Effects of elevated carbon dioxide on arbuscular mycorrhizal fungi activities and soil microbial properties in soybean (*Glycine max* L. Merrill) rhizosphere

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Arbuscular mycorrhizal fungi (AMF) help in promoting plant growth and mediating key belowground processes, however, AMF responses to the continuous increase in the atmospheric carbon dioxide (CO_2) is yet elusive. This has led to considerable interest in the impacts elevated CO_2 on AMF and belowground processes in recent years. The present study investigated the effect of elevated CO_2 on AMF sporulation and root colonization and soil microbial properties in the rhizosphere of soybean. The pot experiment consisted of two levels of CO_2 (ambient; 350 ppm and elevated; 550 ppm) and three soybean cultivars (TGx 1440-1E, TGx 1448-2F and TGx 1480-2F) conducted in open top chambers, laid out in randomized complete block design, replicated thrice. The results showed that elevated CO_2 increased the AMF spore density and root colonization of the soybean cultivars. Elevated CO_2 increased the microbial biomass carbon (34.2–45.4%), microbial biomass nitrogen (44.6–54.9%), soil nitrogen (30.3–50.6%), available phosphorus (20.8–45.7%) in the rhizosphere of the soybean cultivars compared to the ambient CO_2. These could have resulted in increased plant biomass, pod number, 100-seed weight and seed yield under elevated CO_2. From the results of this study, increased atmospheric CO_2 regulates AMF activities, microbial properties and improve soybean performance. Thus, this study may help to a better understanding of the responses of AMF and belowground process with increasing atmospheric CO_2.

Keywords: arbuscular mycorrhizal fungi, climate change, CO_2 enrichment, microbial biomass, open top chambers

1   Introduction

The atmospheric carbon dioxide (CO_2) concentration is constantly increasing since industrial revolution due to anthropogenic activities, and is expected to double by 2050 (IPCC, 2013; Cotton et al., 2015; Kumar et al., 2019). Indeed, elevated CO_2 directly increases the net primary photosynthesis of crops, particularly C_3 plants; stimulating the production of aboveground biomass production (Oliveira et al., 2010) and carbon (C) fluxes from aboveground parts into soil (Ghannoum et al., 2010; McCarthy et al., 2010). Thus, the continuous rising atmospheric CO_2 concentrations may indirectly affect the responses of soil microbial communities and the associated C and nitrogen (N) cycling in the rhizosphere (Bardgett et al., 2008; Butterly et al., 2016). Since plant

mediates belowground processes, the responses of associated root microbes, which serve as sinks for the photoassimilates are likely to be system specific (Carrillo et al., 2014; Goicoechea et al., 2014). Given that soil microbes, particularly arbuscular mycorrhizal fungi (AMF) are actively involved in C and nutrient cycling in the agroecosystems (Cairney, 2012), there is an urgent need to better understand the effects of elevated CO_2 on AMF and soil microbial properties in the rhizosphere of agricultural crops.

AMF are one of the most important soil microbes forming symbiotic association with 90% of terrestrial plants including most agricultural crops (Brundrett, 2009). AMF play an important role in promoting plant growth through nutrient uptake, especially phosphorus, in exchange for

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carbon from the host plant (Wipf et al., 2019). They also account for about 5–50% of microbial biomass and about 80% of fungal biomass (Kabir et al., 1997). The impacts of elevated CO_2 on AMF are often reported by increased root colonisation, which is usually attributed to increased C assimilation rates by the host plant in exchange for increased nutrients demand by the host plant (Johnson and Gehring, 2007; Cairney, 2012). However, the shifts towards greater root colonisation by AMF may have important consequences on soil properties including nutrient supply to the plant, particularly P and N and microbial biomass C and N (Drigo et al., 2013).

Soybean [*Glycine max* (L.) Merrill] like most other legumes is highly mycorrhizal dependent (Adeyemi et al., 2020). Being a C_3 plant and a crop with high phosphorus (P) demand may be more sensitive to elevated CO_2 compared to other non-leguminous crops (Rogers et al., 2006). Soybean is reported to respond positively to elevated CO_2 with higher productivity (Ziska and Bunce, 2000; Sakariyawo et al., 2016; Adeyemi et al., 2017). Previous researchers have focused their studies on above ground changes in response to elevated CO_2, but recently some of the findings revealed that elevated CO_2 could also influence below ground microbial properties, which in turn affects plant productivity (Bhattacharyya et al., 2016). In addition, the alteration of AMF responses in terms of sporulation and colonization of plant roots under elevated CO_2 are yet elusive and there are inconsistences in previous reports (Johnson et al., 2005; Cotton et al., 2015; Parameswaran et al., 2019). Thus, this could hinder the prediction of microbial responses, particularly AMF in agroecosystem functioning (Singh et al., 2011; Johnson et al., 2013). Therefore, the growing awareness of the significant role of AMF for the maintenance of agroecosystem and promoting plant growth has led to considerable interest in the impacts of elevated CO_2 on AMF activities in this present study.

