

IMPACT OF ELEVATED CARBON DIOXIDE AND / OR
TEMPERATURE ON ARBUSCULAR MYCORRHIZAL
FUNGAL COMMUNITY IN GROUNDNUT RHIZOSPHERE
SOIL

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By
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CERTIFICATE

This is to certify that the thesis **entitled "IMPACT OF ELEVATED CARBON DIOXIDE AND /OR TEMPERATURE ON ARBUSCULAR MYCORRHIZAL FUNGAL COMMUNITY IN GROUNDNUT RHIZOSPHERE SOIL"** submitted by **Miss. ASHA A. N.**, for the degree of **MASTER OF SCIENCE in AGRICULTURAL MICROBIOLOGY**, to the University of Agricultural Sciences, Dharwad is a record of research work done by her during the period of her study in this University under my guidance and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles.

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1. INTRODUCTION

Enhancement in concentration of atmospheric carbon dioxide (CO₂) and resultant increasing temperature as a result of the cumulative effects of anthropogenic activities, emissions from fossil fuel combustion, energy use scenario, land clearing and urbanization have created renewed interest in understanding responses of the ecosystem to increased levels of CO₂ and temperature (IPCC, 2007). The atmospheric CO₂ concentration has been steadily rising from pre-industrial era: 280 ppm in the year 1850 to 390 ppm in 2011 (Trans *et al.*, 2011). As a consequence of this rise in CO₂, the global surface temperature is predicted to increase by 1 to 6° C by the year 2100 (IPCC, 2007), leading to enhanced drought in several areas of the world, as a result of increased evapotranspiration.

These climate-changing parameters are known to affect terrestrial plants as well as large varieties of microorganisms which are associated with the host plants; thereby enhancing the plant growth promotion as well as increasing the plant tolerance to biotic and abiotic stresses (Bent, 2006). Many of these plant growth-promoting microorganisms colonize the rhizosphere soil, the portion of soil attached to the root surface and influenced by root exudates (Bent, 2006; Lugtenberg and Kamilova, 2009). Some of the beneficial microorganisms can also enter the root system of their hosts and enhance their beneficial effects with an endophytic lifestyle like plant growth-promoting fungi (PGPF) such as arbuscular mycorrhizal fungi (AMF) as well as plant growth-promoting rhizobacteria (Das and Varma, 2009).

Altered environmental conditions due to climate change are likely to induce changes in plant physiology and root exudation. Elevated CO₂ in particular will lead to increased C allocation to the root zone and potentially also lead to altered composition of root exudates thereby altering the availability of chemoattractants or signal compounds, C/N ratios or nutrient availability (Kandeler *et al.*, 2006; Haase *et al.*, 2007). Further, elevated temperature coupled with drought might lead to change in the composition, abundance and activities of plant associated microbial communities, thus having significant impact on diversity and activities of such plant associated microbial communities (Drigo *et al.*, 2008). Consequently, microorganisms known for their beneficial effects on plant growth or health might

also be impaired in terms of exhibiting their desirable properties and also their colonization capacity under certain conditions.

Among the beneficial soil microorganisms, approximately 90 per cent of the plants form mycorrhizal association and 60 per cent of these plants establish symbiosis with obligate symbionts such as AMF and are known to enhance plant nutrient uptake in exchange for rhizosphere soil carbohydrate compounds. However, due to climate change effects including increasing CO₂ concentrations in the atmosphere, soil warming or drought stress has indirect effect on plant associated AMF (Auge, 2001). Several studies have revealed that elevated CO₂ increased the AMF colonization in comparison with ambient CO₂, due to increased carbon allocation to their external hyphae. However, contrasting results also have been obtained by Gavito *et al.* (2000) who indicated that certain AMF strains did not respond to altered conditions. Hence, different plant growth promoting strains need to be selected for elevated CO₂ and temperature conditions.

In order to overcome, as well as to address the global warming effect on the beneficial microbes, there is a need to study the effect of elevated CO₂ and temperature on beneficial microorganisms. In this regard, an approach has been made to study the effect of elevated CO₂, elevated temperature and combination of elevated CO₂ along with increased temperature on soil biological parameters with a special emphasis on AM fungal community in groundnut rhizosphere soil. This will form a basis for the development of AMF consortium under altered or to be altered climatic conditions. An attempt will also be made to study the genetic variability of the predominant group of the AMF under altered conditions by amplifying the internal transcribed spacer of the ribosomal DNA (ITS rDNA) with the following objectives:

1. To study the effect of elevated carbon dioxide and temperature on physico-chemical and biological properties of soil
2. To study the AM fungal community under elevated carbon dioxide and temperature conditions
3. Genetic characterization of the most predominant arbuscular mycorrhizal (AM) fungi under elevated CO₂ and temperature conditions

2. REVIEW OF LITERATURE

The global climate is predicted to change drastically over the next century and is projected to have an impact on many terrestrial ecosystems including soil microbial communities which, in turn, would modulate nutrient availability and many important soil processes. With this back ground, the literature pertaining to the impact of elevated carbon dioxide and temperature on soil physico-chemical properties, biological properties and particularly on the mycorrhizal community to the altered condition were reviewed in this chapter.

2.1 Impact of elevated carbon dioxide and temperature on soil physico-chemical properties

Abbas *et al.* (2009) studied the soil physico-chemical characteristics under free air CO₂ enrichment technology and revealed that pH drop in the soils exposed to elevated CO₂ under submerged conditions was significantly higher than under un-submerged condition. However unlike pH, the EC of soil was found to be relatively higher under elevated CO₂ as compared to that of ambient CO₂ condition. Furthermore, these soil alteration factors were greatly influenced under elevated CO₂ under the influence of submerged conditions compared to un-submerged soil condition. They have further revealed that elevated CO₂ levels promoted an increased soil organic carbon, phosphorus and potassium in comparison to there in ambient condition.

Stephen *et al.* (2004) showed that elevated atmospheric CO₂ concentration not only resulted in increasing the crop biomass in soybean cropping system but also increased the soil physical properties like soil bulk density, saturated hydraulic conductivity, and aggregate stability compared to the sorghum crop system, indicating greater non yield biomass inputs of soybean under elevated CO₂ resulting in enhanced carbon content, thereby improving the soil physical parameters. Karberg *et al.* (2005) reported an increased dissolved inorganic compound under elevated CO₂ resulting in the alteration of inorganic carbon, thereby increasing the total alkalinity in the soil.

Saurav *et al.* (2011) studied the impact of elevated CO₂ on soil physical parameters in pigeon pea. They concluded that there was no appreciable change in the soil physical properties *viz.*, bulk density and hydraulic conductivity, but oxidizable organic carbon in the soil under elevated CO₂ condition significantly increased by nearly 1.5-fold from initial level of 1.12 per cent to 1.72 per cent under elevated CO₂ condition as compared to ambient condition. The total organic C increased up to 2.15 per cent (1.7-fold increase under elevated CO₂) from its initial level of 1.25 per cent.

Barnard *et al.* (2005) revealed that soil pH did not differ significantly under altered conditions in the beginning and at the end of this study. However, they have observed a significant decrease in the soil nitrate concentration under elevated CO₂ concentration. Hong *et al.* (2007) studied the availability of soil nitrogen in rice-wheat cropping system under elevated CO₂ and revealed that soil nitrogen decreased under elevated CO₂ by 47 and 29 per cent in soils receiving low and normal nitrogen respectively.

2.2 Impact of elevated carbon dioxide and temperature on soil biological properties

It is well known that beneficial plant-associated microorganisms stimulate plant growth and enhance resistance to disease and abiotic stresses. The effects of climate change factors such as elevated CO₂ and temperature on beneficial plant-microorganism interactions are increasingly being explored. The results of studies showing the effects of climate change factors on beneficial microorganisms have been reviewed.

2.2.1 Cyanobacteria

Fu *et al.* (2007) investigated the effect of increased temperature and CO₂ on the growth of marine cyanobacteria. The authors revealed that altered conditions have stimulated the cell division and the photosynthetic rate of the marine cyanobacteria. Similarly, Riebesell *et al.* (1993) have also shown in their laboratory studies that CO₂ enrichment stimulated marine diatoms.

2.2.2 Protozoa

Plants allocate relatively more carbon to below-ground than above ground when grown under elevated atmospheric CO₂ (Rogers *et al.*, 1994). Increased below - ground C under elevated atmospheric CO₂ would be in the form of larger root systems resulting in increased rates of exudation thereby affecting both bacterial population and protozoan number as bacteria are supported by root exudates and dead tissue in turn supporting predatory protozoans (Rogers *et al.*, 1994).

Lussenhop *et al.* (1998) studied the response of protozoa to elevated CO₂ condition in *Populus* rhizosphere soil and documented amoeba, flagellates and few ciliates and their numbers were greater by 2.5 times under elevated CO₂ compared to ambient. Roger *et al.* (2001) examined the densities of protozoa in rhizosphere soil of wheat plants grown under experimentally controlled CO₂ treatments and revealed the highest density of protozoa per gram of soil in wheat rhizosphere soil exposed to elevated CO₂ concentrations (750 ppm) as compared to plants grown at near ambient CO₂ concentrations (365 ppm).

Regin *et al.* (2002) studied the response of free living protozoa to elevated CO₂ in presence of mycorrhizal fungi in pea rhizosphere soil and that elevated CO₂ increased the soil protozoans in the absence of mycorrhizal fungi.

2.3 Microbial biomass and soil enzymes activity

Soil microbial biomass, although very small on an ecosystem basis (1–3% of soil total organic carbon), plays a central role in understanding the changes of C and N cycling, under elevated CO₂. The microbial community is indeed the “eye of the needle” through which virtually all the decomposing of C and nutrients must pass (Coleman and Crossley, 1996). The measure of microbial biomass turnover, provides useful information on soil responses to atmospheric CO₂ enrichment, could therefore be a valid “early warning” of changes in soil organic C and it can help to predict nutrient availability for plant uptake and growth (Insam *et al.*, 1999).

Decomposition processes are the result of a collective activity of microbial communities driven by the acquisition of nutrients released by extracellular degradation of detritus (Sinsabaugh and Moorhead, 1994). Soil enzymatic activities are closely related to microbial activity or biomass as they catalyse biochemical

reactions and nutrient cycling in the soils (Burns, 1982). They also integrate information on the one hand from microbial status, and on the other hand from soil physico-chemical conditions, thus being a candidate “sensors” of ecosystem stress, changing soil properties and soil degradation (Badiane *et al.*, 2001 and Vepsäläinen *et al.*, 2001). Soil enzymes can represent mineralization rates of organic matter as well as creating a longer term legacy of decomposition processes (Frankenberger and Dick, 1983). Furthermore, Kandeler *et al.* (1998) have indicated that the composition of the microbial community determines the potential of that community for enzyme synthesis, and thus any modification of microbial community due to environmental factors should be reflected on the level of soil enzymatic activities. Soil enzymatic activities act as possible tool to study the below - ground processes, induced by CO₂ enrichment (Kang *et al.*, 2005).

Among the diverse soil enzymes, dehydrogenase, phosphatase, urease and cellulase are important in the transformation of different nutrients for plants. The activity of dehydrogenase reflects the total oxidative capacity of the microbial biomass (Nannipieri *et al.*, 1990). Phosphatases are a broad group of enzymes that are capable of catalyzing hydrolysis of esters and anhydrous of phosphoric acid (Schmidt and Lawoski, 1961). In soil ecosystems, these enzymes are believed to play critical roles in P cycles (Speir and Ross, 1978). Urease enzymes predominate among the enzymes involved in the N cycle of the soil (Tabatabai and Bremner, 1972). It catalyzes the hydrolysis of urea into ammonia depending on the soil pH and also responsible for the decomposition of residues. Cellulases are a group of enzymes that catalyse the degradation of cellulose. Cellulose in plant debris has to be degraded into glucose, cellobiose and high molecular weight oligosaccharides by cellulase enzymes (White, 1982). The activity of these enzymes transforms the residue into humus, which is completely decomposed into the free nutrients (Ahmad and Khan, 1988). The microbial biomass is an important labile fraction of the soil organic matter, functioning both as an agent of a source of transformation, recycling of the organic matter, soil nutrients and also of a source of nutrients for plants. The activity of these enzymes is associated with high productivity of the crops.

2.3.1 Dehydrogenase activity

The impacts of elevated CO₂ and temperature on soil enzyme activities have gained wide attention in the past few years. Specific enzyme activities are directly and/ or indirectly affected by elevated CO₂ and/or temperature (Kang *et al.*, 2005).

Bhattacharyya *et al.* (2013) observed that the enzyme activities in soil were strongly influenced by elevated CO₂ as well as by the interactive effect of elevated CO₂ plus temperature in open top chambers (OTCs).

Saurav *et al.* (2011) observed that the dehydrogenase enzyme activity was significantly increased in the soils exposed to elevated CO₂ compared to the ambient condition. Soil dehydrogenase activity increased sharply from the initial value of 34.84 g of TPF g⁻¹ day⁻¹ to 140.59 g of TPF g⁻¹ day⁻¹ in elevated CO₂ as compared to ambient CO₂, which recorded a activity of 45.89 g of TPF g⁻¹ day⁻¹.

Das *et al.* (2011) examined the interactive effect of elevated CO₂ and temperature on soil enzyme activity in tropical rice soils. The highest dehydrogenase activity was recorded in the soils exposed to high concentration of CO₂ (600 ppm), whereas the temperature elevation at 45⁰C recorded the highest dehydrogenase activity. However, the overall interaction of CO₂ and temperature was significantly higher over rest of the altered conditions

Xuefeng *et al.* (2010) reported that dehydrogenase activity was significantly influenced by elevated CO₂ condition, thereby indicating enhanced soil microbial activity under the influence of elevated CO₂.

2.3.2 Phosphatase activity

Phosphatases are involved in transformation of organic and inorganic phosphorous compounds in soil (Amador *et al.*, 1997), and these activities are important factors in maintaining and controlling the rate of P cycling through soils. Elevated levels of CO₂ and temperature independently accelerated phosphatase activities at both the moisture regimes *viz.*, flooded and nonflooded condition. However, the combined effect of elevated levels of CO₂ and temperatures and their interactions had a significant effect on both acid and alkaline phosphatase activities. In general acid phosphatase activity was higher than the alkaline phosphatase activity as the soil pH of the experimental site was closer to the optimal for acid

phosphatase activity viz., pH 6.5 (Das *et al.*, 2011). Similarly, phosphatase activity was influenced in wetlands under elevated CO₂, as the microbes existing in the soil may be stimulated to obtain more phosphate under elevated CO₂ condition, thereby resulting in higher phosphatase activity (Kang *et al.*, 2005). Guenet *et al.* (2012) reported that CO₂ treatment induced a significant increase in both acid and alkaline phosphatase activity; however the acid phosphatase activity was influenced to a greater extent due to the CO₂ elevation.

Kandeler *et al.* (2006) studied the response of soil microbial enzyme activities to elevated CO₂ in semi arid grassland ecosystem of different soil depths. The results revealed that phosphatase activity was significantly increased in the upper 5 cm of the soil and high phosphatase activity was recorded in the upper 20 cm under elevated CO₂ condition. Similarly, Moscatelli *et al.* (2005) revealed that acid phosphatase activity was significantly influenced under elevated CO₂ condition.

2.3.3 Urease activity

The conversion of organic nitrogen to inorganic nitrogen through hydrolysis of urea to ammonia and CO₂ is an important pathway of N transformation in soils. The highest urease activity was recorded at 600 μ mol mol⁻¹ of CO₂ concentration at 45^o C under flooded condition. Compared to non-flooded condition, the increase in urease activity in flooded condition, in relation to elevated CO₂ and temperature interaction was more pronounced (Das *et al.*, 2011). In a long-term CO₂ enrichment study in calcareous grassland, urease activity was faster and was more distinct in spring than in summer (Ebersberger *et al.*, 2003).

Feng *et al.* (2007) investigated the responses of urease activity to simulated climate changes. In this study the combination of elevated temperature plus elevated CO₂ significantly increased the urease activity compared to elevated CO₂ alone.

2.3.4 Cellulase activity

Cellulases are a group of enzymes that catalyze the degradation of cellulose, polysaccharides build up of β -1, 4 linked glucose units (Deng and Tabatabai, 1994).

David *et al.* (2005) revealed that the soil cellulase activity increased steeply with the treatment receiving organic matter under free air CO₂ enrichment compared to the soils under CO₂ enrichment without organic matter incorporation. In another investigation, both cellulase activity and cultivable cellulolytic fungal abundance showed a positive response to the elevated CO₂, reflecting that the higher cellulolytic fungal abundance increased cellulase activity (Xuefeng *et al.*, 2010).

2.4 Microbial biomass carbon and microbial biomass nitrogen

Soil microbial biomass carbon (MBC) is considered to be responsible for regulating nutrient cycling (Singh *et al.*, 1989); is closely linked to the primary productivity of an ecosystem (Marcel *et al.*, 2008) and soil health (Sparling, 1997). Impacts on MBC are believed to be of great importance to understand the below-ground processes in soils exposed to high CO₂ (Drigo *et al.*, 2008). Cheng *et al.* (2000) reported that soil microbial biomass N was significantly increased by elevated CO₂ in a submerged paddy soil. Response of MBC to elevated CO₂ could be further influenced by changes in temperature and soil moisture (Schnurer *et al.*, 1986).

Saurav *et al.* (2011) reported that the microbial biomass carbon in the soil increased significantly in the elevated CO₂ conditions as compared to ambient conditions. Microbial biomass carbon exhibited the highest hike under elevated CO₂ conditions, as it ascended from initial level of 369.47 µg g⁻¹ to 1188.07 µg g⁻¹.

Bhattacharyya *et al.* (2013) reported that microbial biomass carbon content increased significantly under elevated CO₂ alone as well as under elevated CO₂ plus temperature over that of ambient CO₂ conditions. They also observed that soil microbial biomass carbon was influenced by the crop growth stages and maximum MBC was obtained at the panicle initiation stage under elevated CO₂ plus temperature.

Elevated CO₂ concentrations at 600 µ mol mol⁻¹ significantly increased the microbial biomass carbon content in flooded tropical rice soils than under non-flooded conditions. In case of elevated temperature, MBC content significantly increased under both the moisture regimes at 45⁰ C. The interaction of higher CO₂

concentration, temperature and moisture regimes was statistically superior in influencing MBC over other treatments. David *et al.* (2005) observed an increased microbial biomass carbon with the treatment that received soil organic matter under free air CO₂ enrichment (FACE) conditions. Similarly, Moscatelli *et al.* (2005) reported an increased microbial biomass carbon by 16 per cent under FACE elevated CO₂ as compared to ambient conditions.

Rakshit *et al.* (2012) investigated the impact of elevated CO₂ and temperature on microbial biomass carbon under OTC. The highest amount of MBC was recorded with elevated CO₂ at anthesis and maturity stage of the crop (35.2 per cent and 19.5 per cent, respectively) over ambient CO₂ concentration, while MBC decreased by 2–17 per cent at elevated temperature. Further, this study also exhibited an increased microbial biomass nitrogen (MBN) under the influence of elevated CO₂ condition compared to ambient.

2.5 Impact of elevated carbon dioxide and temperature on AM fungi

Approximately 90 per cent of plants form mycorrhizal associations (Smith and Read, 1997), and 60 per cent of these plants establish symbiosis with obligate symbionts such as AMF belonging to the group of PGPF (Fitter and Moyersoen, 1996). AMF are known to enhance plant nutrient uptake (mostly phosphate) or confer other benefits in exchange for rhizosphere soil carbohydrate compounds (Newsham *et al.*, 1995). However, due to climate change effects such as increasing CO₂ concentrations in the atmosphere, soil warming or drought stress, many studies have reported indirect effects of these parameters on these plant-associated fungi (Auge, 2001). Increased ambient CO₂ levels were reported to have effects on hyphal growth and root colonization by AMF in the majority of studies. For instance, Sanders *et al.* (1998) showed that at 600 ml⁻¹ of CO₂, external hyphae of AMF strains showed an increased growth in the rhizosphere soil of *Prunella vulgaris* in comparison with ambient CO₂ (350 ml⁻¹). This was explained to be due to increased C allocation to their external hyphae (Rillig and Allen, 1999). Similarly, internal hyphae of AMF showed the tendency to increase. This might also be due to increased root biomass caused by increased CO₂ levels (Sanders *et al.*, 1998). Contrasting results on CO₂ effects on plant-AMF associations were, however, obtained by Gavito *et al.* (2000), who showed, with *Pisum sp.* as the host

plant, mycorrhizal development of some AMF strains was not affected by CO₂ concentration.

Responses of AMF to increased temperature have been investigated. In the majority of cases, increased temperature had a positive impact on AMF colonization and hyphal length. In some cases, however, no effects or negative effects of enhanced temperature on AMF are reported. AMF respond to higher temperatures with enhanced growth and plant root colonization for majority of the strains (Fitter *et al.*, 2000). This was demonstrated for instance with strains of *Glomus intraradices*, *Glomus mossae* as well as with many others (Monz *et al.*, 1994). Temperature may also significantly alter the structure of the AMF hyphal network and induce a switch from more vesicles in cooler soils to more extensive extraradical hyphal networks in warmer soils (Hawkes *et al.*, 2008). This may be linked to faster carbon allocation to the rhizosphere soil and an increased respiration of the extraradical hyphae at a high soil temperature (Heinemeyer *et al.*, 2006).

Maria *et al.* (2008) observed the response of mycorrhizal fungi to free-air, CO₂ enrichment and nitrogen fertilization. They hypothesized that AMF become more prevalent under elevated CO₂ but they decreased under N fertilization.

Vicca *et al.* (2009) studied the effects of mycorrhizal colonization in model grassland ecosystems. Grassland soil was subsequently inoculated with AMF under ambient conditions and to a combination of elevated CO₂ and temperature conditions. After one growing season, the inoculated soil revealed a positive climate effect on AMF root colonization.

2.6 Methods used to study AMF community

2.6.1 Morphological and phenotypic methods

Classical techniques for AMF identification are based on spore morphology. This approach is time consuming and difficult to master. Spore characteristics, especially spore wall structure, morphology, and ontological changes are the most

important criteria for AMF identification (Morton and Bentivenga, 1994). An illustrated hard copy (Schenck and Perez, 1990) and online electronic taxonomic guides/keys (<http://invam.caf.wvu.edu/>) explain and apply the systematics. Approximately 150 species of AMF are described to date. Undoubtedly, majority of the AMF species remain undescribed (Bever *et al.*, 2001). For example, The International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi (INVAM) maintain approximately 40 isolates that do not belong to any of the currently described species. In field samples, low spore number, parasitization of spores, age and environmental alteration of spores (e.g., discoloration) are known hinder accurate identification (Bever *et al.*, 2001).

2.6.2 AMF communities

Impact of climate change on plant-AMF interactions in natural grassland revealed that the increased temperature significantly affected the relative spore abundance of AMF species, resulting in decreased AMF diversity (Xiu *et al.*, 2013).

2.7 Molecular and genotypic method

The development of molecular biology methods has allowed the circumvention of many of the above limits, first by applying isozyme (Rosendahl and Sen 1992), immunology methods (Sanders *et al.*, 1992), and nucleic acids-based approaches (Clapp *et al.* 2001). Amplification of AM fungal genes from environmental samples with the aid of primers of different specificities now allows fairly accurate detection and identification of AM fungi both in the soil and within plant roots.

Starting with the work of Simon *et al.* (1992), different sets of primers, mostly amplifying nuclear ribosomal genes of AM fungi, have been developed. For research into the natural communities of AM fungi, primers amplifying all AM fungi, but excluding other fungi and plant hosts, are desirable. Fitting these requirements the best is the primer pair NS31 coupled with AM1 (Simon *et al.*, 1992; Helgason *et al.*, 1998), designed to amplify (AM) the fungal and exclude the plant small subunit nuclear ribosomal rRNA gene (SSU rDNA). The AM1 primer was later shown to exclude the deeply branching Archaeosporaceae and Paraglomaceae families (Daniell *et al.*, 2006), but attempts to develop better AM fungi-specific primers have

not been successful. However, this primer pair and the SSU region are the most widely used targets in AM fungal community studies. A range of group specific primers is available (Geue and Hock, 2004).

Other nuclear ribosomal DNA regions have been used in AM research, including the internal transcribed spacer (ITS) flanking the 5.8 S gene, and the large ribosomal subunit (LSU) gene. The ITS region is considered to be an appropriate tool for studying AMF at similar taxonomic level. AM fungi possess divergent copies of the ITS region within an individual spore (Jansa *et al.*, 2002, Pawlowska and Taylor, 2004). Species-specific primers of the LSU region have been useful for detection of AM fungi (Turnau *et al.*, 2001).

Amplification of AM fungal genes from environmental samples with the goal of detection or identification, if using general primers, needs to be followed by an amplicon separation and/or screening step, because the amplicon consists of amplified gene fragments of multiple co-existing organisms. The separation can be achieved via electrophoresis or cloning of PCR products (Husband *et al.* 2002a; Vandenkoornhuyse *et al.* 2002).

3. MATERIAL AND METHODS

An investigation was carried out to study the influence of elevated carbon dioxide, temperature and their combinations on soil physico-chemical and microbiological properties as well as to measure the abundance of the AMF community in groundnut rhizosphere soil in the Open Top Chamber (OTC) facility at the Institute for Agricultural Research on Climate Change (IARCC), University of Agricultural Sciences, Dharwad. The materials used and the methods followed are described below.

3.1 Experimental location

Experiments were conducted in the fields of Main Agricultural Research Station (MARS), University of Agricultural Sciences, Dharwad, located at 15° 29' 44.7" N latitude ; 74° 59' 13.1" E longitude, and at an altitude of 678 m above mean sea level.

3.2 Meteorological conditions

The data on weather variables, namely, rainfall, maximum and minimum temperatures and relative humidity recorded at the meteorological observatory, Main Agriculture Research Station, Dharwad during 2013 are presented in Appendix I.

