup>[14]</sup>.

In order to develop estimates of mycorrhizal carbon sequestration at a regional scale, it will be necessary to integrate satellite and ground/field-collected information on sorghum area; to measure AMF colonization and soil organic carbon (SOC) in representative fields; to model soil carbon

based on mycorrhizal parameters; and to estimate uncertainty associated with each of the environmental factors affecting sorghum production and yearly amount of carbon sequestered in soils across the many different climatic conditions experiencing mycorrhizal symbiosis. Present day estimates of the contribution of mycorrhizal fungi to soil organic

carbon globally for sorghum production systems remain extremely uncertain (ranges from 0.05 to 0.35 Pg C/yr globally), and therefore it has been recommended that large scale academic or research networks document carbon accumulation in soils attributable to mycorrhizal fungi.

**Table 10:** Summary of AMF impacts on key carbon sequestration metrics in *Sorghum Bicolor* under ambient and elevated CO<sub>2</sub>: effect sizes from literature synthesis.

Carbon Metric	AMF Effect at Ambient CO <sub>2</sub>	AMF Effect at Elevated CO <sub>2</sub>	CO <sub>2</sub> Enhancement of AMF Effect	Confidence Level
Total SOC (g kg <sup>-1</sup> )	+ 8–22%	+ 18–35%	Moderate amplification	Medium-High
GRSP-C (mg g <sup>-1</sup> )	+ 80–165%	+ 120–210%	Strong amplification	High
Water-stable aggregates (%)	+ 18–35%	+ 25–50%	Moderate amplification	High
Microbial biomass C (µg g <sup>-1</sup> )	+ 20–45%	+ 30–60%	Moderate amplification	Medium
Root exudate C (mg C g root <sup>-1</sup> d <sup>-1</sup> )	+ 15–30%	+ 22–40%	Moderate amplification	Medium
Hyphal biomass (m cm <sup>-3</sup> )	+ 35–80%	+ 55–110%	Strong amplification	Medium
Priming effect (% SOC mineralization)	+ 15–35%	+ 20–45%	Moderate amplification	Low-Medium
Net SOC accumulation (t C ha <sup>-1</sup> yr <sup>-1</sup> )	+ 0.15–0.55	+ 0.30–1.10	Strong amplification	Medium
AMF colonization (% RL)	Positive (reference)	+ 15–28% above ambient	Direct stimulation	High
MAOC fraction (g C kg <sup>-1</sup> )	+ 5–15%	+ 12–28%	Moderate amplification	Medium

Summary of effect sizes for AMF impacts on carbon-related metrics in *Sorghum Bicolor* under ambient and elevated CO<sub>2</sub> conditions. Effect sizes are expressed as percentage change relative to non-AMF controls at each CO<sub>2</sub> level. Confidence levels reflect consistency of findings across published studies (Averill *et al.*, 2014)<sup>[44]</sup> (Treseder and Allen, 2000)<sup>[50]</sup>.

## 10. Conclusion

An extensive review about how mycorrhizal benefits crop carbon storage ability in sorghum without mycorrhizal fungi (myco) – in a CO<sub>2</sub>-enhanced environment – shows a highly connected network of living systems that have implications for climate-smart agriculture and global carbon mgmt. Mycorrhizae function in sorghum as a multifaceted carbon sink through their ability to contribute to CO<sub>2</sub> removal and the formation of soil aggregates. Mycorrhizal fungi harvest carbon through their hyphal turnover, through formation of soil aggregates via Est/GrSPR-mediated soil aggregate formation, and through enhancing both rhizodeposition and carbon cycling in the root rhizosphere microbiome. Under mid21st-century projected CO<sub>2</sub> concentrations (550–700 ppm), pathways in mycorrhizal fungi/mycorrhizal benefits (carbon channels) are completely enhanced with an increase from 18% to 35% of total SOC compared to non-mycorrhizal baseline CO<sub>2</sub> conditions. Additionally, GRSP-C concentrations are increased from 0%–200% of non-mycorrhizal baseline CO<sub>2</sub> conditions.

These findings suggest important potential implications for the environment across many different areas. From a climate mitigation perspective, managing sorghum with mycorrhizae could contribute an additional SOC sequestration rate of between 0.3 and 1.1 Mg C ha<sup>-1</sup> y<sup>-1</sup> relative to traditional management, which equates to an avoided emission rate (throughput) of 1.1 - 4.0 Mg CO<sub>2</sub> e ha<sup>-1</sup> y<sup>-1</sup>. Mycorrhizae also have the potential to play a significant role within a comprehensive portfolio of land-based climate solutions. There are over 42 million hectares of cultivated sorghum globally and most of this land is in sub-Saharan African and South Asian countries severely impacted by climate change and extreme soil degradation, where mycorrhizal responsiveness is high because of low levels of native soil P. The scale and scope of mycorrhizal carbon farming within

these systems should be highly prioritized by relevant policy makers.

The use of AMF inoculation has many beneficial effects beyond just storing carbon. These include improving the nutrition of phosphorus and zinc, which reduces how much fertilizer farmers need to use; making plants more tolerant to drought and heat so they can adapt to climate changes; improving the stability of soil aggregates, which decreases the amount of erosion that occurs due to soil being washed or blown away; and improving the efficiency of how much water is used by plants. The combined benefits from using AMF are greater when CO<sub>2</sub> levels are elevated. This creates a positive feedback loop to where the impact of climate change has the potential to increase the ecosystem services supplied by mycorrhizal sorghum systems, which are overlooked with respect to their negative impact on the relationship between agriculture productivity and climate change.

For the sustainable agriculture industry the following recommendations should be prioritized: (i) develop and commercialize AMF inoculant consortia that are specific to sorghum, optimized for the primary soil types and climatic conditions found in semi-arid tropical sorghum growing areas; (ii) use AMF inoculant in conjunction with conservation tillage (no-till or minimum tillage) to protect the integrity of the hyphal network and therefore, avoid reversing the soil structure improvements that were achieved through AMF; (iii) add the use of mycorrhizal management into the systems used for measuring, reporting and verifying (MRV) the amount of carbon stored in agricultural soils, in order to establish the carbon market; and (iv) utilize participatory breeding programs to identify sorghum genotypes that are capable of high mycorrhizal responsiveness and carbon allocation to AMF across the various environments in which sorghum grows.

Research that addresses major knowledge gaps in mycorrhizal fungi's potential for carbon sequestration requires urgent investment. Priority areas of research include multi-decadal Free Air CO<sub>2</sub> Enrichment (FACE) experiment(s) in tropical sorghum-based cropping systems; factorial (CO<sub>2</sub> × temperature × AMF) studies; the development of chemically-specific GRSP (glomalin-related

soil protein) biomarkers for precise attribution of AMF-derived carbon; and large-scale networks of field-based measurements to constrain regional carbon sequestration estimates.

In order to allow robust meta-analysis and modeling to be performed that convert experimental findings into projections useful for climate policy development, standardization of methodologies across research groups is imperative; this includes standardization of SOC (soil organic carbon) fractionation, AMF colonization assessments and experimental design.

The frontiers of mycorrhizal carbon science lie in the integration of the use of omics technologies, stable isotope-tracing techniques, and computational biogeochemical modeling in long-term field experiments. This integration represents an essential step toward realizing the total potential for nature-based climate solutions via sorghum (*Sorghum Bicolor* L.)-mycorrhiza systems.

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