Thus, this study aims to investigate the effects of short-term elevated CO_2 on AMF sporulation and root colonization, soil microbial properties in roots of soybean cultivars grown in open top chambers. The results of this study may help to to gain insights into how AMF and belowground process respond to the increasing atmospheric CO_2.

2 Materials and methods

2.1 Experimental treatments and design

The study was conducted in Open Top Chambers (OTCs) at College of Plant Science and Crop Production, Federal University of Agriculture, Abeokuta. The pot experiment consisted of three promiscuous soybean cultivars (TGx 1448-2E, TGx 1440-1E and TGx 1740- 2F) grown at two CO_2

levels (ambient; 350 ppm and elevated; 550 ppm) laid out in completely randomised design, replicated thrice. The soil used for the experiment was a non-sterile obtained from the Teaching and Research Farms of the University, with textural soil of sandy (Table 1). Each pot was filled with 10 kg of soil. The soil in the pots was watered to 60% of the water-holding capacity. Soybean cultivars TGx 1448-2E, TGx 1440-1E and TGx 1740- 2F) were obtained from International Institute of Tropical Agriculture (IITA), Ibadan. Three seeds were sown in each pot on 10th of July, 2015 and emerged seedlings were thinned to two plants per pot. As plants grew bigger, pots were watered to 70% of water-holding capacity. The pots were placed in OTCs at 350 and 550 ppm CO_2 Carbon dioxide enrichment

Table 1 Soil physical and chemical properties of the experimental site

Soil Property	Value
Texture	Sand
Sand (%)	87.9
Silt (%)	7.49
Clay (%)	4.61
pH (H_2O)	6.5
Organic matter (%)	1.12
Nitrogen (mg kg^{-1})	0.80
Available phosphorus (mg kg^{-1})	5.23
Potassium (cmol kg^{-1})	0.44
Calcium (cmol kg^{-1})	2.54
Magnesium (cmol kg^{-1})	0.73
Sodium (cmol kg^{-1})	0.29
Total exchangeable acidity (cmol kg^{-1})	0.11
Exchangeable cation exchange capacity (cmol kg^{-1})	3.92

Each OTC was 3×4 m in cross section and 2.5 m in height. The frame was made of pipes covered with transparent polyvinyl chloride nylon sheet (transparency 85%). CO_2 was produced using a modified method of Saitoh et al. (2004). Yeasts and sugar were used to generate CO_2. Twelve CO_2 generator bottles were placed in the OTC to elevate the CO_2 concentration. The maximum and minimum CO_2 concentration in the OTC was measured daily throughout the enrichment period using a portable CO_2 meter [NDIR Gas Analyzer (Bentech GM8883), China]. To avoid chamber specific bias in the experiment, pots were rearranged in the OTCs every week.

2.2 Data collection

Estimation of AMF spore density and root length colonisation

Rhizosphere soil samples from 0–20 cm depth were collected at 20, 40 and 60 days after planting (DAP) to determine AMF spore density. Extraction of AMF spores from the soil sample was conducted using the modified wet sieving method of Giovannetti and Mosse (1980), followed by centrifugation in 40% sucrose solution to extract the spores. Extracted spores were counted under a dissecting microscope at ×100 magnification.