3.3 OTC system design

Open top chamber (OTC), is one of the prevailing approaches to study plant and soil microbial responses to elevated carbon dioxide and temperature. The OTC structures used were of circular horizontally with 5 m diameter and possessing a height of 4 m (Plate 1). The basic structure of the OTCs was fabricated with galvanized iron pipe and covered with double walled 6 mm thick polycarbonate sheets (Make: Sabic Innovative Plastic, USA), which traps air and also provide thermal insulation. The curved surface (Plenum) at the bottom was covered with 0.5 thick polycarbonate along with one more layer of UV stabilized layer. At 2.4 m height of each chamber, a frustum with an angle, 0.6 m towards inside is maintained, in order to protect the OTC structure against high winds and



Plate 1 General view of the experimental site showing Open Top Chambers covered with poly carbonate sheets

moderate vibrations (Plate.1). The upper portion of the chamber was kept open to maintain the near- natural conditions of temperature and relative humidity (Plate 1).

3.3.1 Supply of CO₂ to OTC

A plenum at the base having radius of 2 feet is provided for CO₂ circulation as well as to arrest vertical diffusion. Inside OTC 99.99 per cent food grade CO₂ gas was used to elevate CO₂ levels. During the investigations, 45 kg capacity gas cylinders were used and the gas was released to the chambers at sonic speed to avoid gradient functions through a manifold fitted with green fit PP-R pipes, with injection points arranged in semicircular shape for elevating CO₂ concentration.

3.3.2 CO₂ control and monitoring

The equipment for monitoring and controlling the levels of CO₂ in OTCs was fully automatic and desired level of CO₂ (550 ppm) was maintained throughout the experimental period in the OTC with the help of NDIR based microprocessor controlled online CO₂ gas analyzer (PP System, USA).

3.3.3 Temperature elevation

Temperature is elevated by deploying octagonal arrays of infrared heaters around the periphery of the OTC at a height of 2.2 m and tilted at 45° toward the center of the plot. Each OTC had 7 number of IR heaters, of 1000 watts each, in order to maintain elevated crop canopy temperature (+2⁰ C) above the reference plot.

3.3.4 CO₂ and temperature analyzer

All OTCs had one suction line for measurement of CO₂ concentration and sensors to monitor soil moisture and temperature (Vegtronics, USA), air temperature, humidity sensor (Rotronic, Switzerland) and canopy temperature. Ambient OTC had one suction line for measurement of CO₂ concentration and sensors to monitor soil moisture, soil temperature, air temperature and humidity. Reference plot had sensors to monitor all these variables soil moisture, soil



Plate 2 Automatic online CO₂ and temperature control model



Plate 3 View of Infrared ceramic heaters to elevate the canopy temperature of the OTC

temperature, air temperature, humidity, canopy temperature and also sensor for PAR radiation (Plate 2).

Information from all the above sensors is received by “OT-MATIC” system and used the information to maintain the desired CO₂ concentration and temperature in OTC chamber. Thermal Mass Flow Meter (Siargo, USA) which is installed in the CO₂ pipe line indicated the current flow rate. Air was sampled from the centre of the OTC chamber and pumped to a non-dispersive infrared gas analyzer (PP System USA) that continuously monitored CO₂ concentration. Pure CO₂ was released near to the sonic velocity, through a large number of small jets, causing rapid mixing between CO₂ and air, thereby avoiding gradient formation inside OTC. Infrared thermometers (Apogee Instruments, USA) were installed to sense canopy temperatures in warmed and in unheated reference plots, wherein a control system modulated power to the heaters to maintain the set level of warming above the reference plot.

3.4 Soil sampling and sampling site conditions

Soil samples were collected at the start of the experiment from each OTC at four positions viz., northwest, northeast, southwest and southeast (each sampling position represented one replication) under five conditions representing elevated carbon dioxide, elevated temperature, elevated carbon dioxide plus elevated temperature plus ambient conditions and one sample from the open ring as reference plot. The soil samples collected were dried at 28°C and passed through 1000 µm sieve to remove the debris and later analyzed for the initial soil physico-chemical properties. However, fresh soil samples were collected to carry out soil microbiological properties.

Each OTC was considered as a treatment for this study, which included

- T₁ - Elevated carbon dioxide upto 550 ppm + elevated temperature of +2°C
- T₂ - Elevated carbon dioxide 550 ppm
- T₃ - Elevated temperature +2°C

- T₄ - Ambient condition (only chamber effect)
- T₅-Reference plot (Open ring)

Groundnut, cultivar GPBD-4, as test crop was sown and grown during kharif 2013 inside the OTC as per package of practice. Rhizosphere soil samples from 0 to 15 cm depth were collected at 30 and 60 DAS to study the soil microbiological properties as well as soil physico-chemical properties.

3.5 Physico-chemical properties of soil

3.5.1 Water stable aggregate analysis.

Per cent water stable aggregate (WSA) was determined by wet sieving method as described by Yoder (1936). Ten grams of air dried soil clads, measuring around 5 mm diameter was placed in upper most set of graduated sieves with mesh size of 2.0, 1.0, 0.5, 0.25 and 0.16 mm. The diameter and height of the sieves were 21 and 5 cm, respectively. The samples were wet sieved in salt free water using Yoder sieving apparatus. The sieves were oscillated vertically for a period of 10 minutes. Then, the set of sieves was removed from the apparatus and the oven dry weight of the material left on each sieve was determined. The PAS was calculated using the following formula.

$$\text{WSA (\%)} = \frac{(\text{Weight of aggregate } > 0.25 \text{ mm}) - \text{Weight of sand}}{\text{Weight of sample} - \text{sand}} \times 100$$

3.5.2 Soil pH

The soil pH was determined by using glass electrode pH meter (Sparks, 1996).

3.5.3 Electrical conductivity

Soil electrical conductivity was determined by using conductivity bridge and expressed in terms of dSm⁻¹ (Sparks, 1996).

3.5.4 Organic carbon (%)

Soil organic carbon was determined by wet oxidation (Wakley and Black) method as outlined by Sparks (1996).

0.5 g of finely ground soil sample was taken in 500 ml conical flask. 10 ml of 1N $K_2Cr_2O_7$ was added and 20 ml of con. H_2SO_4 was added from sides of the flask and kept in shaker for 30 min. 200 ml of distilled water and 10 ml of 85% orthophosphoric acid, 1ml ferroin indicator were added in that order and titrated against 0.5 N ferrous ammonium sulphate (FAS) solution till the color changed from yellowish orange through green to reddish brown color. A blank was also run.

3.5.5 Inorganic carbon (mg/g)

Inorganic carbon was estimated by simple titrimetric method as outlined by Bundy and Bremner (1972).

3.5.6 Available nitrogen ($kg\ ha^{-1}$)

The available soil nitrogen was estimated by alkaline permanganate oxidation method as outlined by Subbiah and Asija (1956).

3.5.7 Available phosphorus ($kg\ ha^{-1}$)

The available soil phosphorus was estimated by Olsen's method as outlined by Sparks (1996).

The available phosphorus in the soil sample was extracted with Olsen's reagent. The phosphorus content in extractant was determined colorimetrically and the intensity of blue colored complex was measured at 660 nm wave length in a spectrophotometer.

3.5.8 Available potassium ($kg\ ha^{-1}$)

The available soil potassium was extracted with neutral normal ammonium acetate and the content of K in the solution was estimated by flame photometer (Sparks, 1996).

3.5.9 Available sulfur (kg ha^{-1})

The available sulfur was determined by extracting the soil with 0.15 per cent $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ solution and the extracted S was quantified by Turbidometric method using spectrophotometer at 420 nm wave length (Sparks, 1996).

3.6 Soil biological properties

3.6.1 Enumeration of rhizosphere soil microflora

3.6.1.1 Enumeration of bacterial population

Enumeration of total soil bacteria was carried out as per the standard aerobic plate count method given by Bunt and Rovira (1955) using nutrient agar medium (Appendix -II). Ten gram of soil sample was transferred into 90 ml sterilized water blank and serially diluted up to 10^{-6} . Further, one ml of aliquot from 10^{-6} diluent was taken and dispensed in sterile petriplate with nutrient agar medium. The bacterial colonies were enumerated after 24-48 hrs of incubation at 37°C and expressed as number of colony forming units (CFU g^{-1}) per dry weight of soil.

3.6.1.2 Enumeration of fungal population

Enumeration of fungi was carried out as per the standard aerobic plate count method (Bunt and Rovira, 1955) using Martin's rose Bengal agar medium (Appendix -II). Ten gram of soil sample was transferred into 90 ml diluent and serially diluted up to 10^{-6} . Further, one ml of aliquot from 10^{-4} diluent was taken and dispensed in sterile petriplate with Martin's rose Bengal medium. The Fungal colonies were enumerated after 6 to 7 days of incubation at 32°C and expressed as number of colony forming units (CFU g^{-1}) per dry weight of soil.

3.6.1.3 Enumeration of actinomycetes

Enumeration of actinomycetes was carried out as per the standard aerobic plate count method (Bunt and Rovira, 1955) using Kuster's agar medium

(Appendix -II). Ten gram of soil sample was transferred into 90 ml diluent and serially diluted up to 10^{-6} . Further, one ml of aliquot from 10^{-4} diluent was taken and dispensed in sterile petriplate with Kuster's agar medium. The actinomycetes colonies were enumerated 6 to 7 days after incubation at 32°C and expressed as number of colony forming units (CFU g^{-1}) per dry weight of soil.

3.6.1.4 Enumeration of free living nitrogen fixers

Enumeration of free living nitrogen fixers was carried out as per the standard aerobic plate count method by Bunt and Rovira (1955) using Norris-N-free agar medium (Appendix -II). Ten gram of soil sample was transferred into 90 ml diluent and serially diluted up to 10^{-6} . Further, one ml of aliquot from 10^{-4} diluent was taken and dispensed in sterile petriplate with Norris-N-free agar medium. The free living nitrogen fixing bacterial colonies were enumerated after 4 to 5 days of incubation at 32°C and expressed as number of colony forming units (CFU g^{-1}) per dry weight of soil.

3.6.1.5 Enumeration of phosphorus solubilizers

Enumeration of total bacteria was carried out as per the standard aerobic plate count method by Bunt and Rovira (1955) using Pikovaskya's agar medium (Appendix -II). Ten gram of soil sample was transferred into 90 ml sterilized water blank and serially diluted up to 10^{-6} . Further, one ml of aliquot from 10^{-4} was taken and dispensed in sterile petriplate with Pikovaskya's agar medium. Colonies of P solubilizer appearing on plate were enumerated after 3 to 4 days of incubation at 32°C and expressed as number of colony forming units (CFU g^{-1}) per dry weight of soil.

3.6.1.6 Enumeration of Fluroscent pseudomonads

Enumeration of Fluroscent pseudomonads was carried out as per the standard plate count method by Bunt and Rovira (1955), using King's B agar medium (Appendix -II). Ten gram of soil sample was transferred into 90 ml sterilized water blank and serially diluted up to 10^{-6} . Further, one ml of aliquot from 10^{-4} diluent was taken and dispensed in sterile petriplate with King's B agar medium. The Fluroscent pseudomonads colonies were enumerated under a

transilluminator after 3 to 4 days of incubation and expressed as number of colony forming units (CFU g⁻¹) per dry weight of soil.

3.6.1.7 Enumeration of protozoa

Enumeration of protozoa in the soil samples was done by following the procedure described by Fisher and Yates (1963). *E. coli* culture was prepared by using Luria broth. One ml dense suspension of the culture was transferred into nine ml sodium chloride broth. Ten fold dilutions of soil sample were prepared and one ml of suspension from each dilution was transferred into each of the dilutions up to 10⁻¹⁰ and incubated for 3-6 days at room temperature. After the incubation, red color turbidity in the extinction dilution was determined and the population quantified as the number by means of MPN technique.

3.6.1.8 Qualitative estimation of algae

Enumeration of algae was carried out by using BG-11 medium (Appendix - II) (Herdman *et al.*, 1979). One gram of soil sample was inoculated into the nitrogen source containing BG-11 medium and kept under the light chamber for two to three weeks. Wet mounts of each sample was prepared and observed under a microscope Janse *et al.* (2006).

3.6.2 Estimation of soil enzyme activities

3.6.2.1 Estimation of dehydrogenase activity

Dehydrogenase activity in the soil samples was determined by following the procedure as described by Casida *et al.* (1964).

Ten grams of soil and 0.2 g CaCO₃ were thoroughly mixed and dispensed in test tubes. To each tube, one ml of 3 per cent aqueous solution of 2, 3, 5-Triphenyl tetrazolium chloride (TTC), one ml of one per cent glucose solution and eight ml of distilled water were added. This was sufficient to leave a thin film of water above the soil layer. The tubes were stoppered with rubber cork and incubated at 30° C for 24 h. At the end of incubation, the contents of the tube were rinsed down into a small beaker and slurry was made by adding 10 ml methanol. The slurry was filtered through Whatman No. 50 filter paper. Repeated

rinsing of soil with one ml methanol was continued till the filtrate ran free of red color. The filtrate was pooled and made up to 50 ml with methanol in a volumetric flask. The intensity of red color was measured at 485 nm against methanol as blank using UV- vis spectrophotometer. (Thermo Scientific, USA). The concentration of formazan in soil samples were determined by referring to a standard curve prepared using graded concentration of formazan. The results were expressed as μg of triphenyl formazan (TPF) formed g^{-1} soil per day.

3.6.2.2 Estimation of total phosphatase activity

The activity of both acid and alkaline Phosphatases in soil samples was determined by following the procedure of Tabatabai and Bremner (1969).

3.6.2.3 Acid phosphatase activity

One gram of soil sample was placed in 50 ml Erlenmeyer flask to which 0.2 ml toluene followed by 4 ml of modified universal buffer (pH 6.5) was added. One ml of 0.05 M para nitrophenol phosphate (pH 6.5) was added the flasks were swirled for few seconds, flasks were covered and kept in incubator for 1 hr at 37° C. After incubation, 1ml of 0.5 M CaCl_2 and 4 ml of 0.5 M NaOH were added to the flask, swirled and filtered through Whatman No. 42 filter paper. The intensity of yellow color developed was measured at 420 nm with a blank reagents using UV- vis spectrophotometer at 420 nm (Thermo Scientific, USA). Controls were maintained for each soil sample and were analyzed by following the same procedure described above except that the para nitrophenol phosphate solution was added after the addition of 0.5 M CaCl_2 and 0.5 M NaOH and just before filtration. The phosphatase activity in the soil samples was expressed as μg para nitrophenol formed per gram soil per hour with reference to the standard curve prepared by using graded concentrations of p-nitrophenol phosphate.

3.6.2.4 Alkaline phosphatase

One gram of soil sample was placed in 50 ml Erlenmeyer flask to which 0.2 ml toluene followed by 4 ml of modified universal buffer (pH 11) was added. One ml of 0.05 M para nitrophenol phosphate (pH 11) the flasks were swirled for few seconds, covered the flasks and kept in incubator for 1 hr at 37°C. After

incubation, 1 ml of 0.5 M CaCl_2 and 4 ml of 0.5 M NaOH were added to the flask, swirled and filtered through Whatman No. 42 filter paper. The intensity of yellow color developed was measured at 420 nm against the reagent blank using UV-vis spectrophotometer at 420 nm (Thermo Scientific, USA). Controls were maintained for each soil sample and were analyzed by following the same procedure described above except that the para nitrophenol phosphate solution was added after the addition of 0.5 M CaCl_2 and 0.5 M NaOH and just before filtration. The phosphatase activity in the soil samples was expressed as μg para nitrophenol formed per gram soil per hour with reference to the standard curve prepared by using graded concentrations of p-nitrophenol phosphate.

3.6.2.5 Estimation of urease activity

Urease activity of soil samples was determined by following the procedure of Tabatabai and Bremner (1972).

Ten gram of soil samples were treated with 1 ml toluene and 10 ml phosphate buffer and incubate at 30°C for 24 h. After incubation 15 ml 1N KCl was added and the contents filtered through Whatman No. 42. The filtrate volume was made up to 100 ml with distilled water. The 1 ml of the extractant was taken 2 ml of 10% sodium tartarate, 0.5 ml Nessler's reagent were added and incubated for 30 min and volume made up to 25 ml with distilled water. Color (yellow) developed was read at 610 nm against blank (without urea solution) using UV- Vis spectrophotometer (Thermo Scientific, USA). The results were expressed as μg $\text{NH}_4\text{-N/g}$ soil/day.

3.6.2.6 Estimation of cellulase activity

Cellulase activity in soil samples was determined by following the procedure of Pancholy and Rice (1973).

Ten gram of soil sample was placed in 50 ml Erlenmeyer flask to which 1 ml toluene followed by 10 ml of 0.5 M acetate buffer was added. Ten ml of 1% carboxymethyl cellulose was added to the flasks and contents of the flasks were mixed by swirling for 2 minutes. The flasks were stoppered and incubated at 30°C for 24 hr. After incubation, samples were centrifuged at 4000 rpm for 20 min.

The supernatant was filtered and aliquots were analyzed for reducing sugar using DNS method of Gascoigne and Gascoigne (1958). 5 ml of filtered aliquot and 5 ml of DNS solution (3, 5, dinitrosalicylic acid) were added and left in boiling water for 5 minutes, the samples were cooled and after centrifugation at 4000 rpm for 10 minutes, absorbance was recorded using UV- vis spectrophotometer at 550 nm (Thermo Scientific, USA). Controls were maintained for each soil sample and were analyzed by following the same procedure described above except that the 10 ml of 1% carboxymethyl cellulose solution. The Cellulase activity in the soil samples was expressed as mg of glucose released g^{-1} soil per 24 hr with reference to the standard curve prepared by using graded concentrations of glucose with DNS solution.

3.6.2.7 Estimation of microbial biomass carbon

Soil microbial biomass carbon estimation was carried out following the fumigation and extraction method described by Carter (1991).

Ten gram of soil was taken in screw cap bottle and fumigated with ethanol free chloroform for five days. On fifth day caps were removed and incubated at 40°C overnight in order to remove the chloroform residues. This sample was extracted with 50 ml of 2M potassium chloride by placing these bottles on a shaker for 30 min. The suspension was filtered through whatman No. 41 filter paper. Similarly, controls without adding chloroform were maintained as unfumigated samples. Then 4 ml of filtrate was taken in test tube and 4 ml of freshly prepared ninhydrin reagent were added and kept in water bath for 20 min to get purple color and allowed to cool. Then add 4 ml of Methyl ethanol (1:1 100 ml methyl ethanol: 100 ml distilled water) was added. Intensity of purple color was read at 570 nm under UV- Vis spectrophotometer (Thermo scientific, USA) and calculated by using the formula.

$$\text{Biomass C mg/g of soil} = \frac{\text{Ninhydrine reactive C in fumigated soil} - \text{Ninhydrin reactive C unfumigated soil}}{\text{Weight of soil sample used}} \times 24$$

3.6.2.8 Estimation of microbial biomass nitrogen

Soil microbial biomass nitrogen was estimated following the fumigation and extraction method described by Carter (1991).

Ten gram of soil was taken in screw cap bottle and fumigated with ethanol free chloroform for 5 days. On the fifth day, caps were removed and kept in oven at 40⁰ C overnight to remove the chloroform. This sample was extracted with 50 ml of 2M potassium chloride by placing these bottles on a shaker for 30 min. The suspension was filtered through whatman No. 41 filter paper. Similarly, controls without adding chloroform were maintained as unfumigated samples. Then 4 ml of filtrate was taken in test tube, add 4 ml of freshly prepared ninhydrin reagent was added and kept in water bath for 20 min to get purple color and allowed to cool. Then add 4 ml of Methyl ethanol (1:1 100 ml methyl ethanol: 100 ml distilled water) was added. Intensity of purple color was read at 570 nm under UV- Vis spectrophotometer in nanometer (Thermo scientific) and calculated by using the formula.

$$\text{Biomass N mg/g of soil} = \frac{\text{Ninhydrine reactive N in fumigated soil} - \text{Ninhydrin reactive N unfumigated soil}}{\text{Weight of soil sample used}} \times 2.8$$

3.6.3 Arbuscular Mycorrhizal spore count

The chlamydospores in groundnut rhizosphere soil of groundnut were determined by using wet sieving and decantation method as outlined by Gerdemann and Nicholson (1963).

AM spores were isolated using wet sieving and decantation method. Hundred gram of soil sample was taken in one liter beaker, made up into 1000 ml with water, stirred well, heavier soil particles were allowed to settle for a few seconds. Then the suspension was passed through a series of different size sieves (250µm, 106µm, 75µm, 45µm, 37µm) arranged in the descending order of their mesh size. Again water was added up to 1000 ml, stirred well and allowed for few seconds this was repeated for five to six times, till the suspension appeared clear. Sievates were collected from each sieve separately in beakers. The supernatant from each beaker was separately filtered through Whatman No.

1 filter paper and the content of the filter papers were examined for spores under stereo zoom microscope (LABOMED).

3.6. 4 Arbuscular Mycorrhizal community study

3.6.4.1 Trap culture technique

Spores extracted directly from the rhizosphere soil can be used for the identification. However, identification of the AM fungi is found to be better after trap pot culturing as not all AM fungal species sporulate at the time soil sampling, resulting in reduced spore number some species in the soil. Furthermore, spores from trap culturing are known to be better state *i.e.*, higher viable percentage of the spores (Patrícia *et al.*, 2009).

The test soil sample was mixed with equal amount of sterilized sand soil mixture (1:1) and suitable host plants (mixture of sorghum and cow pea) were sown. The plants were watered twice weekly. Hoglands nutrient solution was added bi-weekly. After 3-4 months, the potting mix was wet sieved and the spores were counted under a stereomicroscope and grouped according to their morphological characteristics. The richness and relative abundance of each fungal type were calculated.

3.6.4.2 Extraction of AM spores from the soil

AM fungal spore extraction was done as outlined by Gerdemann and Nicholson (1963).

AM spores were isolated using wet sieving and decantation method. Hundred gram of soil was taken in one liter beaker, made up into 1000 ml with water, stirred well and allowed for heavier soil particles to settle for a few seconds. Then the suspension was passed through a series of different sized sieves (250 μm , 106 μm , 75 μm , 45 μm , 37 μm) arranged in the descending order of their mesh size. Again water was added up to 1000 ml, stirred well and allowed for few seconds and this was repeated for five to six times, till the suspension appeared clear. Sievings were collected from each sieve separately in beakers. The collected sieving was subjected to the density gradient centrifugation method.

3.6.4.3 Sucrose density gradient centrifugation

Density gradient centrifugation was done as outlined by Ohms (1957).

Density gradient centrifugation method is most commonly used technique for AM fungal spore extraction. Large or small quantities of soil can be processed rapidly with reasonable efficiency and very little debris remains with the spores. Soil sieving was added to centrifuge tubes and centrifuge (REMI) at 2000 rpm for five minute. Suspension floating on top was removed carefully and 50 % sucrose solution was added in the centrifuge tube containing sample and centrifuged at 2000 rpm for two minutes. The suspension was passed through 37 μ m sieve and AMF spore samples were collected by washing with distilled water and filtered through Whatman No.1 filtered paper.

3.6.4.4 Spore identification

Morphologically similar spores were picked and the population of each spore type was enumerated. Single spore from each morphotypes was brought in to funnel technique after surface sterilization of spore with 200 ppm streptomycin sulphate and 2% chloramineT. Once roots start emerging from the stem-tips of the funnels, the contents are transferred to small plastic cups containing sterilized sand:soil (1:1) after checking the roots for AM colonization (Plate 3). The spores were mounted on a clean glass slide in lacto phenol. The spore identification was mainly based on morphological features, viz., colour, size, wall structure and hyphal attachment according to the outline given by Schenk and Perez, 1990; Rodrigues and Muthukumar, 2009 and INVAM website <http://invam.caf.wvu.edu> by. Finally, the spores were photographed under Olympus research stereomicroscope connected to a computer with digital image analysis software.

3.7 Diversity analysis

Margalef's Species richness index Species Richness is defined as the number of species per specified number of individuals or biomass (Margalef, 1958).

The Margalef's species richness index is the simplest and can be computed based on the formula:

$$\text{Margalef's species richness index} = DMg = (S-1) / \ln N$$

Where,

$\ln$ = Natural Log i.e., $\log_e$

S = Total number of species

N = Total number of individuals summed over all species

Higher the value of the index, higher is the diversity.

3.7.1 Shannon-Wiener index

The Shannon's index has probably been the most widely used index in community ecology. It is a measure of the average degree of "uncertainty" in predicting to what species an individual chosen at random from a collection of S species and N individuals will belong. Shannon and Wiener independently derived the function, which has become known as the Shannon index of diversity (Shannon and Weaver, 1949).

3.7.2 Shannon's index is estimated by using formula:

$$H' = -\sum_{i=1}^S \left[\left(\frac{n_i}{N} \right) \ln \left(\frac{n_i}{N} \right) \right]$$

Where,

n_i = Number of individuals belonging to the i^{th} species

N = Total number of individuals in the sample

The quantity P_i is the proportion of individuals found in the i^{th} species. The higher the value of index, higher is the diversity.

3.7.3 Per cent colonization

Mycorrhizal root colonization was determined as per the procedure proposed by Philips and Hayman (1970). Fresh root samples were cut into 1 cm pieces and placed in screw cap vials. The clearing of the roots was achieved by treating them with 10 percent KOH and leaving them overnight. The KOH solution was poured off and the roots were rinsed with tap water. Later, the roots were treated with 10 per cent HCl for 10 minutes to neutralize the residual effect of alkali and create an acidic environment required for further staining. The root bits were stained with 0.05 per cent trypan blue in lactoglycerol (lactic acid, glycerol and water in the ratio of (40:20:20 respectively) by boiling them at 90° C for 30 minutes. Excess stain was decanted and the root samples were immersed in lactoglycerol for destaining. The stained root bits were placed on a clean glass slide and observed under microscope for colonization. The percentage of roots colonized by mycorrhizae was calculated by the formula:

$$\text{Per cent root colonization} = \frac{\text{Root bits positive for colonization}}{\text{Total number of root bits}} \times 100$$

3.8 Genetic characterization of most predominant species of AMF under five altered environmental conditions

3.8.1 Modified technique for PCR amplification of AMF from the five altered environmental conditions

The most predominant species of AMF from five altered environmental condition were selected. Gene sequences were amplified by using Primer Glo ITS-I 5'-AAGCGAGTCGA GCGTTAAGC-3' Antisense primer Glo ITS-II 5'-ACCGGATGGATGAATTTC-3'. The primer synthesis was done by SIGMA-ALDRICH.