Collected root samples were prepared using the method as described by Phillips and Hayman (1970). Root samples collected were bleached in 10% KOH at 90 °C for 30 minutes and stained in acidic glycerol containing trypan blue lacto-glycerol (1 : 1 : 1 : 0.5 g) at 90 °C for 2 minutes. The roots were viewed under compound microscope to determine percent root colonization using the formula below as described by Adeyemi et al. (2019, 2020):

$$percent\ root\ colonisation = \frac{number\ of\ root\ infected}{total\ number\ of\ roots} \times 100$$

Measurement of soil microbial biomass C and N

Soil microbial biomass C and N were measured by using the chloroform fumigation extraction method (Vance et al., 1987). The dry soil samples were divided into two portions (10 g per portion) to measure the soil microbial biomass carbon (SMBC) and soil microbial biomass nitrogen (SMBN). SMBC in filtrates was determined by the $K_2Cr_2O_7$ wet-oxidation method. Briefly, 2 ml of extract, 2 ml of 1 N $K_2Cr_2O_7$ and 4 ml of 98% H_2SO_4 were reacted at 130 °C for 40 min. Solutions with known concentrations of $C_{12}H_{22}O_{11}$ were included as standards. After cooling, the C concentration in digests was determined spectrophotometrically at 600 nm. The C contained within digested non-fumigated samples

was denoted extractable organic C and the SMBC was estimated as the difference between the fumigated and non-fumigated samples and applying a conversion factor of 0.38 (Singh et al., 2017). SMBN in filtrates was assayed by the semi-micro Kjeldahl method. Specifically, 2.5 ml of extract and digestion mix (50 g $K_2S_2O_8$ and 30 g H_3BO_4 in 100 ml of 3.75 M NaOH were autoclaved (121 °C, 104 kPa) for 30 min and stored at 4 °C until analysis. Solutions with known concentrations of $CO(NH_2)_2$ were included as controls. SMBN was calculated from the differences between the organic N extracted from the fumigated and non-fumigated samples by using conversion factor of 0.54 (Huang et al., 2014).

2.3 Soybean growth

The plants were harvested and oven dried (60 °C, 72 h) prior to weighing to determine dry plant biomass. At 95% harvest maturity, yield attributes and seed yield were collected on the number of pods per plant, number of seeds per pod, 100 seed weight and seed yield per plant.

2.4 Statistical analysis

Data were subjected to analysis of variance (ANOVA). The Duncan's multiple range (DMR) test at $P < 0.05$ probability level was used to determine the differences among mean values. The statistical package used was Genstat 12th Edition.

3 Results and discussion

3.1 Effects of elevated CO_2 on AMF spore density and percent root colonization

The result showed that elevated CO_2 significantly increased the spore density of AMF in rhizospheric soil of the soybean cultivars at 40 and 60 DAP (Table 2). Higher spore density of 166 and 216 spores per 20 g soil was recorded in TGx 1448 2E grown under elevated CO_2 concentration (500 ppm) at 40 and 60 DAS respectively. The percent root colonization of the soybean cultivars

Table 2 Effect of elevated CO_2 on AMF spore density in rhizospheric soil of three soybean cultivars

Cultivars	Atmospheric CO_2	AMF spore density (per 20 g dry soil)		
		20 DAP	40 DAP	60 DAP
TGx 1440 1E	Ambient	30.0[a]	111.7[cd]	143.3[b]
	Elevated	38.3[a]	155.0[ab]	208.3[a]
TGx 1448 2E	Ambient	33.3[a]	115.0[c]	155.0[b]
	Elevated	35.0[a]	166.7[a]	216.7[a]
TGx 1740 2F	Ambient	26.7[a]	86.7[d]	135.0[b]
	Elevated	36.3[a]	128.3[bc]	211.7[a]

Treatments followed by the same letters are not significantly different at $P < 0.05$ level; DAP (days after planting)

was significantly influenced by elevated CO_2 with higher root colonization observed in the cultivars grown under elevated CO_2 (Figure 1a, b, c). The mean percent colonization ranged from 50.7 to 88% at 60 DAP.
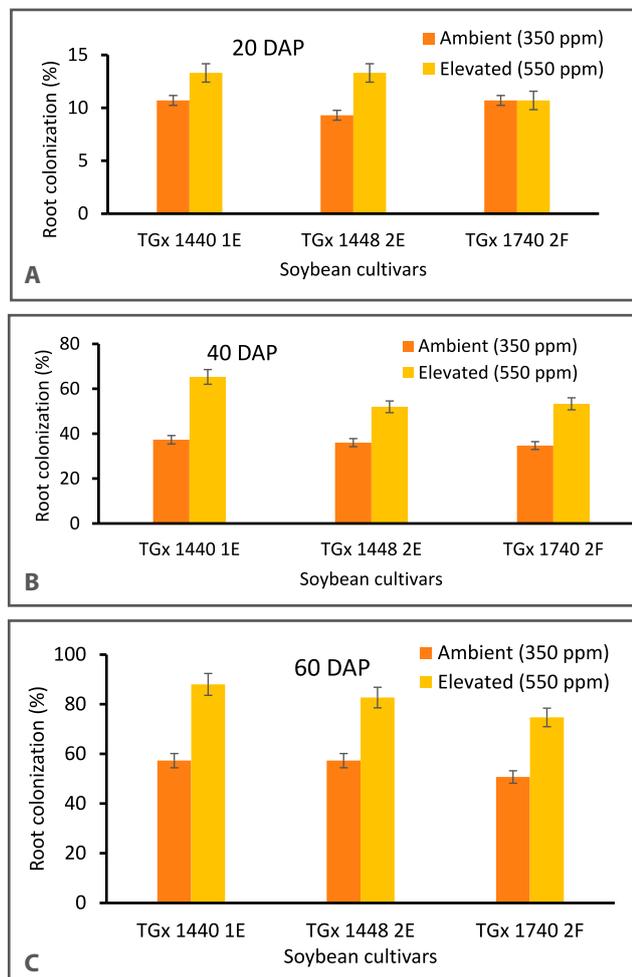

Figure 1 Effect of elevated CO_2 on AMF root colonization of soybean cultivars at 20 DAP (A), 40 DAP (B) and 60 DAP (C)

3.2 Soil microbial biomass C and N

Elevated CO_2 caused significant changes in the soil microbial biomass C and N. Specifically, the SMBC increased significantly under the elevated CO_2 in the rhizospheric soils of the three soybean cultivars. The SMBC in the soil layer of the soybean was in the range of 511 to 659 mg kg^{-1} under the elevated CO_2 compared to range of 336 to 360 mg kg^{-1} under ambient condition. Compared with the ambient treatment, SMBC significantly increased by 40.9%, 45.4%, and 34.2% in the roots of TGx 1440 1E, TGx 1448 2E and TGx 1740 2F cultivars respectively (Figure 2). Moreover, SMBC was higher in TGx 1448 2E than other cultivars under the elevated CO_2. Similarly, SMBN increased with an increased CO_2 (Figure 3). Compared with ambient CO_2,

SMBN significantly increased by 44.6%, 50.8% and 54.9% in the roots of TGx 1440 1E, TGx 1448 2E and TGx 1740 2F cultivars respectively.
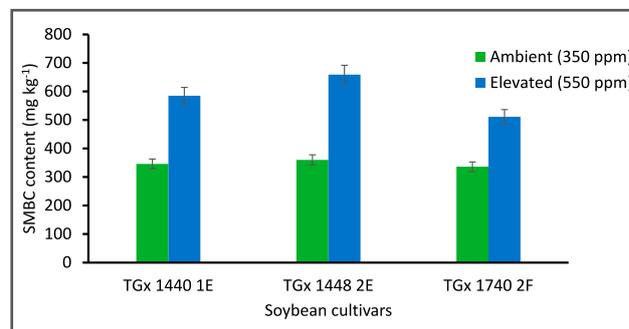

Figure 2 Dynamics of soil microbial biomass carbon (SMBC) in the 0–10 cm soil layer of soybean cultivars as affected by elevated CO_2
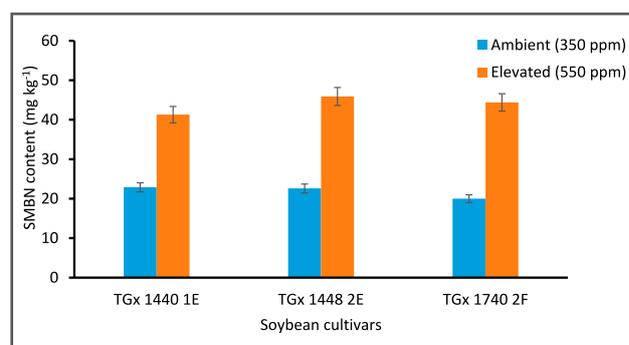

Figure 3 Dynamics of soil microbial biomass nitrogen (SMBN) in the 0–10 cm soil layer of soybean cultivars as affected by elevated CO_2