3.8.2 Amplification of genes from AMF spores

For the PCR amplification, a single surface sterilized spore of AMF was placed in a sterile PCR vial. The presence of single spore in the PCR vial was

confirmed by observing under stereomicroscope and then subjected for a short spin. The vial containing spore was kept in water bath at 95⁰ C for 10 min. PCR reaction mixture containing 0.1 mM dNTPs, 5 pM was made each of primer, 5 U of *Taq* DNA polymerase and the supplied reaction buffer amounting a total volume of 20 µl was made. The reactions were carried out in a polymerase chain reaction (PCR) machine (Eppendorf). The reaction mixture was cycled through the following temperature profile: 94⁰ C for 5 min; 94 °C for 1 min; 54⁰ C for 1 min; 72⁰ C for 45 min and holding at 4⁰ C.

3.8.3 Agarose gel electrophoresis

The PCR samples were subjected to gel electrophoresis (BIO-RAD) using 1.5 per cent agarose gel along with 100 bp molecular marker of λ DNA. Gene amplification was observed under UV transilluminator and documented.

3.9 Statistical analysis

The data were subjected to analysis following Completely Randomized Design (CRD) as described by Yates *et al.* (1937). Further, the data were also subjected to principal component (PCA) analysis with softwares, SPSS and Microsoft Excel for Windows 2007 add-ins with XLSTAT Version 2010.5.05. Statistically significant differences between the treatments were analysed using analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) at 5% significance level. To reveal the similarities and differences between sample plots and the relationships between different variables, principal component analysis (Wold *et al.*, 1987) was performed on all the data. In a PCA model, the objects (soil samples) are represented by their scores and the variables are represented by their loadings. The scores and loadings can be presented in a graph where two components are plotted against each other. In a score plot, similar samples will be positioned close to each other, and in loading plots positively correlated variables will be positioned close to each other and negatively correlated will be opposite to each other. Samples that are high in a specific variable will be pulled towards the area of the score plot where the variable in the corresponding loading plot is located. The number of variables was reduced by excluding those explained to less than 50% by the significant components.

4. EXPERIMENTAL RESULTS

The present investigation was carried out during 2013-2014 for assessing the soil physico-chemical, microbiological properties, AMF community analysis and PCR based molecular approaches to authenticate the predominant AMF species under elevated CO₂, temperature and combination of CO₂ and temperature conditions under open top chamber (OTC) facility available at Institute for Agricultural Research on Climate Change (IARCC), University of Agricultural Sciences, Dharwad. The results obtained during the investigations are documented and presented below.

4.1 Initial soil physico-chemical and biological properties of the experimental site

The preliminary data on soil physico-chemical and biological properties under OTC and reference ring carried out before the startup of the experiment are presented in Table 1.

4.2 Soil physico-chemical properties as influenced by elevated CO₂ and / or temperature in groundnut rhizosphere soil

The observations on soil physico-chemical properties viz., soil water stable aggregates, soil pH, electric conductivity, organic and inorganic carbon under the influence of elevated CO₂, elevated temperature and combination of elevated CO₂ and temperature conditions were recorded at 30 days after sowing (DAS) and 60 DAS. These parameters were retrieved from the rhizosphere soil samples of groundnut crop.

4.2.1 Soil water stable aggregates (per cent)

The observations on soil water stable aggregates (SWSA) made at 30 and 60 DAS as influenced by altered environmental conditions resulted in the following interpretation. Among the altered environmental conditions, the highest SWSA was recorded in the soils exposed to elevated CO₂ levels at 30 DAS (66.48 %) followed by the treatment which received elevated CO₂ and temperature (65.37 %)

Table 1: Initial soil physico-chemical and biological properties of the experimental site

Sl. No.	Soil properties	OTC chamber				Reference ring
		1	2	3	4	
	Physico-chemical properties					
1	WSA (%)	57.38	57.35	57.37	57.38	55.43
2	Soil pH	7.38	7.37	7.38	7.38	7.36
3	Electrical conductivity (dS/m)	0.58	0.57	0.58	0.58	0.58
4	Organic carbon (%)	0.72	0.73	0.72	0.73	0.71
5	Inorganic Carbon (mg/g)	25.6	25.7	25.6	25.7	25.4
6	Available nitrogen (kg/ha)	228	227	227	228	228
7	Available phosphorus (kg/ha)	34	33	34	33	31
8	Available potassium (kg/ha)	276	278	276	276	279
9	Available sulphur (kg/ha)	24.65	24.78	24.81	24.79	27.2
	Biological properties					
1	Bacteria $\times 10^5$ CFU g^{-1} of soil	14	13	14	12	11
2	Fungi $\times 10^3$ CFU cfu g^{-1} of soil	4	5	4	5	6
3	Actinomycets $\times 10^4$ CFU g^{-1} of soil	26	29	29	32	46
4	Free living N_2 fixing bacteria $\times 10^4$ CFU g^{-1} of soil	21	23	25	24	27
5	P- solubilizing bacteria $\times 10^3$ CFU g^{-1} of soil	12	13	15	14	16
6	Fluorescent pseudomonads $\times 10^3$ CFU g^{-1} of soil	8	9	7	8	6
7	Protozoa (Number g^{-1} soil)	2.6	2.5	2.3	2.2	2.2
8	Dehydrogenase activity (μg TPF formed g^{-1} soil d^{-1})	2.55	2.57	2.59	2.53	2.48
9	Acid phosphatase activity (μg pnp released g^{-1} soil h^{-1})	9.45	9.43	9.45	9.46	8.62
10	Alkaline phosphatase activity (μg pnp released g^{-1} soil h^{-1})	24.54	24.57	24.53	24.56	22.79
11	Urease activity (μg $NH^+ N$ g^{-1} soil day^{-1})	0.24	0.24	0.23	0.24	0.21
12	Cellulase activity (μg reducing sugars released g^{-1} soil/ 24 hr)	0.235	0.234	0.236	0.235	0.232
13	Microbial biomass carbon (μg g^{-1} of soil)	191	193	194	192	184
14	Microbial biomass nitrogen (μg g^{-1} of soil)	22.56	22.61	23.45	23.68	21.53
15	Mycorrhizal spore count (No of spores 50 g^{-1})	178	176	179	182	195

Table 2: Qualitative estimation of algae before the start up of the experiment

Sl. No	Probable Genus	OTC chamber				Reference ring
		1	2	3	4	
1.	<i>Achnantheidium</i>	+	+	+	+	+
2.	<i>Anabaena</i>	+++	+++	+++	+++	+++
3.	<i>Chlorococcus</i>	+++	+++	+++	+++	+++
4.	<i>Diatoms</i>	+++	+++	+++	+++	+++
5.	<i>Lyngbia</i>	++	++	++	++	++
6.	<i>Nitzschia</i>	+	+	+	+	+
7.	<i>Oscillatoria</i>	++	++	++	++	++

Note: Low: +; Medium: ++; High: +++

and elevated temperature alone (64.97%). The lowest water stable aggregates (62.33 %) were recorded in the open reference ring.

In general, an increased SWSA was recorded at 60 DAS as compared to 30 DAS in the soil exposed to altered environmental conditions. However, the results were comparable to 30 DAS with respect to SWSA in all the treatments at 60 DAS (Table 3).

4.2.2 Soil pH

The pH of the groundnut rhizosphere soil at 30 and 60 DAS under five environmental conditions are presented in Table 3. The pH of the soil under altered conditions did not differ significantly at 30 and 60 DAS. However, an increased soil pH was noticed at 60 DAS under all the altered conditions.

4.2.3 Soil electrical conductivity

Similar to pH, the EC of the soil also did not differ significantly due to the altered environmental conditions at 30 as well as 60 DAS (Table 3).

4.2.4 Soil organic carbon

The soil organic carbon content at 30 DAS increased significantly under elevated CO₂ (0.78 %) as compared to elevated CO₂ plus temperature (0.75 %) and elevated temperature (0.73 %). The lowest organic carbon content was recorded in the open reference plot (0.68 %) followed by chambered ambient condition (0.70 %). Similar results were also recorded at 60 DAS, with relatively increased percentage of soil organic carbon compared to 30 DAS (Table 3).

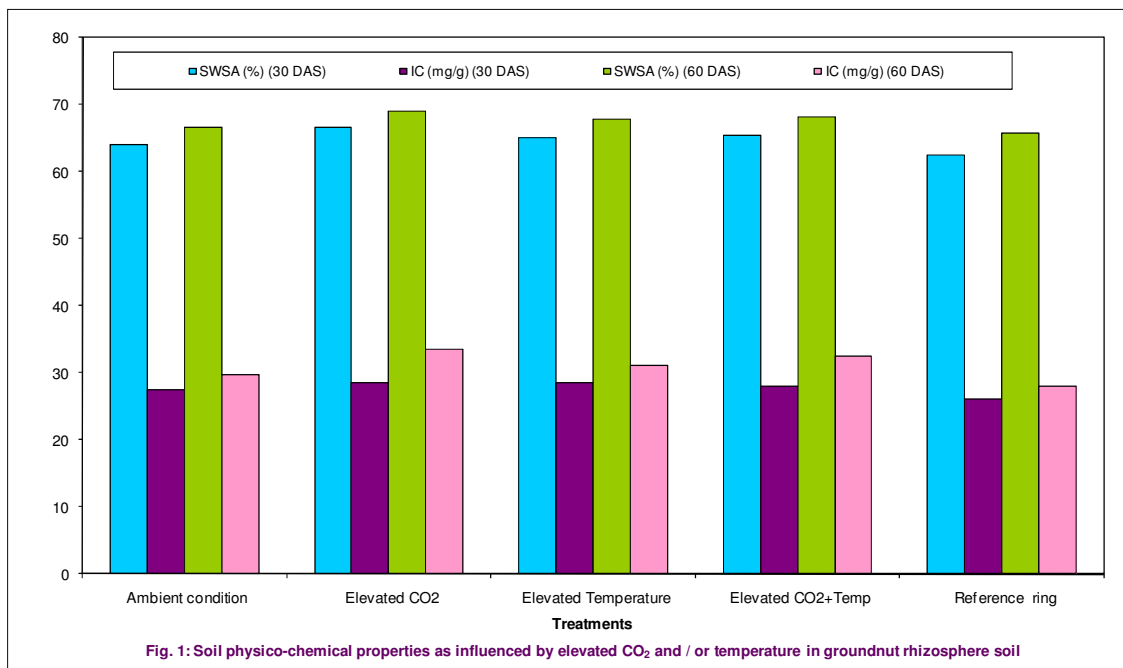
4.2.5 Soil inorganic carbon

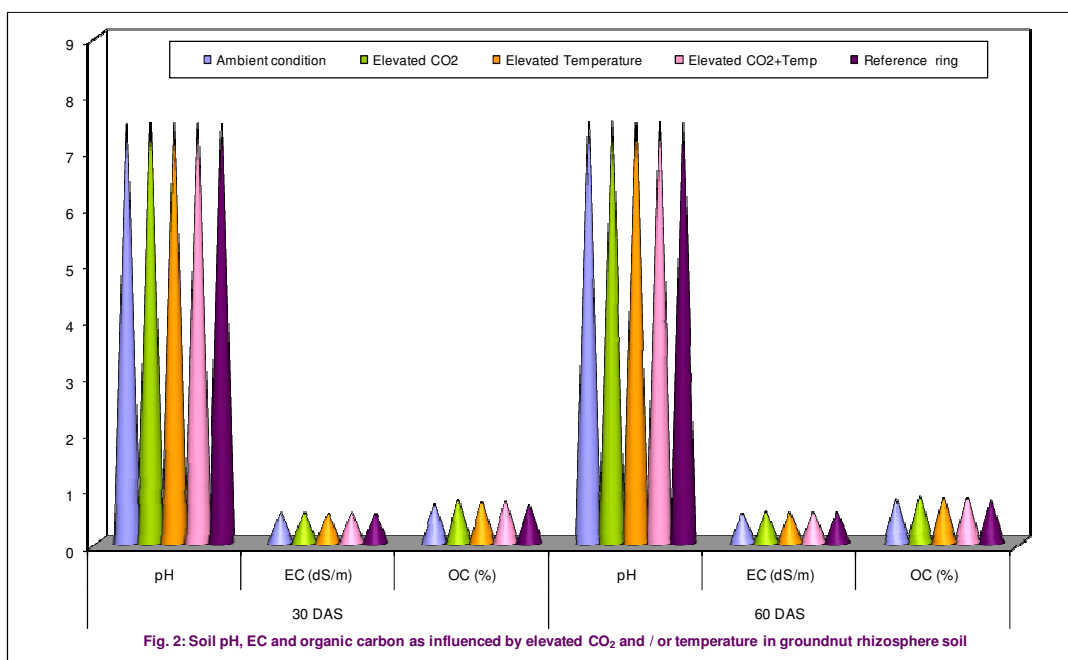
Inorganic carbon content in soil under five altered environmental conditions at 30 DAS and 60 DAS are depicted in Table 3. The highest soil inorganic carbon content was recorded with elevated temperature (28.48 mg g⁻¹) followed by elevated CO₂ (28.38 mg g⁻¹) and elevated CO₂ plus temperature (28.00 mg g⁻¹). However, these results were on par with each other. The lowest inorganic carbon content at 30 DAS was recorded in the reference plot (26.05 mg g⁻¹). At 60 DAS,

Table 3: Soil physico-chemical properties as influenced by elevated CO₂ and / or temperature in groundnut rhizosphere soil

Treatment	30 DAS					60 DAS				
	SWSA (%)	pH	EC (dS/m)	OC (%)	IC (mg/g)	SWSA (%)	pH	EC (dS/m)	OC (%)	IC (mg/g)
Ambient condition	63.94	7.45	0.55	0.70	27.33	66.59	7.49	0.54	0.78	29.69
Elevated CO ₂	66.48	7.47	0.55	0.78	28.38	69.01	7.50	0.57	0.84	33.48
Elevated Temperature	64.97	7.47	0.54	0.73	28.48	67.74	7.48	0.56	0.82	31.03
Elevated CO ₂ + Temperature	65.37	7.47	0.55	0.75	28.00	68.08	7.49	0.56	0.82	32.49
Reference ring	62.33	7.45	0.54	0.68	26.05	65.62	7.47	0.56	0.76	27.90
S.Em±	0.54	0.01	0.01	0.01	0.33	0.51	0.01	0.01	0.01	0.59
CD (0.05)	1.62	0.03	0.03	0.03	1.00	1.53	0.03	0.02	0.04	1.78

Note: **SWSA** – Soil water stable aggregate, **EC**- Electrical conductivity, **OC**-Organic carbon, **IC**- Inorganic carbon





there was a significant difference in inorganic carbon content under the influence of elevated CO₂, temperature and elevated CO₂ plus temperature (33.48, 31.03 and 32.49 mg g⁻¹ respectively) compared to ambient and reference plot.

4.2.6 Available nitrogen

The observation on available nitrogen as influenced by altered environmental conditions was recorded at 30 and 60 DAS. The data are presented in Table 4. The highest available nitrogen was observed in elevated CO₂ condition at 30 and 60 DAS respectively (253.00 and 265.75 kg ha⁻¹) compared to rest of the conditions. The lowest available nitrogen content was observed in the reference plot at 30 and 60 DAS (250.50 and 260.00 kg ha⁻¹ respectively). However, at 60 DAS the results were statistically on par with each other across the altered environmental conditions.

4.2.7 Available phosphorus

The observations on available phosphorus as influenced by altered environmental conditions were recorded at 30 and 60 DAS (Table 4). At 30 DAS the highest available phosphorus in soil was recorded with elevated CO₂ (37.20 kg ha⁻¹) followed by elevated CO₂ plus temperature (36.82 kg ha⁻¹). However, the altered conditions at 30 DAS did not significantly influence the available P. At 60 DAS, the soil exposed to elevated CO₂ exhibited the highest available phosphorus (46.25 kg ha⁻¹) as compared to rest of the altered conditions. The lowest available phosphorus (41.75 kg ha⁻¹) was observed in the open reference plot.

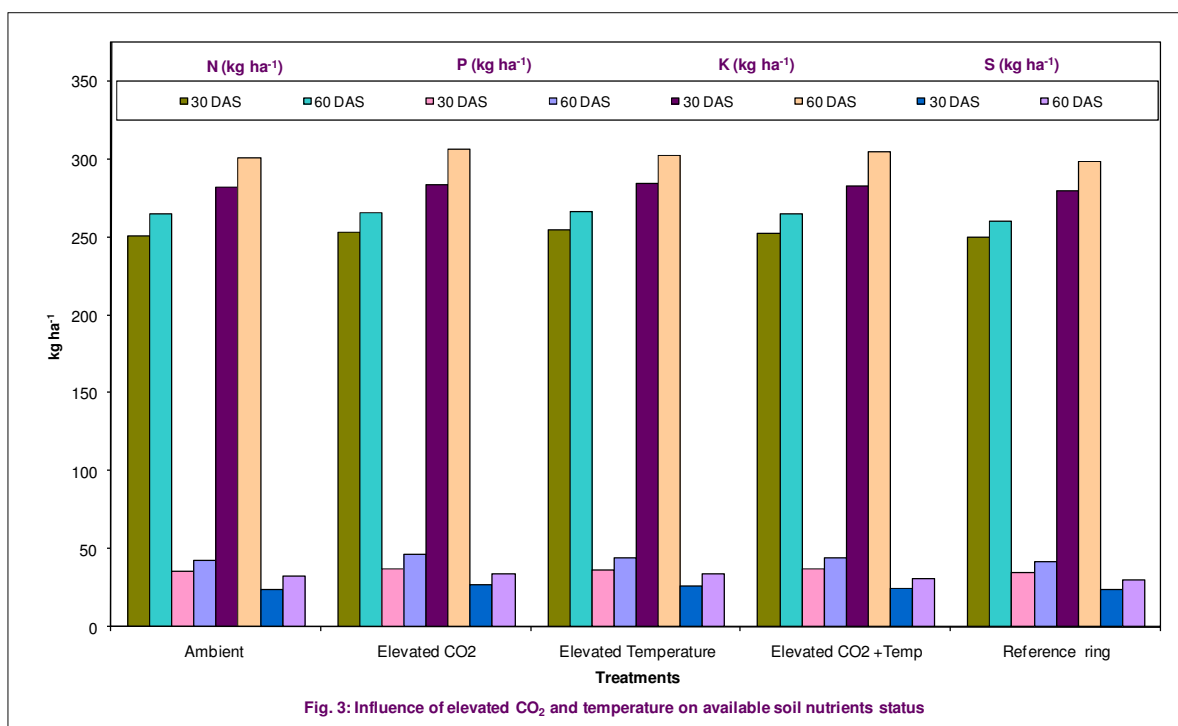
4.2.8 Available potassium

The available potassium (K) was estimated from the soil samples at 30 DAS. The highest amount of available potassium was recorded under elevated temperature followed by elevated CO₂ and were on par with each other (284.50 and 284.00 kg ha⁻¹ respectively). Unlike on 30 DAS, the highest amount of available potassium (306.50 kg ha⁻¹) was recorded in soil under elevated CO₂ condition, which was statistically on par with soil sample exposed to elevated CO₂ and temperature (304.83 kg ha⁻¹) at 60 DAS. The lowest amount of available potassium (279.75 kg ha⁻¹ and 299.00 kg ha⁻¹) respectively at 30 and 60 DAS was recorded in the reference plot (Table 4).

Table 4: Available soil nutrients status as influenced by elevated CO₂ and/ or temperature in groundnut rhizosphere soil

Treatments	N (kg ha ⁻¹)		P(kg ha ⁻¹)		K (kg ha ⁻¹)		S (kg ha ⁻¹)	
	30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS
Ambient	251.25	265.25	35.60	42.75	282.00	300.75	24.25	32.50
Elevated CO ₂	253.00	265.75	37.20	46.25	284.00	306.50	26.63	34.00
Elevated Temperature	254.50	266.75	36.50	44.50	284.50	302.50	26.48	33.75
Elevated CO ₂ + Temperature	252.25	265.00	36.82	44.25	283.25	304.83	25.05	31.18
Reference ring	250.50	260.00	34.65	41.75	279.75	299.00	23.92	30.50
S.Em±	1.03	1.23	0.65	0.73	0.94	1.56	0.41	0.60
CD (0.05)	3.10	3.70	1.95	2.20	2.82	4.69	1.23	1.80

Note: N- Nitrogen, P-Phosphorus, K-potassium, S- Sulphur



4.2.9 Available sulphur

The results recorded on available sulphur (S) as influenced by altered conditions at 30 and 60 DAS are depicted in Table 4. At 30 DAS the available S was increased under elevated CO₂ condition (26.63 kg ha⁻¹) followed by elevated temperature (26.48 kg ha⁻¹) at 30 DAS. At 60 DAS, also the highest amount of available sulphur (34.00 kg ha⁻¹) was observed under elevated CO₂ condition. The lowest sulphur (23.92 kg ha⁻¹ and 30.50 kg ha⁻¹) was recorded under reference plot respectively at 30 as well as 60 DAS.

4.3 Soil biological properties as influenced by elevated CO₂ and/ or temperature in groundnut rhizosphere soil

4.3.1.1 Population of bacteria as influenced by elevated CO₂ and/ or temperature in groundnut rhizosphere soil

The total bacterial population were enumerated and the data are presented in Table 5. The highest bacterial population was observed under elevated CO₂ at both 30 and 60 DAS (61 x 10⁵ and 65 x 10⁵ CFU g⁻¹ dry soil respectively) as compared to elevated CO₂ plus temperature followed by elevated temperature alone and ambient condition. The lowest bacterial population was enumerated in the open reference ring (33 x 10⁵ and 40 x 10⁵ CFU g⁻¹ of dry soil).

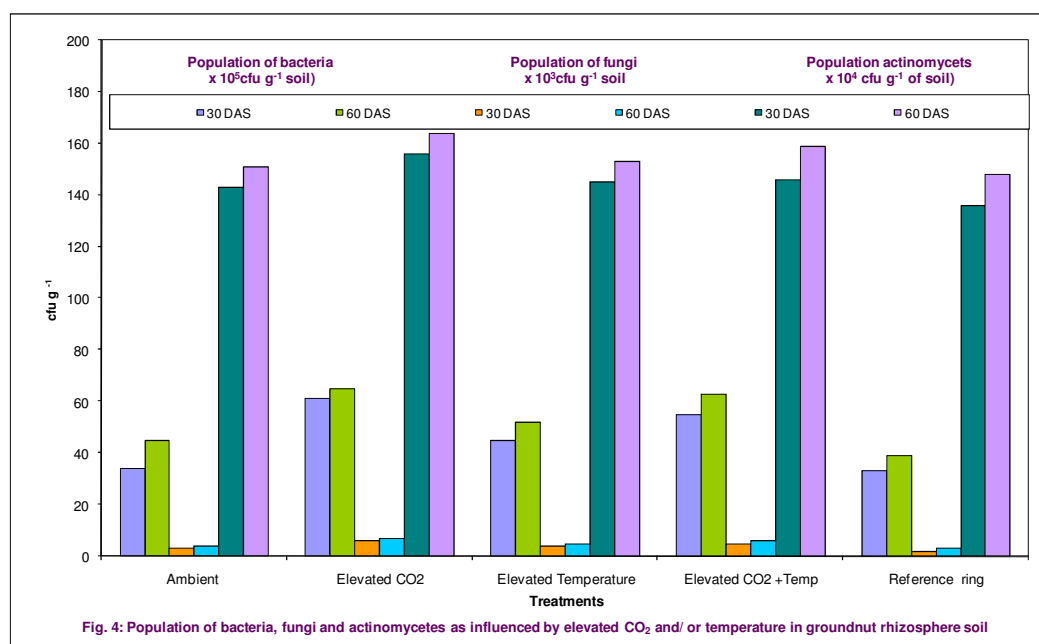
4.3.1.2 Population of fungi as influenced by elevated CO₂ and/ or temperature in groundnut rhizosphere soil

The fungal population were enumerated and the data are presented in Table 5. Significant differences in the fungal populations were recorded in the groundnut rhizosphere soil exposed to varied altered conditions at 30 and 60 DAS. The highest fungal population was observed under elevated CO₂ condition at 30 and 60 DAS (6 x 10³ and 7 x 10³ CFU g⁻¹ soil respectively) as compared to the soil exposed to elevated CO₂ plus temperature followed by elevated temperature. The lowest fungal populations (2 x 10³ and 3 x 10³ CFU g⁻¹ soil) were noticed in reference plot during the crop growing period at 30 and 60 DAS respectively.