3.3 Soil nitrogen and available phosphorus

The result showed that the total nitrogen and available phosphorus in the soil increased significantly with elevated CO_2 in the soil of all the three cultivars. Compared with ambient CO_2, total N significantly increased by
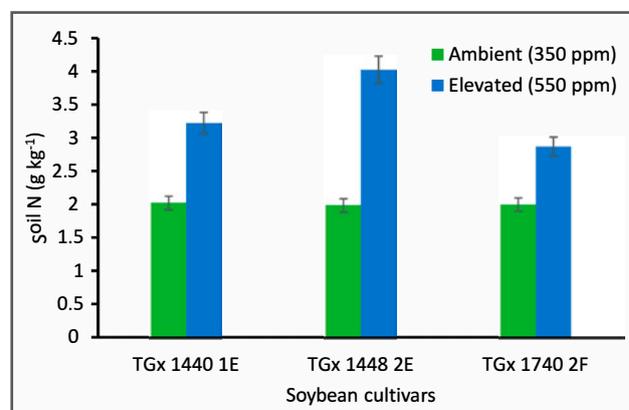

Figure 4 Dynamics of soil total nitrogen (g kg^{-1}) in the 0–10 cm soil layer of soybean cultivars as affected by elevated CO_2

37.2%, 50.6% and 30.3% in the soil of TGx 1440 1E, TGx 1448 2E and TGx 1740 2F cultivars respectively (Figure 4). Similarly, compared with ambient CO_2, the soil available P significantly increased by 45.7%, 20.8% and 39.8% in the roots of TGx 1440 1E, TGx 1448 2E and TGx 1740 2F cultivars respectively (Figure 5).
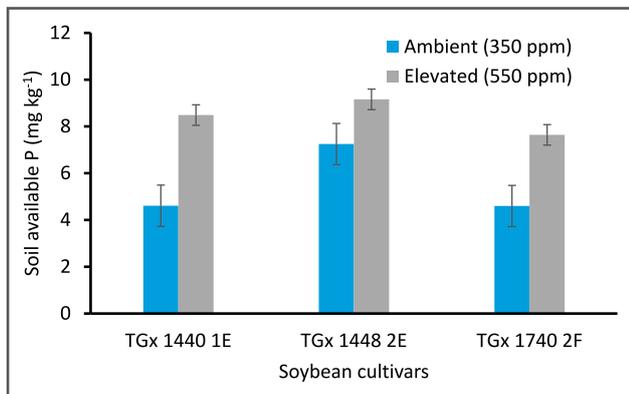

Figure 5   Dynamics of soil available phosphorus (mg kg⁻¹) in the 0–10 cm soil layer of soybean cultivars as affected by elevated CO_2

3.4 Effects of elevated CO_2 on plant biomass and yield components of soybean cultivars

The result showed that the above ground dry biomass and all the yield attributes measured were significantly influenced by elevated CO_2 (Table 3). The soybean dry biomass, number of pod, number of seed per pod, 100-seed weight and seed yield per plant were significantly higher in the three cultivars grown under elevated CO_2 compared to the ambient control.

The effects of the elevated CO_2 on soil microorganisms may be direct or indirect. This present study investigated the effects of elevated CO_2 on AMF sporulation and root colonization and soil microbial properties in roots of soybean cultivars. In the present study, root colonization of the soybean cultivars increased under elevated CO_2. Elevated CO_2 concentrations have been reported to affect the AMF hyphal growth and root colonization. The result of this study agrees with reports of previous studies (Compant et al., 2010). However, Goicoechea et al. (2014), have also reported a contrary result. The increased root colonization of the soybean cultivars may be explained by increase flow of C from the host to AMF being a strong sink of C fixed during photosynthesis by host plants under elevated CO_2 (Smith and Read, 2008; Ghannoum et al., 2010; Cairney, 2012) in exchange for additional nutrients uptake, especially P (Moran and Jastrow, 2010). For example, Rillig and Allen (1999) reported an increased growth of external as well as internal hyphae of AMF in the rhizosphere of *Prunella vulgaris* under elevated CO_2 (600 ppm) due to increased root biomass and higher allocation of fixed C to the external hyphae. Furthermore, in a metal-analysis by Alberton et al. (2005), a significant positive response of AMF to elevated CO_2, causing a 21% increase in root colonization was revealed. However, Gavito et al. (2000) observed no effect of elevated CO_2 (700 ppm) on root colonization in pea (*Pisum sativum* L.). Another mechanism for the increased root colonization of the soybean cultivars could be attributed to rapid release of flavonols needed for chemotactic action on AMF, which stimulate the hyphal growth and root colonization (Bécard et al., 1992).