4.3.1.3 Population of actinomycetes as influenced by elevated CO₂ and/ or temperature in groundnut rhizosphere soil

Table 5: Population of bacteria, fungi and actinomycetes as influenced by elevated CO₂ and/ or temperature in groundnut rhizosphere soil

Treatments	Population of bacteria x 10 ⁵ CFU g ⁻¹ soil)		Population of fungi x 10 ³ CFU g ⁻¹ soil		Population actinomycetes x 10 ⁴ CFU g ⁻¹ of soil)	
	30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS
Ambient	34	45	3	4	143	151
Elevated CO ₂	61	65	6	7	156	164
Elevated Temperature	45	52	4	5	145	153
Elevated CO ₂ + Temperature	55	63	5	6	146	159
Reference ring	33	39	2	3	136	148
S.Em±	0.48	0.52	0.47	0.60	0.57	0.50
CD(0.05)	1.44	1.57	1.40	1.87	1.73	1.51



The actinomycetes population were enumerated and the data are presented in Table 5. Significant differences existed between altered environmental conditions with respect to actinomycetes population both at 30 and 60 DAS. Among the five conditions, the highest actinomycetes population was observed in the treatment exposed to elevated CO₂ condition at 30 as well as 60 DAS (156×10^4 and 164×10^4 CFU g⁻¹ soil respectively) compared to the rest of the altered conditions. However, the lowest actinomycetes population was recorded at 30 and 60 DAS in the reference plot (136×10^4 and 148×10^4 CFU g⁻¹ soil respectively).

4.3.1.4 Population of free living nitrogen fixers as influenced by elevated CO₂ and/ or temperature in groundnut rhizosphere soil

The free living nitrogen fixers populations were enumerated and the data are presented in Table 6. Significant differences in the population of free living nitrogen fixers was observed at 30 and 60 DAS between the treatments. The rhizosphere soil exposed to elevated CO₂ harbored the highest free living nitrogen fixers at 30 and 60 DAS (68×10^4 and 76×10^4 CFU g⁻¹ soil respectively), followed by the treatment exposed to elevated CO₂ plus temperature (66 and 74 CFU g⁻¹ soil respectively), The lowest population of free living nitrogen fixers (55×10^4 and 64×10^4 CFU g⁻¹ of soil) were noticed in the reference plot, which was significantly lower than ambient condition (60×10^4 and 71×10^4 CFU g⁻¹ soil respectively).

4.3.1.5 Population of mineral phosphate solubilizers (MPS) as influenced by elevated CO₂ and/ or temperature in groundnut rhizosphere soil

The population of MPS was enumerated on the Pikovskaya's medium. The altered conditions had a positive influence on population of MPS (Table 7). The highest population of P- solubilizers were recorded in the treatment exposed to elevated CO₂, followed by elevated CO₂ plus temperature and elevated temperature. However, they were statistically on par with each other. (66.50×10^3 and 69.25×10^3 ; 62.25×10^3 and 66.75×10^3 ; 60.75×10^3 and 64×10^3 CFU g⁻¹ respectively at both 30 and 60 DAS).

Table 6: Population of free living N₂ fixing bacteria as influenced by elevated CO₂ and/ or temperature in groundnut rhizosphere soil

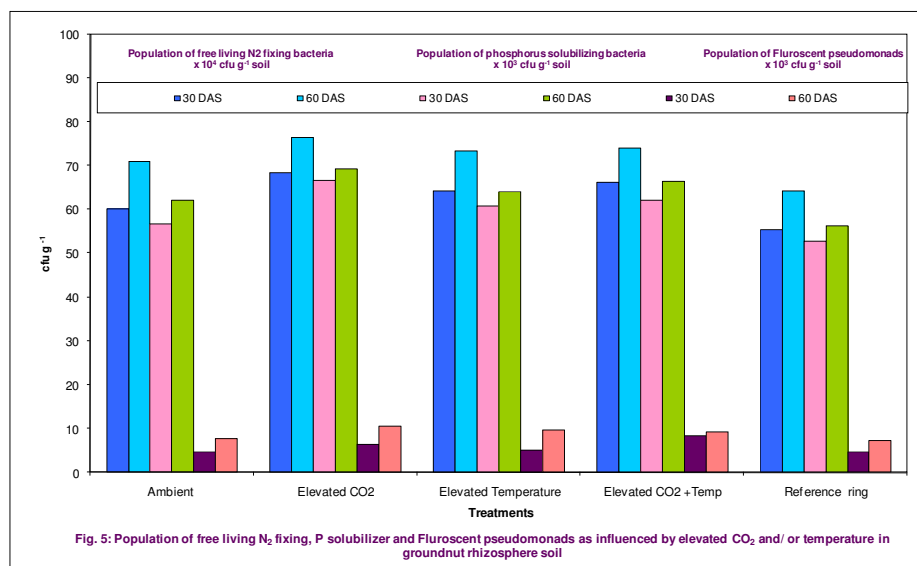
Treatments	Population of free living N ₂ fixing bacteria x 10 ⁴ CFU g ⁻¹ soil	
	30 DAS	60 DAS
Ambient	60.23	71.00
Elevated CO ₂	68.50	76.50
Elevated Temperature	64.25	73.50
Elevated CO ₂ + Temperature	66.25	74.00
Reference ring	55.50	64.25
S.Em±	1.45	1.48
CD(0.05)	4.44	4.46

Table 7: Population of phosphorus solubilizing bacteria as influenced by elevated CO₂ and/ or temperature in groundnut rhizosphere soil

Treatments	Population of phosphorus solubilizing bacteria x 10 ³ CFU g ⁻¹ soil	
	30 DAS	60 DAS
Ambient	56.75	62.25
Elevated CO ₂	66.75	69.25
Elevated Temperature	60.75	64.00
Elevated CO ₂ + Temperature	62.25	66.50
Reference ring	52.75	56.25
S.Em±	1.34	1.50
CD(0.05)	4.03	4.53

Table 8: Population of Fluroscent pseudomonads as influenced by elevated CO₂ and/ or temperature in groundnut rhizosphere soil

Treatments	Population of Fluroscent pseudomonads x 10 ³ CFU g ⁻¹ soil	
	30 DAS	60 DAS
Ambient	4.63	7.75
Elevated CO ₂	6.33	10.50
Elevated Temperature	5.18	9.75
Elevated CO ₂ + Temperature	8.35	9.25
Reference ring	4.75	7.25
S.Em±	0.68	1.05
CD (0.05)	2.04	3.16



4.3.1.6 Population of Fluroscent pseudomonads as influenced by elevated CO₂ and/ or temperature in groundnut rhizosphere soil

The populations of Fluroscent pseudomonads in the groundnut rhizosphere soil were enumerated and the results are depicted in Table 8.

The population of Fluroscent pseudomonads on 30 DAS did not differ significantly under the influence of altered conditions. At 60 DAS, the soil exposed to elevated CO₂ alone harbored the highest Fluroscent pseudomonads population (10.50×10^3 CFU g⁻¹soil) followed by the treatment which received elevated temperature (9.75×10^3 CFU g⁻¹), which was statistically on par with each other. The lowest population of Fluroscent pseudomonads population was observed in the reference plot at 30 and 60 DAS (4.75×10^3 and 7.25×10^3 CFU g⁻¹ soil respectively).

4.3.1.7 Soil protozoa number as influenced by elevated CO₂ and/ or temperature in groundnut rhizosphere soil

Protozoa in the soil samples exposed to altered conditions were enumerated by following MPN method and the data are depicted in Table 9.

The highest protozoan population (5.3 and 8.25number g⁻¹ soil) was observed under elevated CO₂ condition at 30 DAS and 60 DAS crop growing period. The lowest protozoan population (3.2 and 4.9) was recorded in the reference plot compared to other altered conditions.

4.3.1.8 Qualitative estimation of algae as influenced by elevated CO₂ and/ or temperature in groundnut rhizosphere soil

During the present investigation, the visual qualitative observation of algae under altered conditions at 30 and 60 DAS revealed that *Anabaena*, *Chlorococcus*, *Diatoms* were found to be maximum, followed by *Lyngbia*, *Oscillatoria*. However, *Achnanthidium* and *Nitzschia* recorded the lowest at 30 DAS and 60 DAS.

Table 9: Protozoan number as influenced by elevated CO₂ and/ or temperature in groundnut rhizosphere soil

Treatments	Protozoa (Number g ⁻¹ soil)	
	30 DAS	60 DAS
Ambient	3.57	4.59
Elevated CO ₂	5.25	8.25
Elevated Temperature	5.19	5.08
Elevated CO ₂ + Temperature	5.30	6.33
Reference ring	3.20	4.90
SEm±	0.05	0.68
CD (0.05)	0.16	2.06

Table 10: Qualitative estimation of algae as influenced by elevated CO₂ and/ or temperature in groundnut rhizosphere soil at 30 DAS

Sl. No.	Algae	Ambient	Elevated CO ₂	Elevated Temperature	Elevated CO ₂ + Temperature	Referencing
1	<i>Achnantheium</i>	+	+	+	+	+
2	<i>Anabaena</i>	+++	+++	+++	+++	+++
3	<i>Chlorococcus</i>	+++	+++	+++	+++	+++
4	<i>Diatoms</i>	+++	+++	+++	+++	+++
5	<i>Gleocapsa</i>	++	++	++	++	++
6	<i>Lyngbia</i>	++	++	++	++	++
7	<i>Nitzschia</i>	+	+	+	+	+
8	<i>Oscillatoria</i>	++	++	++	++	++

Note: Low: +; Medium: ++; High: +++

Table10 (a): Qualitative estimation of algae as influenced by elevated CO₂ and/ or temperature in groundnut rhizosphere soil at 60 DAS

Sl. No .	Algae	Ambient	Elevated CO ₂	Elevated Temperature	Elevated CO ₂ + Temperature	Referenc e ring
1	<i>Achnanthidium</i>	+	+	+	+	+
2	<i>Anabaena</i>	+++	+++	+++	+++	+++
3	<i>Chlorococcus</i>	+++	+++	+++	+++	+++
4	<i>Diatoms</i>	+++	+++	+++	+++	+++
5	<i>Gleocapsa</i>	++	++	++	++	++
6	<i>Lyngbia</i>	++	++	++	++	++
7	<i>Nitzschia</i>	+	+	+	+	+
8	<i>Oscillatoria</i>	++	++	++	++	++
9	<i>Scendesmus</i>	+	+	+	+	+

Note: Low: + ; Medium: ++ ; High: +++

4.3.2 Soil enzyme activities as influenced by elevated CO₂ and/ or temperature in groundnut rhizosphere soil

4.3.2.1 Dehydrogenase activity as influenced by elevated CO₂ and/ or temperature in groundnut rhizosphere soil

In general, the soil exposed to the environmental conditions was found to be more with influenced respect to dehydrogenase activity as compared to the reference plot and ambient conditions (Table 11).

The highest dehydrogenase activity was recorded in the treatment which received elevated CO₂ plus temperature (3.19 µg TPF formed g⁻¹ soil d⁻¹) followed by elevated CO₂ (3.15 µg TPF formed g⁻¹ soil d⁻¹) conditions alone at 30 DAS. The lowest dehydrogenase activity was observed in the reference plot (2.73 µg TPF formed g⁻¹ soil d⁻¹) and ambient condition (2.79 µg TPF formed g⁻¹ soil d⁻¹). Similar results were also recorded at 60 DAS.

4.3.2.2 Acid phosphatase activity as influenced by elevated CO₂ and/ or temperature in groundnut rhizosphere soil

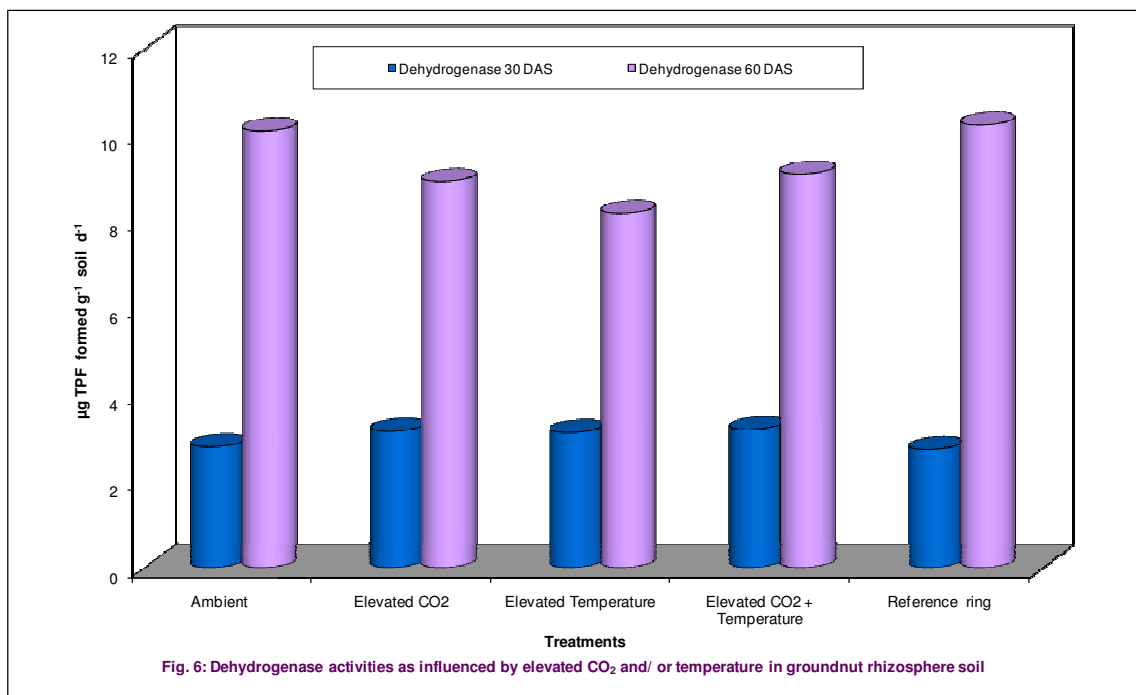
The influence of elevated CO₂ and/ or temperature on acid phosphatase activity in the groundnut rhizosphere soil sample was analyzed at 30 and 60 DAS and the values are furnished in Table 11.

At 30 DAS, the groundnut rhizosphere soil exposed to elevated CO₂ recorded significantly the highest acid phosphatase activity under the influence of elevated CO₂ alone (16.12 µg pnp released g⁻¹ soil h⁻¹), followed by CO₂ plus temperature (15.85 µg pnp released g⁻¹ soil h⁻¹) and temperature alone (14.29 µg pnp released g⁻¹ soil h⁻¹). The lowest acid phosphatase activity (12.95 µg pnp released g⁻¹ soil h⁻¹) was observed in the reference plot, which was at par with ambient condition (13.89 µg pnp released g⁻¹ soil h⁻¹).

At 60 DAS, maximum acid phosphatase was measured in the groundnut rhizosphere soil exposed to elevated CO₂ (20.57 µg pnp released g⁻¹ soil h⁻¹). This was followed by soil exposed to elevated CO₂ plus temperature (18.70 µg pnp released g⁻¹ soil h⁻¹) and the elevated temperature alone (18.65 µg pnp released g⁻¹ soil h⁻¹).

Table 11: Dehydrogenase and phosphatase activities as influenced by elevated CO₂ and/or temperature in groundnut rhizosphere soil

Treatments	Dehydrogenase activity ($\mu\text{g TPF formed g}^{-1} \text{ soil d}^{-1}$)		Phosphatase activity ($\mu\text{g pnp released g}^{-1} \text{ soil h}^{-1}$)			
			Acid phosphatase		Alkaline phosphatase	
	30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS
Ambient	2.79	10.10	13.89	17.63	30.40	43.53
Elevated CO ₂	3.15	8.92	16.12	20.57	34.33	52.68
Elevated Temperature	3.12	8.19	14.29	18.65	31.92	48.66
Elevated CO ₂ + Temperature	3.19	9.1	15.85	18.7	32.59	48.66
Reference ring	2.73	10.23	12.95	16.96	29.69	39.96
SEm$\pm$	0.07	0.18	0.36	0.47	0.75	0.76
CD(0.05)	0.20	0.54	1.09	1.43	2.27	2.29



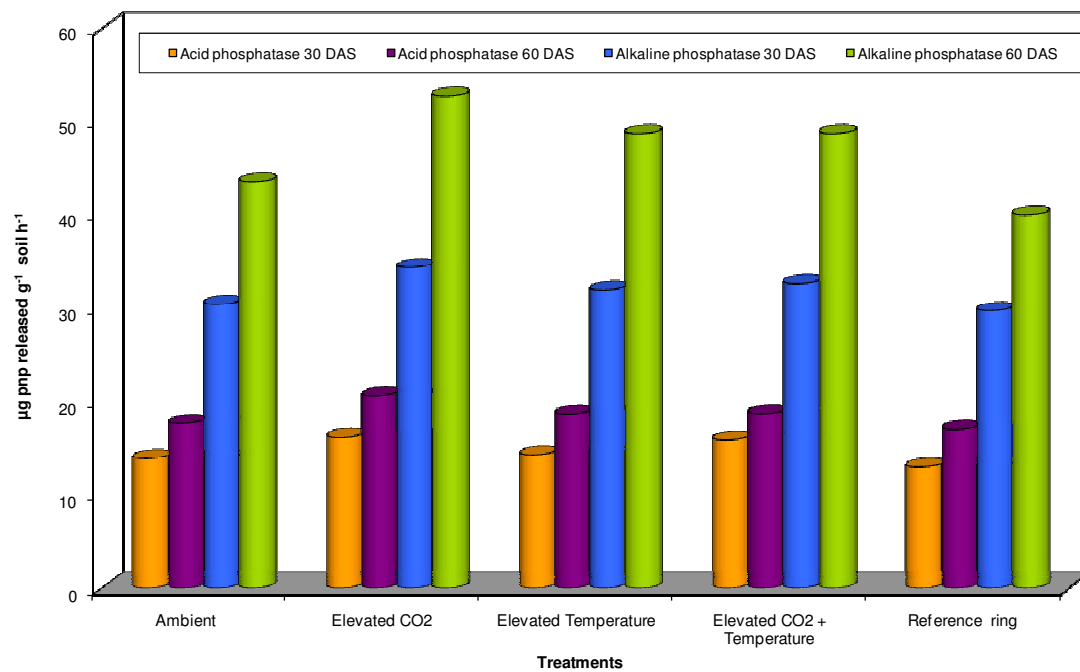


Fig. 7: Phosphatase activities as influenced by elevated CO₂ and/ or temperature in groundnut rhizosphere soil

$^1 \text{ soil h}^{-1}$). The lowest activity was measured in the soil under open reference plot ($16.96 \mu\text{g pnp released g}^{-1} \text{ soil h}^{-1}$).

4.3.2.3 Alkaline phosphatase activity as influenced by elevated CO_2 and/ or temperature in groundnut rhizosphere soil

The impact of elevated CO_2 and/or temperature on alkaline phosphatase activity in groundnut rhizosphere soil was analyzed and the results are presented in Table 11. Significant differences were observed due to altered conditions at 30 and 60 DAS. The alkaline phosphatase activity in the rhizosphere soil ranged from 29.69 to $34.33 \mu\text{g pnp released g}^{-1} \text{ soil h}^{-1}$ at 30 DAS, while it ranged from 39.96 to $52.68 \mu\text{g pnp released g}^{-1} \text{ soil h}^{-1}$ at 60 DAS. The highest alkaline phosphatase activity at 30 DAS was observed under elevated CO_2 condition ($34.33 \mu\text{g pnp released g}^{-1} \text{ soil h}^{-1}$) followed by elevated CO_2 plus temperature ($32.59 \mu\text{g pnp released g}^{-1} \text{ soil h}^{-1}$) and elevated temperature ($31.92 \mu\text{g pnp released g}^{-1} \text{ soil h}^{-1}$). The lowest alkaline phosphatase activity ($29.69 \mu\text{g pnp released g}^{-1} \text{ soil h}^{-1}$) was observed in the reference plot.

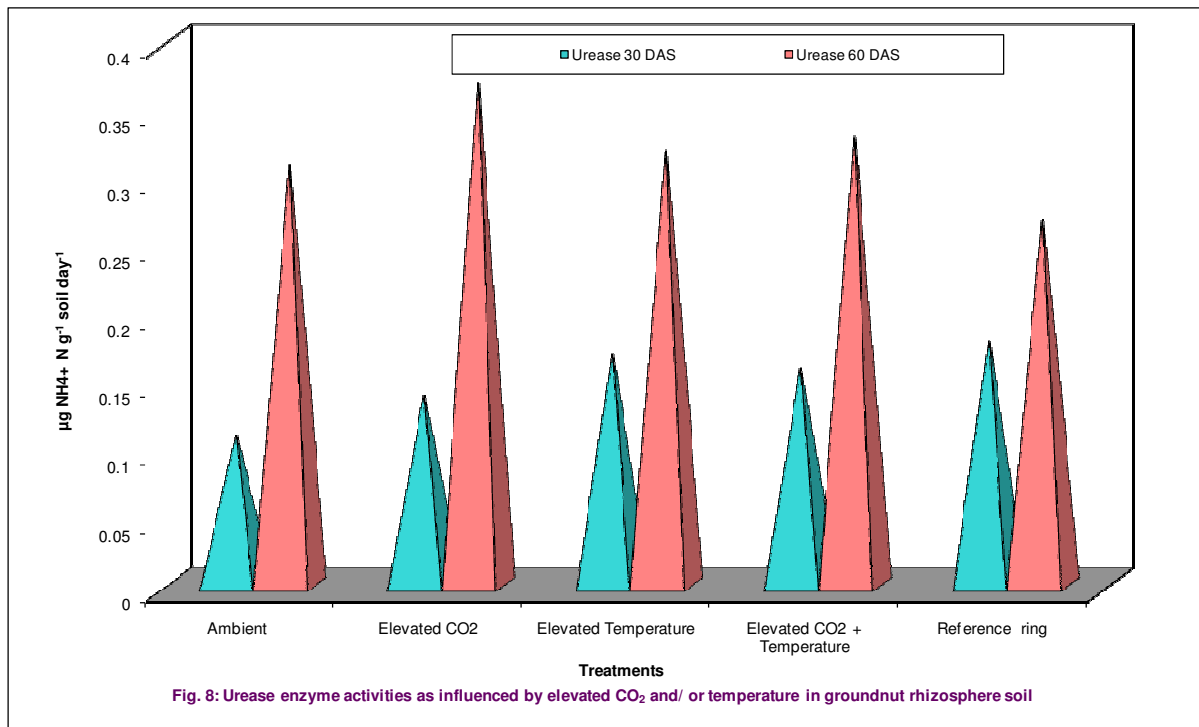
At 60 DAS, the alkaline phosphatase content ranged from 39.96 to $52.68 \mu\text{g pnp released g}^{-1} \text{ soil h}^{-1}$. The maximum activity was recorded in elevated CO_2 ($52.68 \mu\text{g pnp released g}^{-1} \text{ soil h}^{-1}$) followed by elevated CO_2 plus temperature and elevated temperature alone ($48.66 \mu\text{g pnp released g}^{-1} \text{ soil h}^{-1}$). The lowest phosphatase activity was recorded in the reference plot ($39.66 \mu\text{g pnp released g}^{-1} \text{ soil h}^{-1}$).

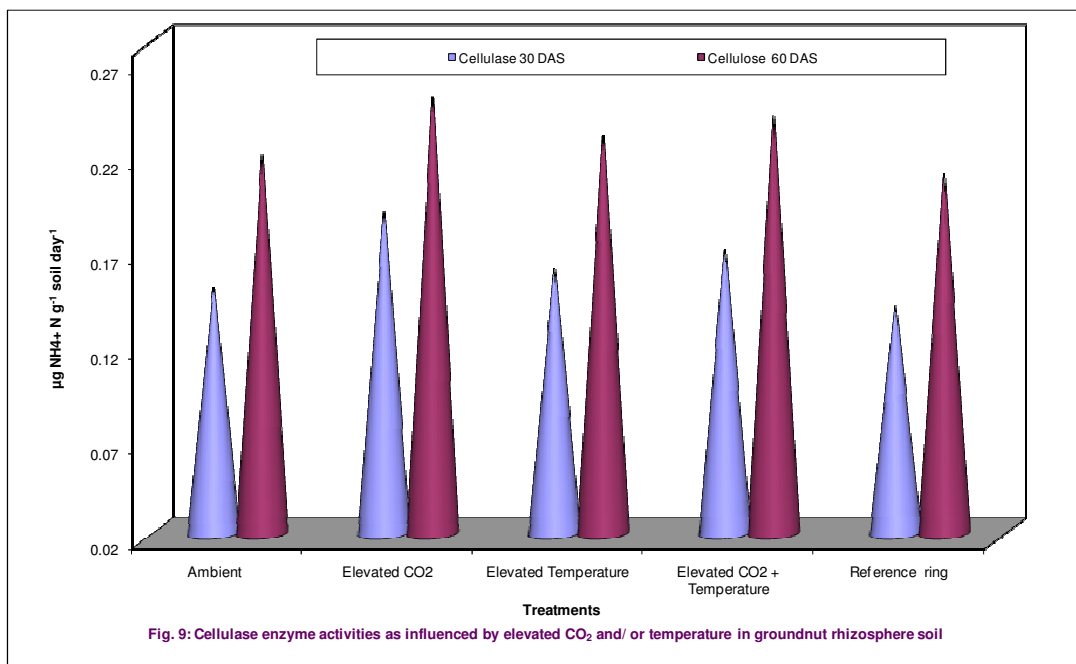
4.3.2.4 Urease activity as influenced by elevated CO_2 and/ or temperature in groundnut rhizosphere soil

The influence of elevated CO_2 and temperature on urease activity in the rhizosphere soil sample was analyzed and the results are furnished in Table 12. At 30 DAS, all the five conditions were statistically on par with each other in influencing the urease activity. However at 60 DAS, significantly higher urease activity was observed under elevated CO_2 condition ($0.37 \mu\text{g NH}_4^+ \text{N g}^{-1} \text{ soil day}^{-1}$) followed by interaction of elevated CO_2 plus temperature condition ($0.33 \mu\text{g NH}_4^+ \text{N g}^{-1} \text{ soil day}^{-1}$) and elevated temperature alone ($0.32 \mu\text{g NH}_4^+ \text{N g}^{-1} \text{ soil day}^{-1}$), which

Table 12: Urease and cellulase enzyme activities as influenced by elevated CO₂ and/or temperature in groundnut rhizosphere soil

Treatment	Urease activity ($\mu\text{g NH}_4^+ \text{N g}^{-1} \text{soil day}^{-1}$)		Cellulase activity (μg reducing sugar released $\text{g}^{-1} \text{soil day}^{-1}$)	
	30 DAS	60 DAS	30 DAS	60 DAS
Ambient	0.11	0.31	0.15	0.22
Elevated CO ₂	0.14	0.37	0.19	0.25
Elevated Temperature	0.17	0.32	0.16	0.23
Elevated CO ₂ + Temperature	0.16	0.33	0.17	0.24
Reference ring	0.18	0.27	0.14	0.21
SEm$\pm$	0.01	0.01	0.01	0.01
CD(0.05)	0.04	0.03	0.02	0.02





was at par with values recorded under ambient conditions ($0.31 \mu\text{g NH}_4^+ \text{N g}^{-1} \text{ soil day}^{-1}$). The lowest soil urease activity was recorded in the reference plot ($0.27 \mu\text{g NH}_4^+ \text{N g}^{-1} \text{ soil day}^{-1}$).

4.3.2.5 Cellulase activity as influenced by elevated CO_2 and/ or temperature in groundnut rhizosphere soil

The influence of elevated CO_2 and temperature on cellulase activity in soil samples was analyzed and depicted in Table 12. The cellulase activity at 30 DAS ranged between 0.14 to 0.18 mg of glucose released $\text{g}^{-1} \text{ soil day}^{-1}$. The highest cellulase activity was observed under the influence of elevated CO_2 (0.19 mg of glucose released $\text{g}^{-1} \text{ soil day}^{-1}$) as compared to elevated CO_2 plus temperature (0.17 mg of glucose released $\text{g}^{-1} \text{ soil day}^{-1}$) while treatment with increased temperature alone recorded cellulase activity of 0.16 mg of glucose released $\text{g}^{-1} \text{ soil day}^{-1}$. The lowest cellulase activity was recorded with reference plot (0.14 mg of glucose released $\text{g}^{-1} \text{ soil day}^{-1}$) which was at par with those observed with ambient condition (0.15 mg of glucose released $\text{g}^{-1} \text{ soil day}^{-1}$).

At 60 DAS, an increased cellulase activity was recorded in the groundnut rhizosphere soil compared to 30 DAS. It ranged from 0.21 to 0.25 mg of glucose released $\text{g}^{-1} \text{ soil day}^{-1}$ at 60 DAS. While the values varied from 0.14 to 0.17 mg glucose released $\text{g}^{-1} \text{ soil day}^{-1}$. The highest cellulase activity was observed under elevated CO_2 condition (0.25 mg of glucose released $\text{g}^{-1} \text{ soil day}^{-1}$). This was followed by treatment with elevated CO_2 plus temperature conditions, which was on par with the treatment received elevated temperature alone (0.23 mg of glucose released $\text{g}^{-1} \text{ soil day}^{-1}$). The lowest cellulase activity of 0.22 mg of glucose released $\text{g}^{-1} \text{ soil day}^{-1}$ in soil rhizosphere soil was measured under ambient conditions followed by the reference plot (0.21 mg of glucose released $\text{g}^{-1} \text{ soil day}^{-1}$).

4.3.3.1 Soil microbial biomass carbon (MBC) as influenced by elevated CO_2 and/ or temperature in groundnut rhizosphere soil

The measure of microbial biomass turn over provides an useful information on soil response to atmospheric CO_2 enrichment and was estimated by following fumigation extraction method and the results are furnished in Table 13. The highest values for microbial biomass carbon were recorded at 30 DAS in the

treatment which received elevated CO₂ (272.8 µg g⁻¹ soil) followed by that with elevated temperature treatment (262.5 µg g⁻¹ soil) which was statistically at par with the soil exposed to elevated CO₂ and temperature (259.5 µg g⁻¹ soil). In general, MBC recorded in the ambient (225.5 µg g⁻¹ soil) and reference plot (211.8 µg g⁻¹ soil) always were lower as compared to the other altered conditions.

Similar trend was observed at 60 DAS with respect to the MBC. The highest MBC was observed in elevated CO₂ (353.5 µg g⁻¹ soil), whereas, unlike on 30 DAS, the second highest MBC was recorded with the treatment which received combinations of elevated CO₂ and temperature (345.75 µg g⁻¹ soil). The lowest MBC (326 µg g⁻¹ soil) was observed in the reference plot which was on par with the values recorded under ambient condition (336 µg g⁻¹ soil).

4.3.3.2 Soil microbial biomass nitrogen (MBN) as influenced by elevated CO₂ and/or temperature in groundnut rhizosphere soil

The soil microbial biomass nitrogen also responded to altered conditions similar to MBC (Table 13). There were significant differences observed due to the altered conditions in influencing the MBN at 30 DAS. The highest microbial biomass nitrogen was recorded in the treatment with elevated CO₂ (31 µg g⁻¹ soil) which was significantly superior to elevated temperature and interactive effect of CO₂ plus temperature (30.75 µg/g soil and 29.25 respectively). The lowest soil microbial biomass nitrogen was observed in the reference plot, which was significantly lower to the ambient environment condition (23 and 26.50 µg/g soil respectively).

At 60 DAS, the treatment with elevated CO₂ alone exhibited significantly influenced the MBN over elevated temperature (44.42 and 41.45 µg g⁻¹ soil respectively). However, the results obtained with elevated CO₂ and the combination of CO₂ plus temperature was found to be at par with each other. The MBN was significantly higher in the rhizosphere soil under ambient condition (37.65 µg g⁻¹ soil) soil compared to the soil under reference ring (35.45 µg g⁻¹ soil).

Table 13: Soil microbial biomass carbon and nitrogen as influenced by elevated CO₂ and/ or temperature in groundnut rhizosphere soil

Treatment	Biomass carbon (µg/g soil)		Biomass nitrogen (µg/g soil)	
	30 DAS	60 DAS	30 DAS	60 DAS
Ambient	225.50	336.00	26.50	37.65
Elevated CO ₂	272.80	353.50	31.00	44.42
Elevated Temperature	262.50	344.75	30.75	41.45
Elevated CO ₂ + Temperature	259.55	345.75	29.25	42.52
Reference ring	211.83	326.00	23.00	35.45
SEm±	1.40	1.04	0.44	0.72
CD(0.05)	4.21	3.13	1.36	2.17

Table 14: Mycorrhizal spore count as influenced by elevated CO₂ and/ or temperature in groundnut rhizosphere soil

Treatments	Mycorrhizal spore count (Number of spore 50 g ⁻¹)	
	30 DAS	60 DAS
Ambient	221.50	324.75
Elevated CO ₂	227.00	336.50
Elevated Temperature	223.25	328.75
Elevated CO ₂ + Temperature	224.75	330.00
Reference ring	219.50	318.75
S.E.m±	1.29	2.09
CD (0.05)	3.88	6.31

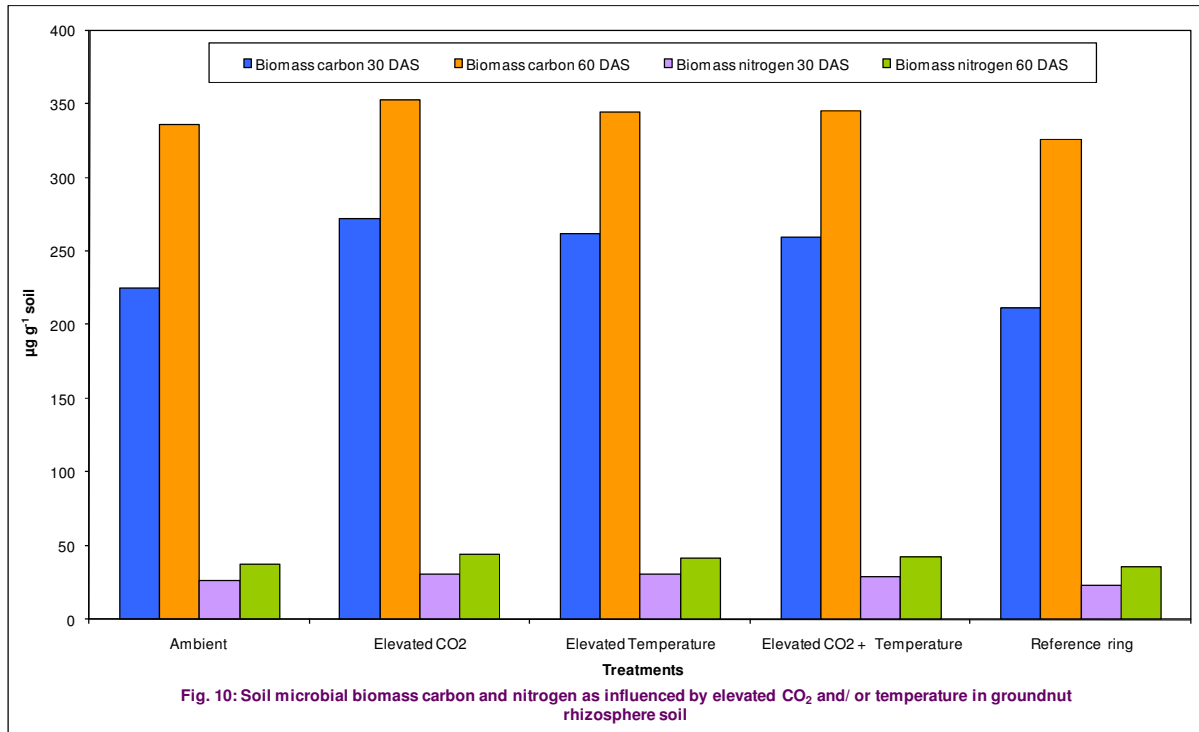


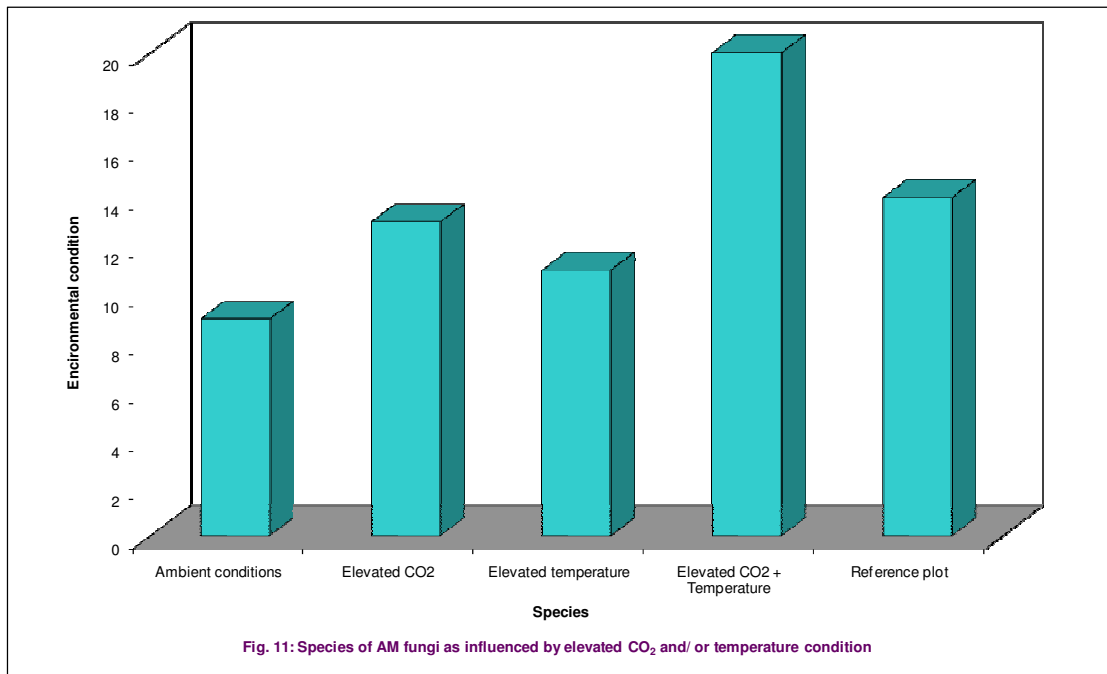
Table 15: Mycorrhizal root colonization as influenced by elevated CO₂ and/ or temperature in groundnut rhizosphere soil

Treatments	Percent root colonization
Ambient	50.50
Elevated CO ₂	55.75
Elevated Temperature	51.50
Elevated CO ₂ + Temperature	54.00
Reference ring	47.00
SEm±	0.50
CD (0.05)	1.49

Table 16: Species of AM fungi as influenced by elevated CO₂ and/ or temperature condition

SI No	Ambient conditions	Elevated CO ₂	Elevated temperature	Elevated CO ₂ + Temperature	Reference plot
1	<i>A. lacunosa</i>	<i>A. lacunosa</i>	<i>A. mellea</i>	<i>A. appendicula</i>	<i>A. dilatata</i>
2	<i>A. morrowae</i>	<i>Acaulospora</i>	<i>A. rugosa</i>	<i>A. lacunose</i>	<i>A. lacunosa</i>
3	<i>A. scrobiculata</i>	<i>Gigaspora</i>	<i>A. scrobiculata</i>	<i>A. longula</i>	<i>A. morrowe</i>
4	<i>G. etunicatum</i>	<i>G. diaphanum</i>	<i>A. spinosa</i>	<i>A. marrowe</i>	<i>A. sporocarpum</i>
5	<i>G. fasciculatum</i>	<i>G. etunicatum</i>	<i>Acaulospora spp</i>	<i>Acaulospora</i>	<i>Acaulospora spp</i>
6	<i>G. lacteum</i>	<i>G. farosanum</i>	<i>G. fasciculatum</i>	<i>G. ambisporum</i>	<i>E. singulae</i>
7	<i>G. multicaulis</i>	<i>G. fasciculatum</i>	<i>G. lacteus</i>	<i>G. constrictum</i>	<i>G. ambispora</i>
8	<i>G. mosseae</i>	<i>G. foevata</i>	<i>G. macrocarpum</i>	<i>G. etunicatum</i>	<i>G. farosanum</i>
9	<i>G. versiformae</i>	<i>G. lacteum</i>	<i>G. mosseae</i>	<i>G. fasciculatum</i>	<i>G. fasciculatum</i>
10		<i>G. macrocarpum</i>	<i>G. radiata</i>	<i>G. gerdimanii</i>	<i>G. macrocarpum</i>
11		<i>G. mosseae</i>	<i>Glomus sp.</i>	<i>G. geosporum</i>	<i>G. mosseae</i>
12		<i>Glomus</i>		<i>G. globifrum</i>	<i>G. multicaulis</i>
13		<i>S. heterogama</i>		<i>G. leptotichum</i>	<i>G. occultum</i>
14				<i>G. macrocarpum</i>	<i>G. radiatum</i>
15				<i>G. mosseae</i>	
16				<i>G. pubescens</i>	
17				<i>Glomus radiata</i>	
18				<i>G. radiates</i>	
19				<i>Glomus spp</i>	
20				<i>Scutellospora</i>	
Total	9	13	11	20	14

Note: A – *Acaulospora*, G- *Glomus*, S – *Scutellospora*, E- *Entrophospora*



4.4 Influence of elevated CO₂ and/ or temperature in groundnut rhizosphere soil on Mycorrhizal parameters

4.4.1 AM fungal spore count as influenced by elevated CO₂ and/ or temperature in groundnut rhizosphere soil

The mycorrhizal spore load was calculated for 50 g of soil and is presented in Table 14. The highest mycorrhizal spore count was recovered with treatment exposed to elevated CO₂ condition at 30 DAS (227.00 spore/ 50 g soil) followed by the treatment which received elevated CO₂ plus temperature (224.75 spore/ 50 g soil), which was statistically on par with the soil exposed to elevated temperature (223.25 spore/ 50 g soil). The lowest spore load was observed in the reference plot (210.50/50 g soil), which was statistically on par with ambient condition (221.50 /50 g soil). Similar trend was also recorded at 60 DAS. However, the mycorrhizal spore count recorded with ambient conditions were found to be statistically greater than the reference plot.

4.4.1.1 Per cent root colonization as influenced by elevated CO₂ and/ or temperature in groundnut rhizosphere soil

Colonization of mycorrhizal fungi in groundnut roots was analyzed at 45 DAS and the data is depicted in Table 15. In general the per cent root colonization ranged from 47.00 to 55.75 per cent. The maximum per cent root colonization was recorded in the plants exposed to elevated CO₂ (55.75 per cent) followed by elevated CO₂ plus temperature (54.00 per cent) and elevated temperature alone (51.50 per cent). Similar to mycorrhizal spore count, significantly higher root colonization was recorded in ambient condition over reference ring (50.50 per cent and 47.00 per cent) respectively.

4.4.2 Influence of elevated CO₂ and/ or temperature in groundnut rhizosphere soil on AMF community

4.4.2.1 Isolation and characterization of AM fungi from five altered conditions

AM fungi were isolated from five environmental conditions *viz.* elevated CO₂ and temperature, elevated CO₂, elevated temperature, ambient condition and

reference plot. These isolates were identified up to species level based on morphological characteristics of the chlamydospores (Table 18 and Plate 4).

The AM fungal species identified during the investigations are given in Table 16. As many as 20 species of AMF were identified in the elevated CO₂ and temperature condition belonging to three different genera. Among them, 15 species belonged to the genus *Glomus*, five species were representatives of *Acaulospora* and one of *Scutellospora*. In reference plot a total number of 14 species were identified which included eight species from *Glomus*, five species from *Acaulospora* and one species from *Entrophospora*. Thirteen species were observed from elevated CO₂ chamber which included 10 species of *Glomus*, two species of *Acaulospora* and one species of *Scutellospora*. In elevated temperature condition, 11 species were identified. Among them, five species belonged to *Acaulospora*, six species belonged to the genus *Glomus*. In the ambient condition, nine species of AMF were identified. Among them, six belonged to *Glomus* and three to *Acaulospora*.

4.4.2.2 Abundance of AM fungal spores of different species as influenced by elevated CO₂ and/ or temperature condition

The spore abundance of AM fungal species is given in Table 17.

The spores of *G. fasciculatum* were found to be more (35 spores/50 g soil), followed by *G. mosseae* and *G. macrocarpum* respectively (32 spores/50 g soil and 29 spores/50 g soil). The lowest spore load was found (*G. leptotichum* 12 spores/50 g soil) in elevated CO₂ plus temperature conditions. In elevated CO₂ condition, spores of *G. fasciculatum* were found to be more abundant (37 spores/50 g soil) followed by *G. mosseae* and *G. macrocarpum* (35 spores/50 g soil and 26 spores/50 g soil respectively). However, *G. farnosum* recorded the lowest spore abundance (12 spores/50 g soil). In elevated temperature condition, maximum spore load was observed in case of *G. mosseae* (26 spores/50 g soil) followed by *Glomus sp* and *Acaulospora sp.* respectively (22 spores/50 g soil) and the least spore load was noticed in the AM fungal species *S. heterogama* (11 spores/50 g soil). Rhizosphere soil from ambient condition recorded the maximum spore abundance with respect to the species of *G. mosseae* (28 spores/50 g soil),

Table 17: Abundance of AM fungal spores of different species as influenced by elevated CO₂ and/ or temperature condition

Sl. No.	AM fungal species	Mycorrhizal spore load (No. of spores per 50 g of soil)				
		Ambient condition	Elevated CO ₂	Elevated temperature	Elevated CO ₂ + Temperature	Reference plot
1	<i>A. appendicula</i>	-	-	-	20	-
2	<i>A. dilatata</i>	-	-	-	-	15
3	<i>A. lacunose</i>	18	21	-	25	16
4	<i>A. longula</i>	-	-	-	23	-
5	<i>A. marrowe</i>	23	-	-	18	21
6	<i>A. mellea</i>	-	-	17	-	-
7	<i>A. rugosa</i>	-	-	12	-	-
8	<i>A. scrobiculata</i>	14	-	16	-	-
9	<i>A. spinosa</i>	-	-	18	-	-
10	<i>A. sporocarpum</i>	-	-	-	-	19
11	<i>Acaulospora spp</i>	-	18	23	21	21
12	<i>E. singulae</i>	-	-	-	-	12
13	<i>G. ambisporum</i>	-	-	-	23	-
14	<i>G. diaphanum</i>	-	24	-	-	-
15	<i>G. constrictum</i>	-	-	-	16	-
16	<i>G. etunicatum</i>	18	15	-	22	-
17	<i>G. farmosanum</i>	-	12	-	-	14
18	<i>G. fasciculatum</i>	16	37	-	35	18
19	<i>G. foevata</i>	-	17	-	-	-
20	<i>G. gerdimanii</i>	-	-	-	19	-

Contd...

Sl. No.	AM fungal species	Mycorrhizal spore load (No. of spores per 50 g of soil)				
		Ambient condition	Elevated CO ₂	Elevated temperature	Elevated CO ₂ + Temperature	Reference plot
21	<i>G.geosporum</i>	-	-	-	15	-
22	<i>G. globifrum</i>	-	-	-	17	-
23	<i>G.infrequens</i>	-	-	-	21	-
24	<i>G.lacteum</i>	20	26	-	-	-
25	<i>G.lacteus</i>	-	-	15	-	-
26	<i>G. leptotichum</i>	-	-	-	12	-
27	<i>G. macrocarpum</i>	-	23	-	25	21
28	<i>G. mosseae</i>	28	35	26	32	33
29	<i>G.multicaulis</i>	15	-	-	-	17
30	<i>G. occultum</i>	-	-	-	-	13
31	<i>G. pubescens</i>	-	-	-	23	-
32	<i>G.radiata</i>	-	-	19	26	-
33	<i>G. radiates</i>	-	-	-	21	-
34	<i>G.radiatum</i>	-	-	-	15	-
34	<i>G.versiformae</i>	23	-	-	24	-
36	<i>Glomus sp.</i>	-	-	22	26	-
37	<i>Gigaspora</i>	15	-	18	-	-
38	<i>S.heterogama</i>	-	-	11	-	-
39	<i>Scutellospora</i>	-	-	-	14	-
	Total	190	228	197	493	220

Note: A – Acaulospora, G- Glomus, S – Scutellospora, E- Entrophospora

Table 18: Tentative identification of AM fungal native morphotypes from altered environmental condition

Sl. No.	Isolates	Spore Size (µm)	Colour	Spore wall size (µm)	Spore surface	Hyphal length (µm)	Probable species identified
1	AMF A1	99.5	Yellow	3.4	Smooth	-	<i>Acaulospora</i>
2	AMF A2	210.2	Brown	3.7	Rough	-	<i>Acaulospora</i>
3	AMF A3	138.4	Brown	3.5	Rough	-	<i>Acaulospora</i>
4	AMF A4	148.5	Yellow	3.4	Rough	67.6	<i>Glomus</i>
5	AMF A5	135.3	Yellow	3.2	Smooth	-	<i>Glomus constrictum</i>
6	AMF A6	110.4	Brown	3.3	Rough	30.66	<i>Glomus radiata</i>
7	AMF A7	184.9	Yellow	3.7	Smooth	-	<i>Glomus etunicatum</i>
8	AMF A7 (a)	117.8	Yellow	3.4	Smooth	90.3	<i>G. leptotichum</i>
9	AMF A8	171.2	Brown	3.1	Smooth	74.2	<i>G. globifrum</i>
10	AMF A9	135.4	Yellow	3.6	Rough	120.3	<i>A. longula</i>
11	AMF A10	148.4	Dark brown	3.8	Smooth		<i>G.geosporum</i>
12	AMF A11	308.4	Yellow	3.3	Smooth	-	<i>A. appendicula</i>
13	AMF A12	164.3	Black	3.2	Rough	-	<i>G.infrequens</i>
14	AMF A13	148.4	Yellow	4.3	Rough and granular	70.8	<i>A. lacunose</i>
15	AMF A14	110.6	Yellow	3.4	Smooth	67.3	<i>G. fasciculatum</i>
16	AMF A15	180.1	Yellow	3.9	Rough	-	<i>G. pubescens</i>
17	AMF A16	152.6	Yellow	3.2	Rough	52.4	<i>Scutellospora</i>
18	AMF A17	185.3	Black	3.4	Smooth	-	<i>Glomus</i>
19	AMF A18	184.1	Brown	3.6	Smooth	29.3	<i>G.gerdimanii</i>

Contd...

Sl. No.	Isolates	Spore Size (µm)	Colour	Spore wall size (µm)	Spore surface	Hyphal length (µm)	Probable species identified
20	AMF A19	214.3	Yellow	4.3	Rough	56.8	<i>G. macrocarpum</i>
21	AMF A20	176.4	Black	-	Smooth	-	<i>G. ambisporum</i>
22	AMF A21	91.1	Dark brown	2.2	Smooth	-	<i>Glomus</i>
23	AMF A23	86.3	Yellow	3.2	Smooth	-	<i>G. radiates</i>
24	AMF A24	143.6	Yellow	4.1	Rough	-	<i>G.fasciculatum</i>
25	AMF A25	92.3	Black	-	Smooth	-	<i>G. ambisporu</i>
26	AMF A28	118.5	Yellow	4.2	Smooth	43.6	<i>G. fasciculatum</i>
27	AMF A29	143.3	Brown	4.4	Smooth		<i>Glomus</i>
28	AMF A32	157.8	Yellow	3.5	Rough	-	<i>A. marrowe</i>
29	AMF C1	153.2	Yellow	3.6	Smooth	-	<i>G.etunicatum</i>
30	AMF C3	163.9	Yellow	5.7	Rough	-	<i>G. lacteum</i>
31	AMF C4	157.8	Brown	-	Rough	-	<i>Sporocarp</i>
32	AMF C5	107.5	Yellow	5.2	Rough	-	<i>Glomus</i>
33	AMF C6	163.7	Yellow	4.6	Rough and granular	43.5	<i>G. mosseae</i>
34	AMF C7	167.4	Brown	4.3	Rough and granular	-	<i>G.farmosanum</i>
35	AMF C9	183.2	Yellow	3.7	Rough and granular	45.2	<i>Gigaspora</i>
36	AMF C10	108.3	Yellow	5.4	Smooth	-	<i>G.fasciculatum</i>
37	AMF C13	162.3	Yellow	3.4	Rough	35.6	<i>Gigaspora</i>
38	AMF C14	208.3	Yellow	3.7	Rough	-	<i>A.lacunosa</i>
39	AMF C15	154.7	Brown	7.2	Smooth	49.72	<i>Acaulospora</i>

Contd...