In this study, microbial biomass C and N increased under elevated CO_2, which is in agreement with some elevated CO_2 studies (Jin et al., 2013; Butterly et al., 2016; Panneerselvam et al., 2019). The increase in the microbial biomass C have been associated with higher C inputs or rhizodeposition (Drissner et al., 2007; Cheng et al., 2011), which stimulates microbial growth and increases biomass. A recent review of 68 studies revealed that the average microbial biomass C increase is 14% and N is 7.4% (Liu et al., 2018). Thus, the effects depend on the specific soil conditions, which are mainly controlled by N limitation (Sillen and Dieleman, 2012). However, elevated CO_2 did not influence the microbial biomass C in some other studies (Weigel et al., 2005; Reinsch et al., 2013;

Table 3   Effect of elevated CO_2 on dry biomass, pod number, seed number per pod, seed weight 100 grain⁻¹, and seed weight per plant of soybean cultivars

Cultivars	Atmospheric CO_2	Dry biomass (g plant⁻¹)	Pod number (n plant⁻¹)	Seed number (n pod⁻¹)	Seed weight (g 100 grain⁻¹)	Seed yield (g plant⁻¹)
TGx 1440 1E	Ambient	24.7[d]	41.0[c]	2.4[c]	9.73[b]	9.69[d]
	Elevated	38.4[ab]	57.0[a]	2.7[ab]	11.3[a]	17.5[b]
TGx 1448 2E	Ambient	30.9[c]	48.4[b]	2.6[b]	9.9[b]	12.4[c]
	Elevated	41.3[a]	61.0[a]	2.8[a]	11.4[a]	19.5[a]
TGx 1740 2F	Ambient	19.7[d]	31.6[d]	2.3[c]	8.22[b]	6.13e
	Elevated	35.2[b]	55.2[a]	2.7[ab]	10.6[a]	15.8[b]

Treatments followed by the same letters are not significantly different at *P* <0.05 level

Haugwitz et al., 2014). The increased microbial biomass N observed in this study could be associated with the activities of the rhizobia in the roots of the soybean, which helps in N-fixation in the rhizosphere (Kuzyakov et al., 2018). However, in some studies, microbial biomass N was not affected by elevated CO_2 (Schortemeyer et al., 2000; Zak et al., 2000). Furthermore, the higher soil N in this study may be attributed to reduced nitrate leaching under elevated CO_2 (Cannell and Thornley, 1998; Matamala and Drake, 1999). In contrary, elevated CO_2 has been observed to increase denitrification or N_2O fluxes, therefore, potentially reduce N retention (Ineson et al., 1995). Generally, the increased microbial biomass properties (C and N), soil N and P could also be explained by the functional role of AMF for nutrients cycling particularly C, N and P, which is more intensified under elevated CO_2 (Denef et al., 2007; Drigo et al., 2013; Fang et al., 2015). Thus, the microbial responses to elevated CO_2 are expected to vary among ecosystems, since responses to elevated CO_2 are positively related to the plants or crops grown, soil-type specific and microbial communities in the soil (Nie et al., 2013; Pendall et al., 2013; Procter et al., 2014),

The present study also revealed increased seed yield and yield attributes of the soybean cultivars under elevated CO_2. The result was in consistent with previous reports (Sakariyawo et al., 2016; Adeyemi et al., 2017). This positive response could be attributed to enhance net photosynthesis and dry matter accumulation prior to the formation of the yield attributes, resulting in improve seed yield. Results of several other studies have suggested that elevated CO_2 will result in increased photosynthesis of C_3 plants by 30–50% (Yang et al., 2006; Ainsworth and Rogers, 2007). In addition, the increased yield performance in this study could also be attributed to the enhanced AMF root colonization, which helps in nutrients uptake, particularly P and water, as well as improved soil properties in terms of soil N and P and microbial C and N.

4 Conclusions

The results of this study demonstrates that elevated CO_2 concentration had significant effect on AMF activities in the rhizosphere as well as the soil microbial biomass by increasing the root colonization, soil N and P and microbial biomass C and N. This resulted in higher seed yield and yield attributes of the soybean cultivars. Further investigation is required to ascertain the responses of AMF and other soil microbial communities in the cycling of C, N and P under different crops in response to elevated CO_2. This study may help to better forecast how the AMF and soil microbes respond with increasing atmospheric CO_2.

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