Sl. No.	Isolates	Spore Size (µm)	Colour	Spore wall size (µm)	Spore surface	Hyphal length (µm)	Probable species identified
40	AMF C16	203.4	Yellow	5.3	Smooth	-	<i>G.lacteum</i>
41	AMF C17	152.7	Yellow	5.7	Rough	52.7	<i>G.foevata</i>
42	AMF C18	112.8	Yellow	3.7	Rough and granular	67.4	<i>Scutellospora heterogama</i>
43	AMF C20	103.5	Brown	6.4	Smooth	31.9	<i>G.mosseae</i>
44	AMF C21	88.7	Yellow	3.6	Smooth	-	<i>G.diaphanum</i>
45	AMF C22	94.7	Yellow	6.7	Rough		<i>G.macrcarpum</i>
46	AMF C23	99.8	Yellow	5.2	Smooth	-	<i>G.mosseae</i>
47	AMF C24	104.4	Yellow	7.1	Rough	-	<i>Glomus</i>
48	AMF C25	80.9	Yellow	4.6	Smooth	-	<i>G.fasciculatum</i>
49	AMF C26	116.3	Yellow	7.1	Smooth	57.9	<i>G.fasciculatum</i>
50	AMF C28	81.3	Yellow	3.6	Smooth	-	<i>G.lacteum</i>
51	AMF C29	94.8	Brown	5.3	Smooth	-	<i>G.fasciculatum</i>
52	AMF C31	99.2	Brown	2.7	Smooth	-	<i>G.fasciculatum</i>
53	AMF D2	171.6	Yellow	4.4	Smooth	-	<i>Glomus sp.</i>
54	AMF D3	193.5	Yellow	3.4	Smooth	-	<i>A.rugosa</i>
55	AMF D4	147.3	Brown	7.3	Smooth	22.2	<i>G.radiata</i>
56	AMF D6	181.4	Yellow	4.8	Rough and granular	-	<i>A.scrobiculata</i>
57	AMF D8	139.6	Yellow	3.8	Smooth	-	<i>A.spinosa</i>
58	AMF D9	132.1	Yellow	5.4	Rough and granular	-	<i>A.spinosa</i>
59	AMF D12(d)	135.6	Yellow	8.3	Rough and granular	-	<i>A.scrobiculata</i>

Contd...

Sl. No.	Isolates	Spore Size (µm)	Colour	Spore wall size (µm)	Spore surface	Hyphal length (µm)	Probable species identified
60	AMF D13(b)	109.3	Yellow	7.1	Rough and granular	-	<i>A.scrobiculata</i>
61	AMF D14(a)	159.1	Yellow	3.8	Rough and granular	-	<i>G.lacteus</i>
62	AMF D14(b)	132.4	Yellow	4.5	Smooth	-	<i>A.spinosa</i>
63	AMF D14(d)	125.7	Yellow	3.6	Rough and granular	-	<i>A.mellea</i>
64	AMF D14(e)	124.8	Yellow	4.1	Rough and granular	-	<i>Acaulospora</i>
65	AMF D15	90.5	Yellow	3.2	Rough and granular	-	<i>Acaulospora</i>
66	AMF D16(a)	158.1	Yellow	6.5	Rough and granular	-	<i>Acaulospora</i>
67	AMF D16(b)	120.6	Yellow	4.3	Rough and granular	-	<i>Acaulospora</i>
68	AMF D16(e)	124.8	Yellow	4.7	Rough and granular	-	<i>A.scrobiculata</i>
69	AMF E1	152.7	Yellow	3.2	Smooth	30.2	<i>A.morrowae</i>
70	AMF E3	151.5	Brown	5.6	Smooth	28.9	<i>A.lacunosa</i>
71	AMF E4	152.3	Yellow	4.5	Smooth	-	<i>G.lacteum</i>
72	AMF E5	98.8	Yellow	4.7	Smooth	-	<i>G.mosseae</i>
73	AMF E7(a)	98.2	Brown	3.6	Rough	-	<i>A.scrobiculata</i>
74	AMF E8	103.8	Brown	4.5	Rough	-	<i>G.fasciculatum</i>
75	AMF E10	140.6	Yellow	6.3	Smooth	46,3	<i>G.mosseae</i>
76	AMF E11	105.3	Yellow	3.2	Smooth	67.8	<i>G.fsciculatum</i>
77	AMF E12	121.8	Yellow	5.7	Smooth	-	<i>G.etunicatum</i>
78	AMF E13	196.3	Brown	8.4	Smooth	-	<i>G.versiformae</i>
79	AMF E15	86.8	Yellow	3.4	Rough	15.2	<i>G.multicaulis</i>

Contd...

Sl. No.	Isolates	Spore Size (µm)	Colour	Spore wall size (µm)	Spore surface	Hyphal length (µm)	Probable species identified
80	AMF E16	83.5	Yellow	3.2	Rough	-	<i>G.mosseae</i>
81	AMF B1	132.5	Brown	5.3	Smooth	-	<i>G.fasciculatum</i>
82	AMF B2	112.3	Yellow	4.8	Smooth	-	<i>G.mosseae</i>
83	AMF B3	82.3	Yellow	3.7	Rough	-	<i>G.occultum</i>
84	AMF B4	153.3	Yellow	9.6	Smooth	45.7	<i>Entrophospora singulae</i>
85	AMF B5	158.6	Yellow	5.7	Smooth	-	<i>Acaulospora</i>
86	AMF B7	152.6	Yellow	9.3	Smooth	-	<i>G.radiatum</i>
87	AMF B9	99.6	Brown	3.2	Smooth	-	<i>G.macrocarpum</i>
88	AMF B10	185.2	Yellow	6.3	Rough	-	<i>A.lacunosa</i>
89	AMF B11	160.4	Yellow	9.7	Smooth	10	<i>Acaulospora</i>
90	AMF B12	84.5	Yellow	8.9	Smooth	-	<i>G.multicaulis</i>
91	AMF B17	174.7	Yellow	8.9	Smooth	-	<i>A.morrowe</i>
92	AMF B18	213.8	Black	7.2	Smooth	-	<i>G.farosanum</i>
93	AMF B22	173.3	Brown	5.3	Rough	-	<i>Acaulospora</i>
94	AMF B22(a)	208.6	Brown	5.6	Rough	-	<i>Acaulospora</i>
95	AMF B23	86.2	Black	8.2	Smooth	-	<i>G.ambispora</i>
96	AMF B24	109.2	Yellow	5.7	Smooth	-	<i>Acaulospora</i>
97	AMF B26	92.5	Yellow	-	Rough	-	<i>Acaulospora</i>
98	AMF B27	75.6	Yellow	4.5	Rough	-	<i>A.sporocarpum</i>
99	AMF B29	70.9	Brown	3.8	Smooth	23.9	<i>G.mosseae</i>
100	AMF B32	93.5	Yellow	4.4	Smooth	-	<i>A.dilatata</i>

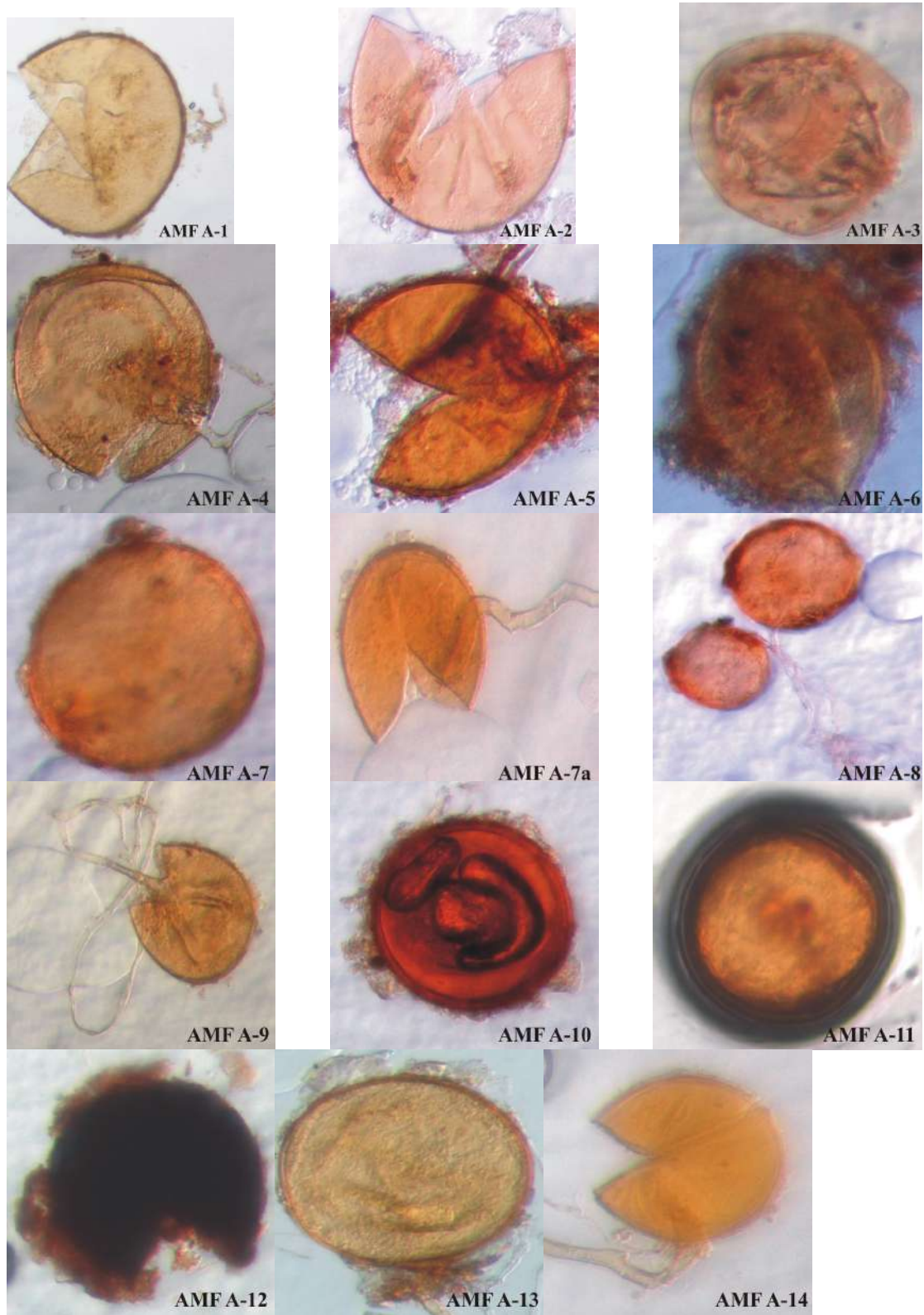


Plate 4 Tentative identification of AM fungal native morphotypes from five altered environmental condition

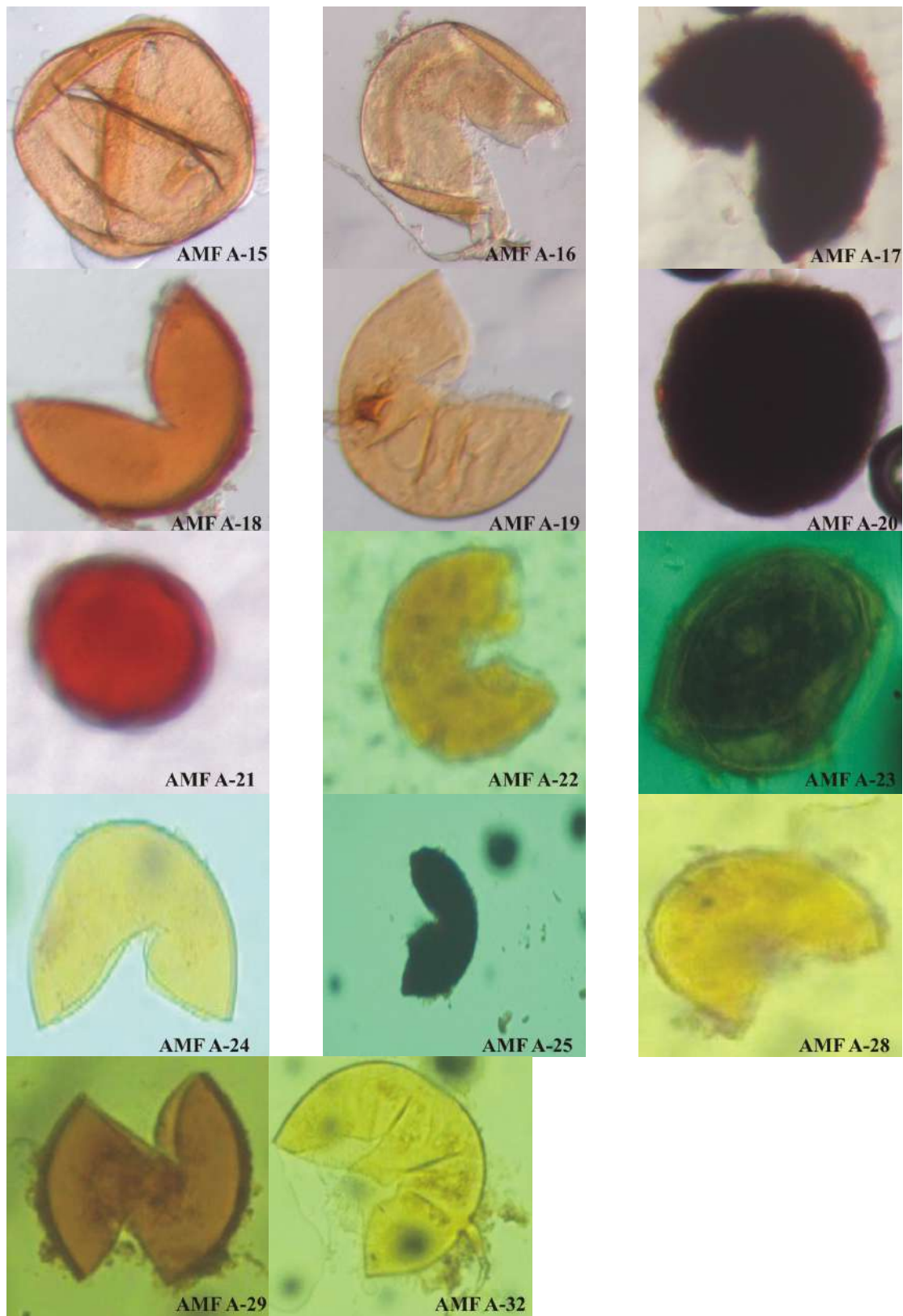


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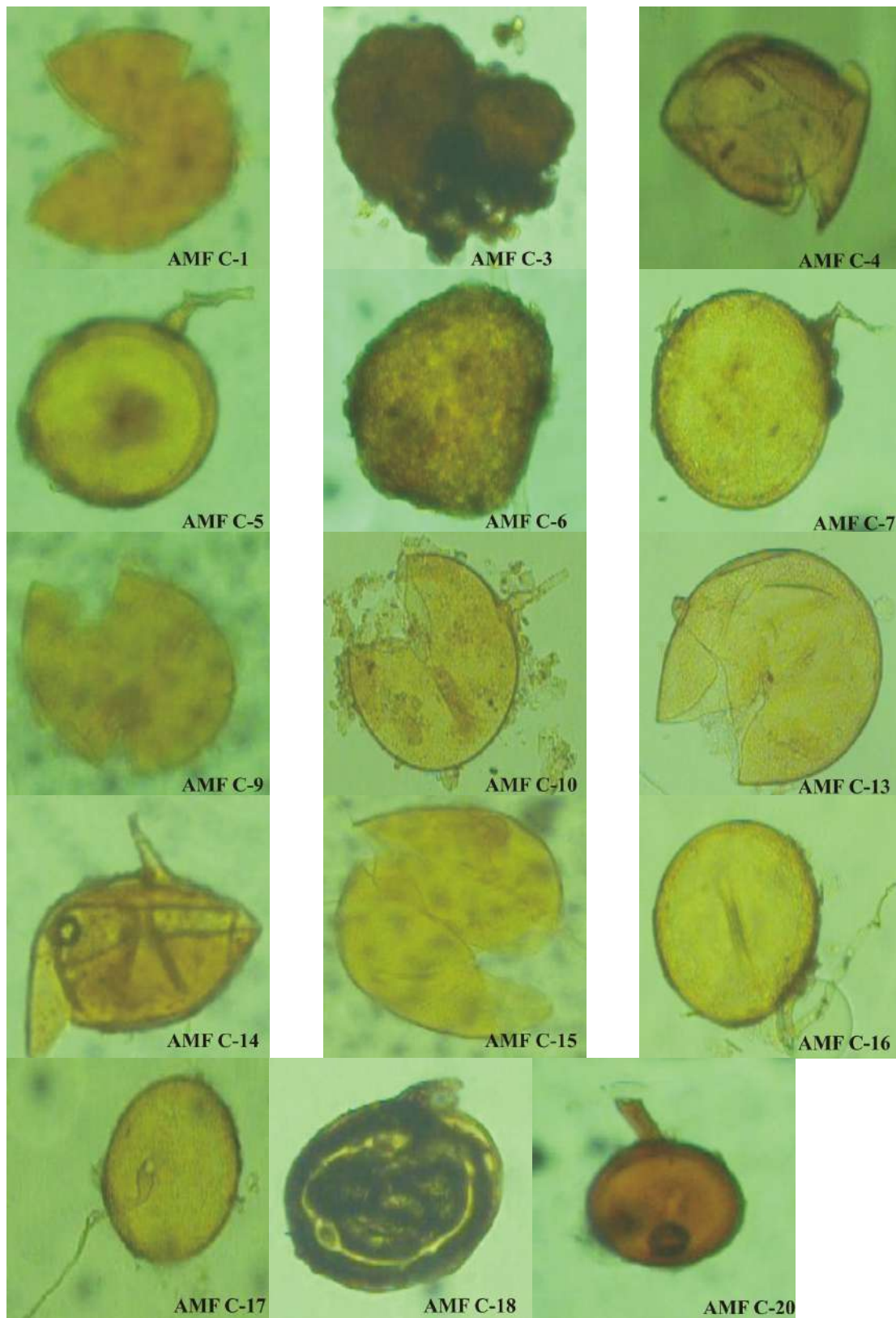


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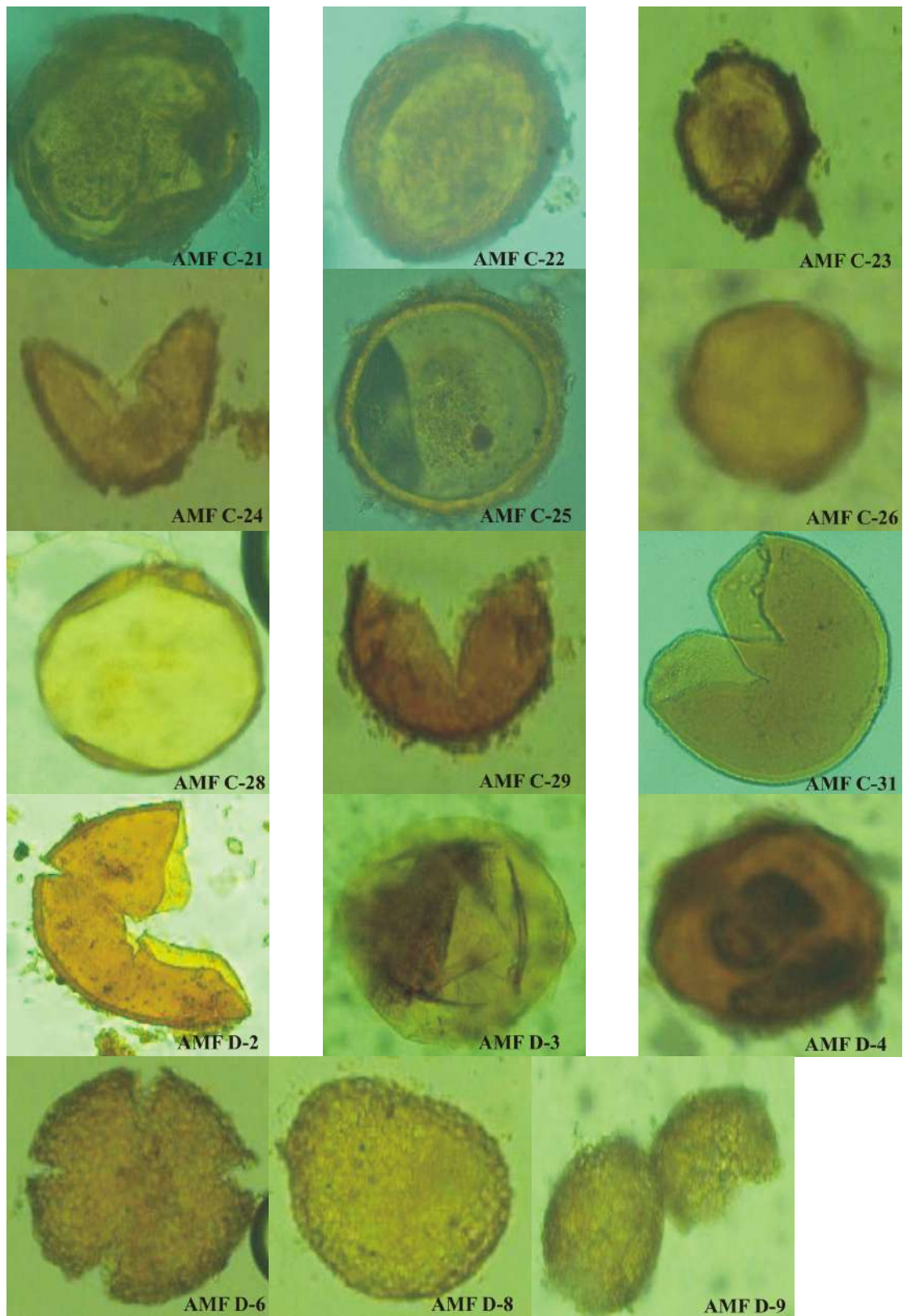


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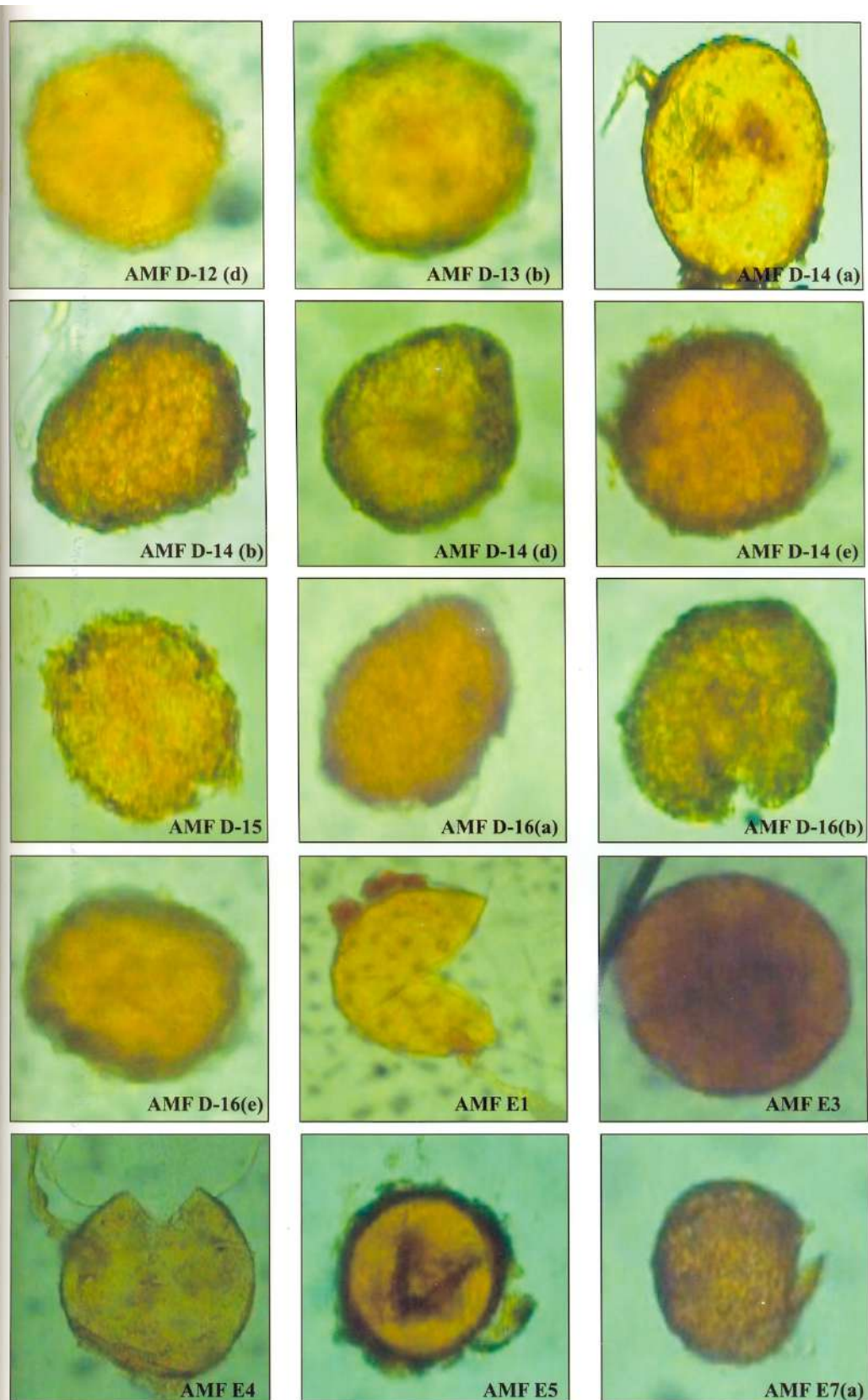


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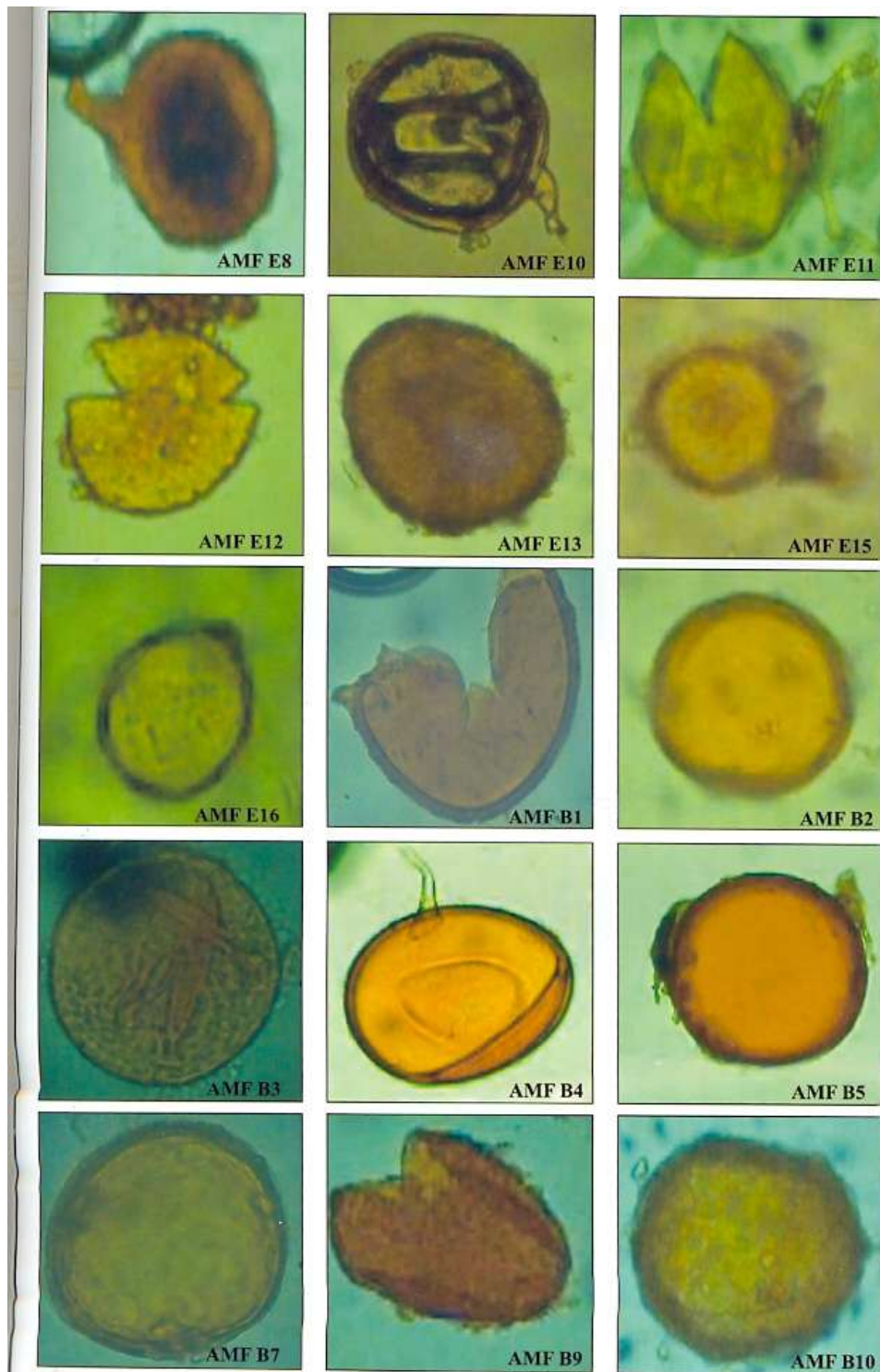
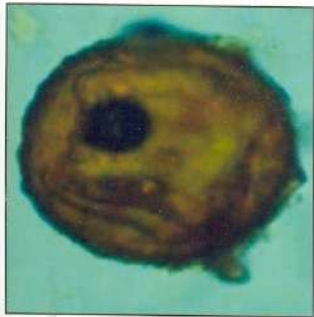


Plate 4: Contd...



AMF B11



AMF B12



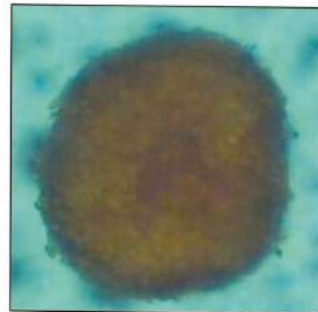
AMF B17



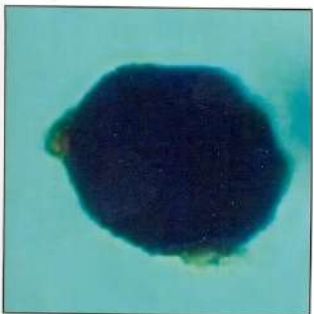
AMF B18



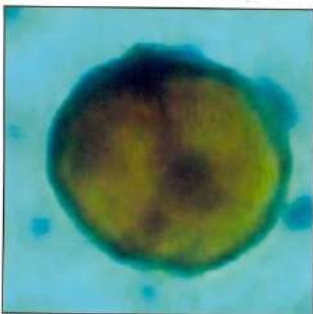
AMF B22



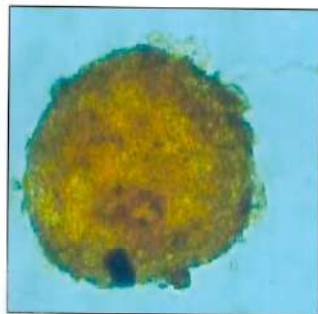
AMF B22(a)



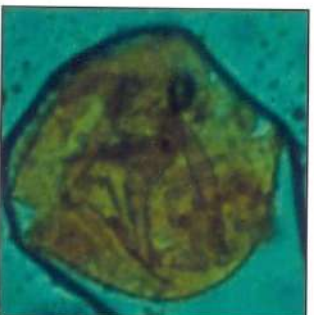
AMF B23



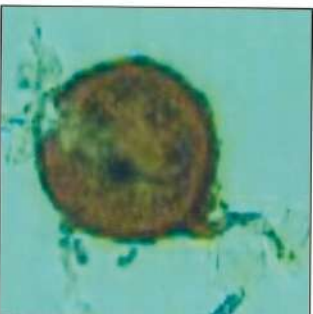
AMF B24



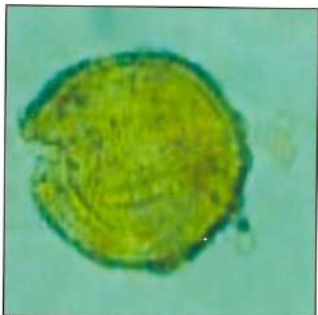
AMF B26



AMF B27



AMF B29



AMF B32

Table 19: Diversity of AMF as influenced by elevated CO₂ and/ or temperature based on spore count

Treatments	Number of species (S)	Total number (N)	Shannon's diversity Index (H)	Margalef's Species richness Index (Dmg)
Ambient	9	190	2.28	1.37
Elevated CO ₂	13	228	2.25	1.54
Elevated temperature	11	197	2.37	1.48
Elevated CO ₂ + Temperature	20	493	3.10	2.66
Reference ring	14	220	2.45	1.86

followed by *G. versiformae* and *A. marrowe* (23 spores/50 g soil) respectively. However, *A. scrobiculata* recorded the lowest spore abundance (14 spores/50 g soil). In the open reference ring, the spore abundance was maximum in the species *G. mosseae* (33 spores/50 g soil) followed by *G. macrocarpum* (27 spores/50 g soil), *Acaulospora sp* and *A. marrowe* (21 spores/50 g soil). The least spore abundance was recorded in the species *E. singulae* (12 spores/50 g soil).

4.4.2.3 Diversity of AMF as influenced by elevated CO₂ and/ or temperature based on spore count

The diversity of AM fungi under elevated CO₂, elevated CO₂ plus temperature, elevated temperature condition, ambient condition and open reference ring based on occurrence and spore count of AMF are given in Table 19.

The highest diversity of AMF was found in elevated CO₂ and temperature condition.

Considering the spore count, the elevated CO₂ plus temperature had higher number of species (20). The Shannon diversity index was higher in elevated CO₂ plus temperature condition ($H'=3.10$) followed by open reference plot ($H'=2.45$); elevated temperature condition ($H'=2.37$) and under elevated CO₂ recorded poorer with respect to AMF diversity ($H'=2.25$).

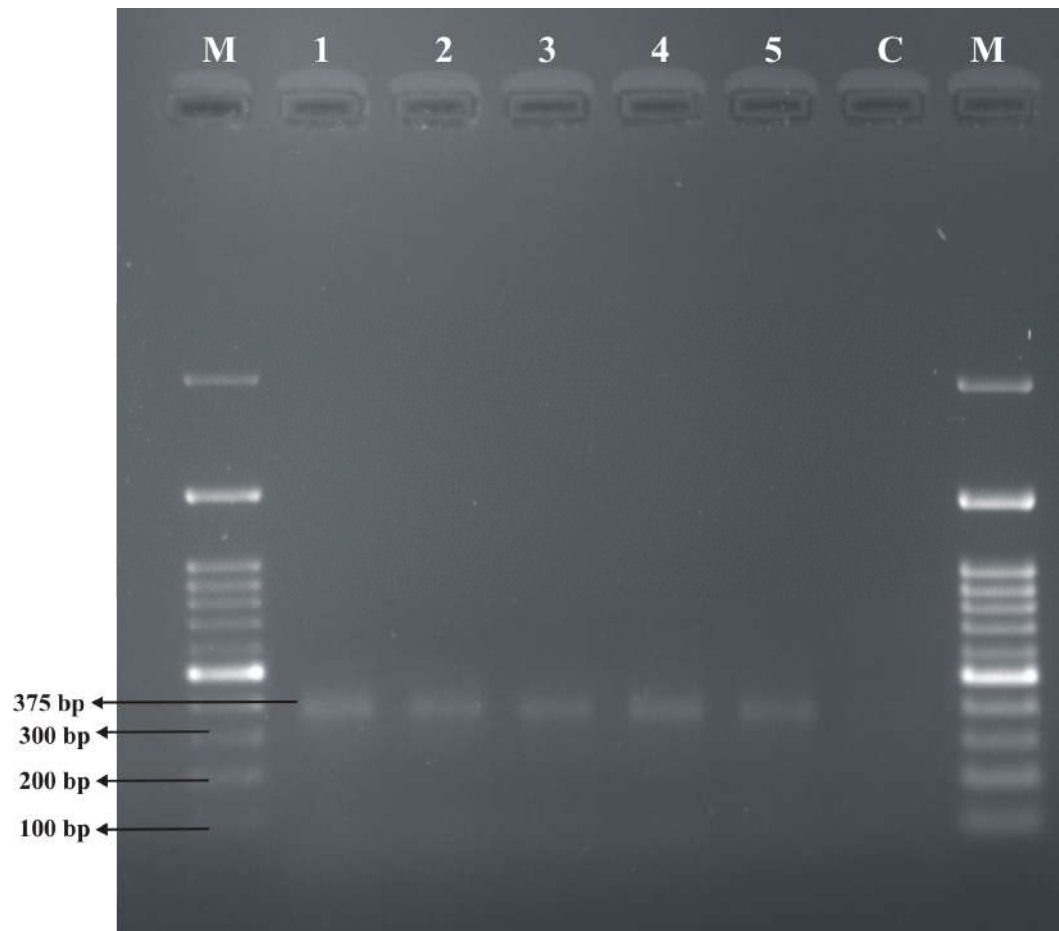
The Margalef's index of species richness for AMF based on spore count was the highest in elevated CO₂ plus temperature (2.66) compared to reference plot (1.86) and elevated CO₂ condition (1.54). The lowest species richness was recorded in ambient condition (1.37) compared to elevated temperature condition (1.48) (Table 19).

4.5 Molecular studies in am fungi

The results of the morphological characterization of the native isolates under five environmental conditions have clearly revealed that *Glomus mossae* was the most predominant species in all the five conditions based on their occurrence and abundance. Further, the native *Glomus mossae* isolates were genetically authenticated by the amplification of 375 bp specific DNA band in the ITS region of AM fungi.

4.6 Principal component analysis of soil physico - chemical and biological properties

The obtained results were subjected to principal component analysis. PCA is the statistical method used to analyze similarities and differences between the samples as well as variables with respect to five altered atmospheric conditions at 30 and 60 DAS. The PC1 and PC2 contributed for 90.77% and 4.64% of the total variation and with eigen values of 21.78 and 1.11 respectively (Table 20). Principal component scores were plotted in the scoring plot. A total of 95 per cent variability was observed among the soil samples and all the assessed variables have sufficiently contributed for that 95 per cent variability. Soil physico-chemical parameters, microbial population, soil enzymes, microbial biomass carbon and nitrogen and AMF spore counts significantly influenced the principal component scores as a soil variable characterized for the influence of elevated CO₂, interaction of elevated CO₂ and temperature, elevated temperature, ambient condition and reference plot. The score plot showed that data of the soil samples clustered among the five altered environment conditions.



M - Marker
 1 - Elevated CO₂ + Temperature condition
 2 - Elevated CO₂
 3 - Elevated temperature
 4 - Ambient
 5 - Reference plot
 C - Control

Plate 5 PCR amplification of ITS region from single spore of native AMF isolates

Table 20: Loading values and per cent contribution of variables on the axis identified by the principal component analysis for soils under different environmental stress conditions

Variables	PC1		PC2	
	Loading value	Contribution (%)	Loading value	Contribution (%)
WSA	1.00	4.55	-0.05	0.26
Ph	1.00	4.56	-0.08	0.55
EC	0.84	3.24	-0.22	4.29
SOC	0.99	4.49	0.00	0.00
SIC	0.97	4.36	-0.20	3.48
N	0.97	4.32	0.09	0.72
P	0.99	4.53	0.05	0.23
K	0.97	4.28	-0.24	5.22
S	0.85	3.30	0.51	23.17
B	0.97	4.36	-0.20	3.64
F	0.91	3.82	0.30	8.29
Act	0.97	4.31	0.09	0.74
FLN	0.98	4.40	-0.12	1.22
PSB	0.99	4.53	0.05	0.26
FP	0.90	3.74	0.21	3.84
Pro	0.94	4.07	-0.26	5.95
DHY	0.77	2.72	0.39	13.92
ACP	0.91	3.79	0.40	14.45
ALP	1.00	4.58	-0.01	0.00
URE	0.98	4.37	-0.21	3.79
CEL	0.95	4.10	-0.23	4.66
SBC	1.00	4.56	0.00	0.00
SBN	0.99	4.49	-0.12	1.31
AMF	0.99	4.53	0.00	0.00
Eigenvalue	21.78		1.11	
Variability (%)	90.77		4.64	
Cumulative %	90.77		95.41	

Note: WSA –Water stable aggregates; SOC – Soil organic carbon; SIC – Soil inorganic carbon; N – Soil available nitrogen; P – Soil available phosphorus; K – Soil available potassium; S – Sulphure; B – Bacteria; F – Fungi; Act –Actinomycets; FLN – Free living N₂ fixer; PSB – Phosphate solubilizing bacteria; FP – Fluroscent psuedomonads; Pro- Protozoa; DHY – soil dehydrogenase; ACP – Acid phosphatase; ALP – Alkaline phosphatase; URE – Urease; CEL – Cellulase; SBC – Soil biomass carbon; SBN – Soil biomass nitrogen; AMF– AMF spore count.

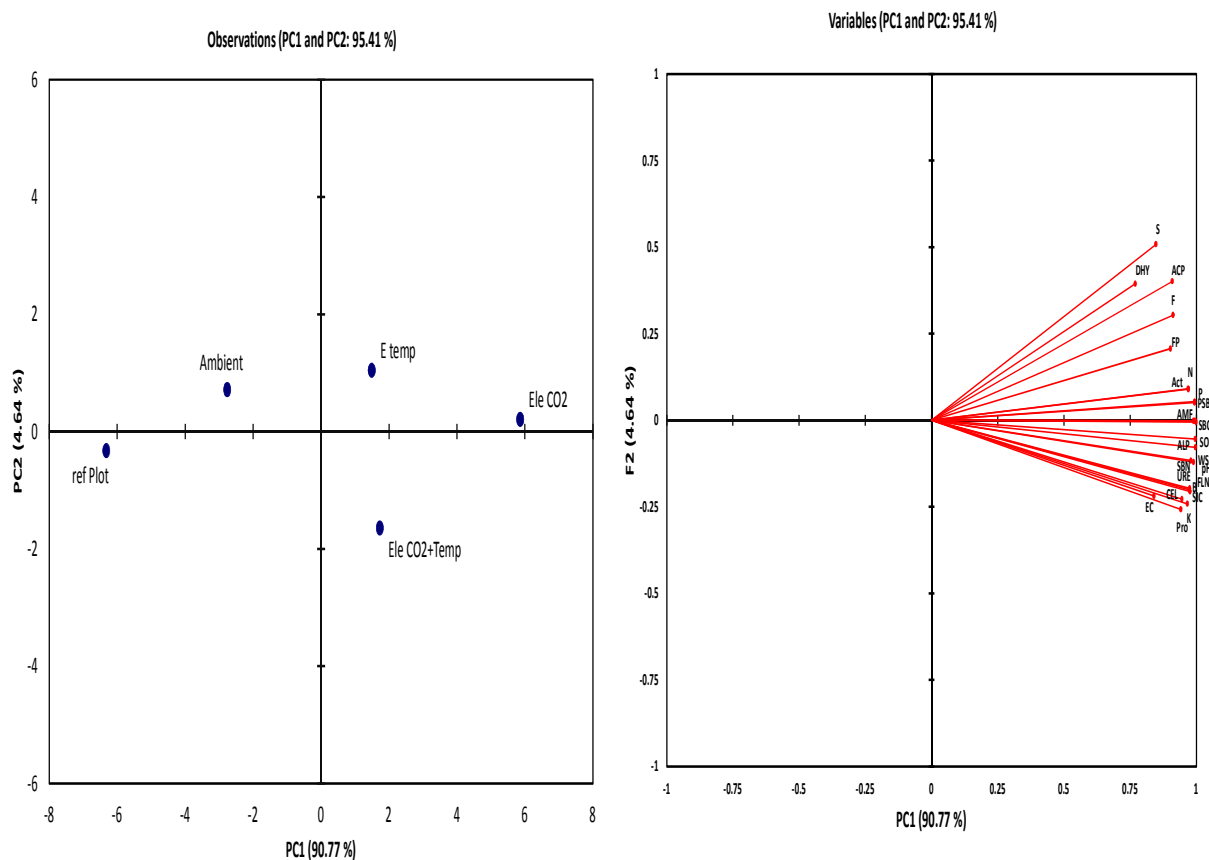


Fig. 12: Scoring plot of samples (A) and loading plot of variables (B) ordinate in PCA

In scoring plot five samples were denoted as EleCO₂ + Temp, Ele CO₂, E temp, Ambient, Ref Plot.

In loading plot, WSA –Water stable aggregates; SOC – Soil organic carbon; SIC – Soil inorganic carbon; N – Soil available nitrogen; P – Soil available phosphorus; K – Soil available potassium; S – Sulphure; B – Bacteria; F – Fungi; Act – Actinomycets; FLN – Free living N₂ fixer; PSB – Phosphate solubilizing bacteria; FP – Fluroscent psuedomonads; Pro- Protozoa; DHY – soil dehydrogenase; ACP – Acid phosphatase; ALP – Alkaline phosphatase; URE – Urease; CEL – Cellulase; SBC – Soil biomass carbon; SBN – Soil biomass nitrogen; AMF– AMF spore count were denoted.

5. DISCUSSION

Global climate change has emerged as an important environmental challenge due to its potential impact on soil physico-chemical and soil biological properties. This may result in impaired activities of beneficial microorganisms in terms of exhibiting their desirable functional properties. Hence, it is important to understand the response of soil physico-chemical and biological properties to elevated CO₂ and/or temperature. With this background, the present investigation was conducted to analyze the influence of altered conditions on physico-chemical, biological properties and AMF community. The results obtained on the above aspects are discussed in this chapter.

5.1 Soil physico-chemical properties as influenced by elevated CO₂ and / or temperature in groundnut rhizosphere soil

5.1.1 Soil water stable aggregates (per cent)

Soil water stable aggregate is an indicator of organic matter content, biological activity and nutrient cycling. In the present investigation, the soil water stable aggregates were indistinctly increased under elevated CO₂ condition at 30 and 60 DAS compared to rest of the altered conditions *viz.*, interaction of elevated CO₂ plus temperature; elevated temperature alone; ambient condition and open reference plot. Similar results have been reported by Stephen *et al.* (2004). They have observed significantly highest soil water stable aggregate in an agro ecosystem exposed to elevated atmospheric CO₂ condition. This may be attributed to enhanced soil carbon content due to CO₂ enrichment (Stephen *et al.*, 2004).

5.1.2 Soil pH and EC

The findings of the present investigation revealed that the soil pH and EC in the groundnut rhizosphere soil at 30 and 60 DAS did not differ significantly under five altered conditions. However, the observation at 60 DAS revealed, relatively a higher soil pH and EC in the soils exposed to elevated CO₂. The increased below ground biomass will have increased the pH and EC at latter stage of the crop

growth. While, Abbas *et al.* (2009) revealed that the pH and EC of soil were greatly influenced by elevated CO₂ under the influence of submerged conditions as compared to un-submerged soil condition. Karberg *et al.* (2005) reported an increased dissolved inorganic compound under elevated CO₂ resulting in the alteration of inorganic carbon, thereby increasing the total alkalinity in the soil. However, several investigators have revealed that the elevated CO₂ did not significantly influence the soil pH and EC in the beginning of the investigation (Das *et al.*, 2011 and Barnard *et al.*, 2005).

5.1.3 Soil organic carbon

Soil organic carbon (SOC) is one of the major components of soil organic matter. It is extremely important in all soil processes *viz.*, nutrient availability, soil structure and soil biological activities. In the present study, increased organic carbon content was recorded in the soil exposed to elevated CO₂ compared to the other altered conditions. This is in line with the findings of Saurav *et al.* (2011) who reported that organic carbon content was significantly increased under elevated CO₂ conditions compared to the ambient conditions. Increased photosynthetic activity and plant growth under elevated CO₂, could have resulted in increased seizure of SOC in the soil (Wolf *et al.*, 2007).

5.1.4 Soil inorganic carbon

Soil inorganic carbon (SIC) is one of the largest carbon pools (Eswaran *et al.*, 2000) and plays a significant role in carbon sequestration in soil (Lal, 2008). In this study SIC was greater in the elevated CO₂ treatment on all sampled dates (Karberg *et al.*, 2005 and Stephen *et al.*, 2004). Our findings are also in accordance with the previous studies conducted by several workers.

5.1.5 Available nitrogen

The highest available nitrogen content in the soil was observed under the influence of elevated CO₂, which was on par with the rest of the altered conditions on both days of sampling. Barnard *et al.* (2005) reported that there was a significant decrease in soil nitrate concentration under elevated CO₂ condition. Hong *et al.* (2007) reported that elevated CO₂ did not significantly

affect soil N availability. The decreased soil N availability elevated CO₂ could be attributed to increased root growth and biomass, which led to higher N uptake by the plant (Stephen *et al.* 1994). Cheng *et al.* (2001) further confirmed the decreased soil N availability was also due to increased N mineralization, resulting in increased root biomass and thereby increased uptake of the nutrients by the plants.

5.1.6 Available phosphorus

In the present investigation, soil available phosphorus content was comparatively higher in elevated CO₂ condition than that of elevated CO₂ plus temperature, elevated temperature alone, ambient and open reference plot the at 30 and 60 DAS. Similarly, Abbas *et al.* (2009) also revealed that under free air CO₂ enrichment technology, soil phosphorus content was relatively higher as compared to ambient condition due increased soil P mineralization and thereby increased availability of soil P. Some evidences were shown in wheat experiment under FACE, such as higher root biomass and exudates, which might have enhanced the availability of soil P (Xie *et al.*, 2002 and Ma *et al.*, 2005). Barrett *et al.* (1998) also showed that elevated CO₂ increased phosphatase activity in the rhizosphere soil. This may be one of the reasons why soil extractable P was increased under elevated CO₂. The results of the present investigation have clearly shown that an increased level of both acid and alkaline phosphatase under the influence of elevated CO₂.

5.1.7 Available potassium

Available soil potassium content was higher under elevated temperature condition than that of elevated CO₂ condition at 30 DAS as compared to other altered conditions. Similar trend was also recorded at 60 DAS with elevated CO₂. Our results are in accordance with the findings of Abbas *et al.* (2009), who reported that CO₂ enrichment promoted soil potassium content as compared to ambient condition.

5.1.8 Available sulphur

Sulphur is one of the important secondary elements, which is important to the groundnut crop for peg formation and pod development. In the present investigation, available sulphur content under the influence of elevated CO₂ condition at 30 and 60 DAS was significantly higher as compared to ambient CO₂ condition. This may be due to higher mineralization of S by the microbial activity.

5.2 Soil biological properties as influenced by elevated CO₂ and/ or temperature in groundnut rhizosphere soil

5.2.1 Population of bacteria, fungi and actinomycetes as influenced by elevated CO₂ and/ or temperature in groundnut rhizosphere soil

Microbial populations in the rhizosphere soil determine the soil fertility and nutrient availability to crops. In the present investigation, the total bacterial, fungal and actinomycetes populations associated with groundnut rhizosphere soil was comparatively higher under elevated CO₂ condition than that of elevated CO₂ plus temperature, elevated temperature, ambient and open reference plot at 30 and 60 DAS. Similarly, Drigo *et al.* (2008) studied the impact of elevated CO₂ on rhizo-bacterial communities and revealed that elevated CO₂ significantly influenced the bacterial population. Interestingly, within the bacterial community, the genera *Burkholderia* and *Pseudomonas* were strongly influenced by elevated CO₂. Marilley *et al.* (1999) also reported that elevated CO₂ increased the dominance of *Pseudomonas* sp. over ambient condition. It is very well documented by several workers that plant ecosystems respond to rising CO₂ concentrations with increased photosynthesis, growth and resource allocation (Jablonski *et al.*, 2002). Thus, an increase in the atmospheric CO₂ concentration might have increased the production of organic compounds by plants root and also root exudation, altered resource allocation, and this might have affected biomass, community structure, and the activity of soil microbes through quantitative alterations in C nutrient availability (Haase *et al.*, 2007). Xuefeng *et al.* (2010) reported that the fungal population was amplified under elevated CO₂ condition than that of ambient condition due to the increased rhizodeposition under elevated CO₂ condition. Fungal growth may have been enhanced by the elevated CO₂ treatment as a result of the increased primary production in these plots (Kammann *et al.*, 2005). The new C entering the soil under elevated CO₂

might favour organisms with lower nutrient requirements. Fungi are characterised by a high C: N ratio compared to bacteria, explaining why elevated CO₂ might affect fungi more readily than bacteria (Blagodatskaya *et al.*, 2010).

5.2.2 Functional groups as influenced by elevated CO₂ and/ or temperature in groundnut rhizosphere soil

Biological nitrogen fixation is an energy demanding process and is catalyzed by nitrogenase enzyme complex. Findings of present study revealed that the population of free living nitrogen fixers was significantly increased under the influence of elevated CO₂ condition compared to other conditions at 30 and 60 DAS. These results are in accordance with the findings of Xuefeng *et al.* (2010). Elevated CO₂ influenced the soil organic matter decomposition as well as N mineralization ensuing increased activity of N₂-fixing bacteria (Zak *et al.*, 2000; Allard *et al.*, 2006 and Langley *et al.*, 2009). Another important functional group of agriculturally important microorganisms is phosphate solubilizers. Phosphate solubilizing microorganisms are known to improve P-solubilization, thereby improving crop growth and yield. In the present investigation, there was a significant increase in the abundance of P- solubilizers in the groundnut rhizosphere soil exposed to elevated CO₂ at both the sampling dates. The overall increase in P-solubilizers may be correlated to increased root exudation under elevated CO₂ condition (Haase *et al.*, 2007).

5.2.3 Soil protozoa number as influenced by elevated CO₂ and/ or temperature in groundnut rhizosphere soil

Protozoans are ubiquitous and are abundant in virtually all natural soils (Ekelund *et al.*, 1994). In the present investigation, the protozoa were significantly influenced under elevated CO₂ conditions compared to other environmental condition at 30 and 60 DAS of crop growth period. The results of our findings are supported by several authors, stating that of density of protozoan population significantly increased under elevated CO₂ condition and documented amoeba, flagellates and few ciliates and their numbers were greater by 2.5 times under elevated CO₂ as compared to ambient condition. The increasing protozoan population under elevated CO₂ might be linked to increased carbon availability

(Roger *et al.*, 2001 and Regin *et al.*, 2002). This may also be related to higher bacterial population on which protozoa depend on.

5.2.4 Qualitative estimation of algae as influenced by elevated CO₂ and/ or temperature in groundnut rhizosphere soil

In the present investigation, the qualitative assay has confirmed the occurrence of the following cyanobacterial groups viz., *Achnanthydium*, *Anabaena*, *Chlorococcus*, *Diatoms*, *Gleocapsa*, *Lyngbia*, *Nitzschia*, and *Oscillatoria* under all the conditions. However, the visual rating through microscopic observations have revealed the abundance of *Anabaena*, *Chlorococcus*, *Diatoms* under elevated CO₂ as well as elevated CO₂ plus temperature conditions indicating the higher requirement of CO₂ by these selective prokaryotic photosynthetic algal groups (Albert *et al.*, 2011).

5.3 Soil enzyme activities as influenced by elevated CO₂ and/ or temperature in groundnut rhizosphere soil

5.3.1 Dehydrogenase activity as influenced by elevated CO₂ and/ or temperature in groundnut rhizosphere soil

Soil enzymes play a key role in controlling the decomposition of soil organic matter and have been widely accepted as indicators of changes in below ground processes (Sinsabaugh and Moorhead, 1994). Elevated CO₂ influenced the soil enzyme activities through altering the quantity and quality of plant-derived substrates entering the soil (Zak *et al.*, 2000). The impacts of elevated CO₂ on soil enzyme activities have gained wide attention in the last few years. Elevated CO₂ compounded by associated rise in ambient temperature and less water availability resulted in a profound impact on soil enzyme activities (Aranjuelo *et al.*, 2007).

Dehydrogenase activity is considered as an indicator of the oxidative metabolism in soils and thus of the microbiological activity (Włodarczyk *et al.*, 2002). Das *et al.* (2011), while evaluating the interactive effects of elevated CO₂ and temperature on soil enzymes in the tropical soils, have recorded significant influence of elevated CO₂, compounded by elevated temperature and soil

moisture stress greatly influencing the soil enzymes. Even in the present investigation, similar results have been recorded, where in the dehydrogenase activity was significantly higher under elevated CO₂ plus temperature condition at 30 and 60 DAS. Similarly, Bhattacharyya *et al.* (2013) also revealed higher dehydrogenase activity under elevated CO₂ plus temperature condition. The higher dehydrogenase activity under the exposure to elevated CO₂ condition may be correlated to higher organic matter, total microbial population and MBC content (Saurav *et al.*, 2011 and Xuefeng *et al.*, 2010). In the present investigation also the highest MBC was recorded under the influence of elevated CO₂ condition compared to ambient condition.

5.3.2 Phosphatase activity as influenced by elevated CO₂ and/ or temperature in groundnut rhizosphere soil

The enzyme phosphatase play an important role in the transformation of organic phosphorus into inorganic phosphorus in soil and these activities are the important factors in maintaining as well as controlling of the phosphorus cycle. In the present investigation, both acid and alkaline phosphatase activities were significantly higher under elevated CO₂ condition, which is followed by elevated CO₂ plus temperature at 30 and 60 DAS. Similar results were also documented by several workers (Kang *et al.*, 2005; Das *et al.*, 2011; Kang *et al.*, 2005 and Guenet *et al.*, 2012). This result might be explained by the fact that increased phosphatase under elevated CO₂, would be benefitting the soil microorganisms in order to use the energy present in the fresh C inputs to obtain the P they needed (Reich *et al.*, 2006).

5.3.3 Urease activity as influenced by elevated CO₂ and/ or temperature in groundnut rhizosphere soil

The conversion of organic N to inorganic N through hydrolysis of urea to ammonia and CO₂ is an important pathway of N transformation in soils. Das *et al.*, (2011) and Feng *et al.*, (2007) unambiguously confirmed that elevated CO₂ condition recorded the highest urease activity in paddy rhizosphere soil and concluded that elevated CO₂ condition favoured conversion of organic N to inorganic N through hydrolysis of urea by urease activity. Our findings of the

present investigations are also in agreement with the finding of the previous workers as mentioned above, although not under paddy soils.

5.3.4 Cellulase activity as influenced by elevated CO₂ and/ or temperature in groundnut rhizosphere soil

Cellulose is one of the most important organic compounds in soil, which is metabolized by the action of enzyme cellulase. Cellulase plays an important role in recycling of polymers. In the present investigation, results have clearly revealed that cellulase activity was significantly influenced under elevated CO₂ condition as compared to other condition *viz.*, elevated CO₂ plus temperature, elevated temperature, ambient condition and open reference at 30 and 60 DAS. David *et al.* (2005) reported that the cellulase activity was significantly increased under free air CO₂ enrichment condition compared to ambient condition. Similarly, Xuefeng *et al.* (2010) revealed that the cellulase activity exhibited positive response to the elevated CO₂ condition as compared to ambient condition. The cellulase activity and cultivable cellulolytic fungal abundance showed similar responses to elevated CO₂, suggesting that the higher cellulolytic fungal abundance might have augmented the activity of cellulase under elevated CO₂ (Xuefeng *et al.*, 2010).

5.3.5 Soil microbial biomass carbon (MBC) and soil microbial biomass nitrogen (MBN) as influenced by elevated CO₂ and/ or temperature in groundnut rhizosphere soil

Microbial biomass is an important liable fraction of the soil organic matter, functioning as driving force in recycling organic matter. In the present investigation, the microbial biomass carbon was increased under the influence of elevated CO₂ condition at 30 and 60 DAS of crop growth period. Microbial biomass carbon was more responsive under elevated CO₂ condition as compared to ambient condition (Saurav *et al.*, 2011; Rakshit *et al.*, 2012 and Bhattacharyya *et al.*, 2013). The increase in MBC under elevated CO₂ may be due to the enhanced quantity of root exudates that acted as C-substrates for microbes which reflected in increased MBC (Emmerling *et al.*, 2000).

5.4 Influence of elevated CO₂ and/ or temperature in on Mycorrhizal parameters

5.4.1 AM fungal spore count as influenced by elevated CO₂ and/ or temperature in groundnut rhizosphere soil

Elevated CO₂ often increases mycorrhizal colonization of plant roots (Treseder, 2004), since elevated CO₂ enhances C allocation to roots (Rillig and Allen, 1998). Further, AM fungi rely on host plant carbon (Smith and Read, 1997) and mycorrhizal colonization increases sink strength of the root. Our experiment revealed that the AM fungal spore count as well as root colonization was significantly influenced by elevated CO₂ condition as compared to other altered conditions. A positive feedback loop can be envisaged, in which plants respond to elevated CO₂ by increased C fixation followed by the transfer of more carbon to their root systems. Consequently, mycorrhizal fungi might grow more and capture more phosphate (Maria *et al.*, 2008).

5.4 AM fungal community as influenced as influenced by elevated CO₂ and/ or temperature

In this study, as many as 39 AM fungal morphotypes belonging to four genera were isolated and tentatively identified. The highest number of native AMF species was recorded with elevated CO₂ plus temperature (20.00), followed by reference plot (14), elevated CO₂ (13), elevated temperature (11) and the lowest number of species was recorded under ambient condition (9). Similar variation in AMF species composition has been reported by Anita *et al.* (2011).

5.4.3 Abundance of AM fungal spores of different species as influenced by elevated CO₂ and/ or temperature condition

AM fungal abundance was measured by enumerating the spore density in rhizosphere soil the soil sample. In the present investigation, spores of *Glomus fasciculatum* were the most abundant under elevated CO₂ plus temperature condition (35 spores 50 g⁻¹soil) and also under elevated CO₂ condition (37

spores 50 g^{-1} soil). While *Glomus mossae* was the most abundant type under elevated temperature, ambient and in open reference ring (26; 28 and 33 spores 50 g^{-1} soil respectively). Anita *et al.* (2011) recorded three significant indicator species of elevated CO_2 belonging to *Glomus microaggregatum*; *Glomus constrictum* and *Entrophospora. infrequens* compared to ambient condition, indicating the diverse response of mycorrhizal strains towards altered conditions (Stephane *et al.*, 2010). Suggesting that AMF belonging to *Glomus* are likely to be more abundant under elevated environmental condition

5.4.4 Diversity of AMF as influenced by elevated CO_2 and/ or temperature based on spore count

In the present study, variation in Shannon diversity index of AM fungal species in altered environmental conditions viz., elevated CO_2 plus temperature, elevated CO_2 , elevated temperature, ambient condition and reference plot was observed based on the spore load.

The Shannon diversity index of AM fungi was more in elevated CO_2 plus temperature condition (3.10), followed by reference plot (2.45), and elevated temperature condition (2.37) based on the spore count. Suggesting that altered environmental conditions positively influence AMF diversity.

In the present investigation, it was observed that there was a clear difference in the AM fungal species richness between the altered conditions. The Margalef species richness was greater under the influence of elevated CO_2 and temperature (2.66) followed by reference plot (1.86) and the lowest species richness was recorded in ambient condition (1.37). Similar variation in AMF species composition has been reported by Anita *et al.* (2011). Combination of elevated CO_2 plus temperature condition could promote specific AM fungal groups reflecting in species richness.

5.5 Molecular characterization of efficient native AMF by amplification of its region of 18s rDNA subunit

As many as five morphotypes were chosen based on the abundance in the all five altered conditions compared to other morphotypes. These five AM

fungal morphotypes were genetically characterized by PCR amplification of internal transcribed species of the ribosomal DNA (ITS18S rDNA). The obtained morphotype was confirmed as *Glomus mosseae* by amplifying with the *Glomus* specific primers GloITS-I and GloITS-II (Jansa *et al.*, 2002 and Jones *et al.*, 2012).

6. SUMMARY AND CONCLUSIONS

The present investigation was undertaken during kharif 2012-13 to explore the possible effects of elevated CO₂ and/ or temperature on soil physico-chemical and biological properties under altered conditions at Institute for Agricultural Research on Climate Change, Main Agricultural Research Station, University of Agricultural Sciences, Dharwad

1. The per cent soil water stable aggregates were increased under elevated CO₂ condition at 30 and 60 DAS. Whereas, the lowest SWSA was recorded in the open reference ring.
2. Soil pH and soil EC in the groundnut rhizosphere soil did not vary significantly under altered condition at both cropping period. However, the observation at 60 DAS revealed that, relatively higher soil pH and EC in the soils that were exposed to elevated CO₂
3. The soil organic carbon content at 30 DAS increased significantly under elevated CO₂ compared elevated CO₂ plus temperature and elevated temperature. Similar results were also recorded at 60 DAS, with relatively increased percentage of soil organic carbon compared to 30 DAS.
4. The highest soil inorganic carbon content was recorded with elevated temperature followed by elevated CO₂ and elevated CO₂ plus temperature. However, the results were on par with each other.
5. Highest available nitrogen was observed in elevated CO₂ condition at 30 and 60 DAS. The lowest available nitrogen content was observed in the reference plot. However, the results were statistically on par with each other across the altered conditions.
6. Soil available potassium exhibited significant difference in the elevated temperature condition compared to elevated CO₂ conditions at 30 and 60 DAS.
7. Sulphur content in the soil exhibited significant increase under the influence of elevated CO₂ condition.

8. The bacterial, fungal and actinomycetes population was significantly influenced with elevated CO₂ condition.
9. Elevated CO₂ condition significantly influenced the functional groups viz., free living nitrogen fixers and P solubilizers.
10. The population of Fluorescent pseudomonads at 30 DAS did not differ significantly under the influence of altered conditions. At 60 DAS, the soil exposed to elevated CO₂ alone harbored the highest Fluorescent pseudomonads population.
11. Soil protozoan density was increased under elevated CO₂ condition compared to rest of the altered condition.
12. In the present investigation the qualitative assay have confirmed that the occurrence of the following cyanobacterial groups viz., *Achnanthidium*, *Anabaena*, *Chlorococcus*, *Diatoms*, *Glyocapsa*, *Lyngbia*, *Nitzschia*, and *Oscillatoria* under all the conditions. However, the visual rating through microscopic observations have revealed that abundance of *Anabaena*, *Chlorococcus*, *Diatoms* under elevated CO₂ as well as elevated CO₂ plus temperature conditions.
13. The highest dehydrogenase activity was recorded in the treatment which received elevated CO₂ plus temperature followed by elevated CO₂ alone, while reference plot recorded lowest activity.
14. Highest activity under the influence of elevated CO₂ alone followed by CO₂ plus temperature and temperature alone.
15. The highest acid and alkaline phosphatase activity was observed under elevated CO₂ condition followed by elevated CO₂ plus temperature.
16. At 30 DAS, all the five conditions were statistically on par with each other in influencing the urease activity. However at 60 DAS, significantly higher urease activity was observed under elevated CO₂ condition followed by interaction of elevated CO₂ plus temperature condition and elevated temperature alone. The lowest urease activity was recorded in the reference plot.

17. Cellulase activity was significantly influenced under elevated CO₂ condition at both 30 and 60 DAS. The lowest cellulase activity was measured under ambient condition followed by the reference plot.

18. The greatest values for microbial biomass carbon were recorded at 30 DAS in the treatment which received elevated CO₂ followed by elevated temperature, which is statistically at par with the soil exposed to elevated CO₂ and temperature. In general, MBC in the ambient and reference plot always recorded the lowest values compared to the other altered conditions.

19. The soil microbial biomass nitrogen also responded to altered conditions similar to MBC. There were significant differences observed between the altered conditions in influencing the MBN

20. Thirty nine species of AM fungi were identified from five altered condition. Twenty species were recorded under elevated CO₂ plus temperature condition; fourteen species were recorded in reference plot; thirteen species were recorded under elevated CO₂ condition; eleven species were recorded under elevated temperature condition and lowest AMF species were recorded under ambient condition

21. *Glomus fasciculatum* spores were most abundant in elevated CO₂ plus temperature condition. Elevated CO₂ condition harbored maximum *Glomus fasciculatum*, while *Glomus mossae* recorded maximum spore abundance in elevated temperature, ambient and in open reference ring

22. Highest Shannon diversity index was recorded under elevated CO₂ plus temperature condition based on spore count. However, highest species richness was recorded in the soil received elevated CO₂ plus temperature condition.

23. The results of the morphological characterization of the native isolates under five altered conditions have clearly revealed that *Glomus mossae* was the most predominant species in all the five conditions based on their occurrence and abundance. Further, the native *Glomus mossae* isolates were genetically authenticated by the amplification of 375 bp specific DNA band in the ITS region of AM fungi.

24. The present investigation will form a base to understand the altered conditions on soil physico-chemical and biological properties. Furthering, this study will also bring out an efficient climate resilient AMF culture collection to sustain the soil health under the anticipatory climate change.

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**Appendix I: Monthly meteorological data for the year 2013 at Main
Agricultural Research Station, Dharwad**

Months	Temperature		RH%	Rainfall (mm)	No. of rainy days
	Max. °C	Min. °C			
January	31.2	14.5	47	0.0	-
February	32.6	16.8	51	02.2	-
March	35.3	19.2	48	42.0	1
April	36.9	20.2	51	10.0	1
May	35.5	21.8	61	124.6	6
June	28.0	20.8	82	75.4	10
July	25.4	20.3	89	177.8	17
August	26.7	19.9	85	97.2	10
September	27.9	20.2	81	133.6	13
October	28.8	19.4	76	75.4	5
November	29.0	15.8	64	2.2	-
December	28.4	12.7	53	0	-
Total				740.4	63

Appendix II. Composition of growth media/reagents/indicators/stains used

Growth medium	Qty,(g/l)	Growth medium	Qty,(g/l)
A) Nutrient agar		C) Pikovskaya agar	
Peptone	5.00	Glucose	10.00
Beef extract	3.00	MgSO ₄ (2.5%)	10.00
Sodium chloride	5.00	CaCl ₂ (1%)	10.00ml
Agar	18.00	Tricalcium phosphate	5.00
pH	6.8-7.00	Agar	18.00
		pH	7.00
B) Rose Bengal agar		D) NaCl agar	
Glucose	10.00	NaCl	5.00
Peptone	5.0	Agar	18.00
KH ₂ PO ₄	1.0	Distilled water	1000 ml
MgSO ₄	0.05		
Streptomycin	15.0	E) NaCl broth	
Agar	0.035	NaCl	5.00
Rose Bengal	18.08	Distilled water	1000ml
Distilled water	1000 ml		
pH	5.5		

Reagent	Qty,(g)	Reagent	Qty,(g)
F) Kuster's Agar		H) Norris N-free agar	
Starch	10.00	K ₂ HPO ₄	1.00
Casein	0.3	MgSO ₄	0.20
KNO ₃	2.0	CaCO ₃	1.00
NaCl	2.0	NaCl	0.20
K ₂ HPO ₄	2.0	Glucose	10.00
MgSO ₄	4.3	Agar	18.00
CaCO ₃	0.20	pH	7.2
FeSO ₄ .7H ₂ O	0.001	Distilled water	1000 ml
Agar	18		
pH	7.2		
Distilled water	1000 ml		
G) King's B agar		I) Luria broth	
Peptone	16.00	Tryptone	10.00
MgSO ₄	1.60	Yeast extract	5.00
K ₂ HPO ₄	1.60	NaCl	5.00
Glycerol	10 ml	Distilled water	1000ml
Agar	18.00		
pH	7.00		

Growth medium	Qty,(g/l)	Growth medium	Qty,(g/l)
J) EMB agar		L) BG-11 media	
Peptone	10.0	NaNO ₃	1.5
Lactose	5.0	K ₂ HPO ₄	0.04
Sucrose	5.0	MgSO ₄	0.075
K ₂ HPO ₄	2.0	CaCl ₂	0.036
Eosin Y	0.4	Citric acid	0.006
Methylene blue	0.06	Ferric ammonium citrate	0.006
Agar	18.0	EDTA	0.001
Distilled water	1000ml	NaCO ₃	0.02
pH	7.0	Trace element	1 ml
		Distilled water	1000ml
K) Phosphate buffer		Trace element	2.86
Monobasic sodium phosphate	27.8	H ₃ BO ₃	1.81
Distilled water	1000 ml	MnCl ₂	0.22
Di basic sodium phosphate	53.65	ZnSO ₄	0.39
Distilled water	56.5+43.5 ml	NaMOO ₄ .2H ₂ O	0.07
MSP+DSP	200 ml	CuSO ₄	0.494
Volume make up		CO(NO ₃) ₂ .6H ₂ O	1000ml
		Distilled water	

Reagent	Qty,(g)	Reagent	Qty,(g)
N)Hogland nutrient solution		O) MUB	
Ca(NO ₃)	0.940	Tris hydroxy methyl amino methane	12.1
KNO ₃	0.615	Maleic acid	11.6
MgSO ₄ .7H ₂ O	0.520	Citric acid	14.00
EDTA	0.0373	Boric acid	6.30
FeSO ₄ .7H ₂ O	0.0278	NaOH (1 N)	488
Micronutrients		P) Acetate buffer	
H ₃ BO ₃	0.028	Acetic acid	15.72 ml
MnSO ₄	0.034	CH ₃ COONa	34.02
ZnSO ₄ .7H ₂ O	0.022	Distilled water (volume make up)	1000
(NH ₄) ₆ MO ₇ O ₂₄ .4H ₂ O	0.001	Q) Citrate buffer	
CuSO ₄ .5H ₂ O	0.001	Citric acid (0.1 M)	21.03
H ₂ SO ₄	5ml	Distilled water	1000 ml
Distilled water	1000	Sodium citrate (0.1 M)	29.41
N) DNS		Distilled water	1000 ml
3,5-Dinitro salysilic acid	1g	CA+SC	16 ml +34 ml
Distilled water	50 ml	Distilled water	50 ml
NaOH (16%)	10 ml	SnCl ₂	160 mg
Potassium sodium tartarate	30 g		
Volume makeup	100 ml		

Reagent	Qty,(g)
R) Trypan blue stain	
Trypan blue	0.05
Lactic acid	50 ml
Glycerin	10 ml
water	40 ml

Appendix III. Stock solution for molecular characterization

	Reagent/stock solution	Method of preparation	
1	1M Tris HCl (pH8.0)	Tris base	121.1 g
		water	800 ml
		HCl	42 ml
		Final volume	1000 ml
2	0.5M EDTA (pH8.0)	Disodium EDTA.2H ₂ O	186.1g
		water	800 ml
		NaOH	20 g
		Final volume	1000 ml
3	5M NaCl	NaCl	292 g
		water	800 ml
		Final volume	1000 m
4	10N NaOH	NaOH	400 g
		water	800 ml
		Final volume	1000 ml
5	Potassium acetate buffer	5M Potassium acetate	60 ml
		Glacial acetic acid	11.5 ml
		Final volume	100 ml
6	Ethidium bromide	Ethidium bromide	1g
		water	100 ml
7	1% agarose gel	agarose	1.5 g
		1XTAE	100 ml
		Et Br(10mg/1000μl)	8 μl

8	Loading dye	Bromophenol blue	0.25%
		Xylene cyanol	0.25%
		Glycerol	30% in water
9	50X TAE buffer	Tris base	242 g
		Acetic acid	57.1 ml
		0.5M EDTA	100 ml
		Final volume	1000 ml

IMPACT OF ELEVATED CARBON DIOXIDE AND/OR
TEMPERATURE ON ARBUSCULAR MYCORRHIZAL FUNGAL
COMMUNITY IN GROUNDNUT RHIZOSPHERE SOIL

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2014

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ABSTRACT

Investigations were carried to study the impact of elevated carbon dioxide and temperature on arbuscular mycorrhizal fungal community in groundnut rhizosphere under the open top chamber facility at Institute for Agricultural Research on Climate Change, University of Agricultural Sciences, Dharwad. The results of the investigations have revealed that the higher soil water stable aggregate, organic carbon, inorganic carbon, available P, K and S were recorded with elevated CO₂. However, soil pH, EC and available nitrogen did not vary significantly. The population of soil bacteria, fungi, actinomycetes, free living nitrogen fixers, fluorescent pseudomonads and P- solubilizers was significantly influenced due to elevated CO₂ compared to other altered conditions. The soil enzyme activity viz., dehydrogenase, urease, cellulase, acid phosphatase, alkaline phosphatase, soil biomass carbon and nitrogen were greatly influenced by elevated CO₂ condition. The Shannon diversity index was the highest with elevated CO₂ plus temperature ($H'=3.10$), while elevated CO₂ recorded ($H'=2.25$). The Margalef's index of species richness for AMF was the highest in elevated CO₂ plus temperature (2.66) compared to ambient condition (1.37). The most predominate AM fungal species, *Glomus mosseae* from all the five altered conditions were genetically characterised by amplifying a single fragment of 375 bp in the ITS region.