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RESEARCH PAPER

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Influence of cyanobacterial inoculants, elevated carbon dioxide, and temperature on plant and soil nitrogen in soybean

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Abstract

Climate change affects nitrogen dynamics in crops and diazotrophic microorganisms with carbon dioxide (CO_2) sequestering potential such as cyanobacteria can be promising options. The interactions of three cyanobacterial formulations (*Anabaena laxa, Calothrix elenkinii* and *Anabaena torulosa–Bradyrhizobium japonicum* biofilm) on plant and soil nitrogen in soybean, were investigated under elevated CO_2 and temperature conditions. Soybean plants were grown inside Open Top Chambers under ambient and elevated (550 ± 25 ppm) CO_2 concentrations and elevated temperature (+2.5–2.8°C). Interactive effect of elevated CO_2 and cyanobacterial inoculation through *A. laxa* and *Anabaena torulosa–B. japonicum* biofilm led to improved growth, yield, nodulation, nitrogen fixation, and seed N in soybean crop. Nitrogenase activity in nodules increased in *A. laxa* and biofilm treatments, with an increase of 55% and 72%, respectively, over no cyanobacterial inoculation treatment. Although high temperature alone reduced soil microbial biomass carbon, dehydrogenase activity, and soil available N, the combined effect of CO_2 and temperature were stimulatory; cyanobacterial inoculation further led to an increase under all the conditions. The highest seed N uptake (758 mg plant^{-1}) was recorded with cyanobacterial biofilm inoculation under elevated CO_2 with control temperature conditions. The positive interactions of elevated CO_2 and cyanobacterial inoculation, particularly through *A. laxa* and *A. torulosa–B. japonicum* biofilm inoculation highlights their potential in counteracting the negative impact of changing climate along with enhancing plant and soil N in soybean.

Abbreviations: ARA, acetylene reduction assay; CFU, colony forming units; DAP, diammonium phosphate; FACE, free air carbon dioxide enrichment; GC, gas chromatograph; GI, galvanized iron; ICAR-IARI, Indian Council of Agricultural Research-Indian Agriculture Research Institute; IPCC, Intergovernmental panel on climate change; IRGA, Infrared gas analyser; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; MOP, muriate of potash; OTC, Open top chambers; PGPR, Plant Growth Promoting Rhizobacteria; PVC, polyvinyl chloride; TPF, triphenylformazan; TTC, triphenyltetrazolium chloride.

KEYWORDS
cyanobacteria, elevated CO_2, high temperature, soil N, soybean

1 | INTRODUCTION

The last few decades have witnessed a rapid rise in the earth's surface temperatures, along with an increase in the atmospheric concentrations of greenhouse gases (GHGs) due to different anthropogenic activities. Carbon dioxide (CO_2) concentration in the atmosphere has increased from 280 to 400 ppm [1], and the Intergovernmental Panel on Climate Change (IPCC) report has reiterated that the warming of the climate system is unequivocal [2]. The increased concentration of atmospheric CO_2 has a fertilization effect on plants while an increase in temperature shortens the crop growth period and reduces yield [3–6]. A rise in temperature also hastens organic matter mineralization [7] and elevated CO_2 concentrations might increase nutrient requirements thereby causing N limitation in crop plants [8,9]. This is supported by reports that many C3 crops grown under elevated CO_2 concentrations have less protein content [10,11]. Leguminous crops have an advantage, due to their ability to fix atmospheric nitrogen (N_2), hence, can use the additional carbon for nitrogen fixation under elevated CO_2 concentration more effectively [12,13]. In cropland and other managed ecosystems, leguminous crops are known to respond to high CO_2 levels, as compared with other crops such as rice, maize, sorghum, and so on [14].

Soybean (*Glycine max* (L.) Merrill) is an important leguminous crop, being the richest and cheapest source of high-quality proteins and fats. Soybean grows well in a warm and moist climate. Free air carbon dioxide enrichment (FACE) studies showed an increase in aboveground biomass in soybean under elevated CO_2 conditions [15] and also a significant yield increase under elevated CO_2 and temperature than ambient conditions [16]. Rogers et al. [13] reported that under elevated CO_2 concentration, leaf nitrogen in soybean decreased at the initial growth stages, but once the N_2 fixation process was initiated, nitrogen levels were moderated. A study conducted in central India showed that the N requirement in soybean will be more under elevated CO_2 conditions [17]. The legume–rhizobia symbiosis is sensitive to temperature, and root temperature affects the formation and development of nodules as well as its activity [18]. Although rhizobial inoculants are widely used for enhancing the yields of leguminous crops, recent reports illustrate the significant role of augmenting with Plant Growth Promoting Rhizobacteria (PGPR) to alleviate the negative effects of abiotic stresses like high-temperature stress, sodicity, water stress, etc, and stimulating crop yields [19].

Cyanobacteria are known for their diazotrophic potential and are widely used as inoculants in different crops, besides rice [20,21]. Biofilms represent robust assemblages, with the mucilaginous sheath serving as a habitat for one or more types of organisms, belonging to the same/different phyla, which can survive and proliferate even under extremes of environment and facilitate better nutrient translocation [22]. Beneficial biofilms, comprising fungi/cyanobacteria/bacteria have been developed which can grow and colonize plant roots to facilitate more efficient nutrient cycling and act as biocontrol agents [23–25]. With their CO_2 sequestering ability, resilience and adaptation to abiotic stresses, and nitrogen-fixing potential [26], cyanobacteria represent ideal candidates for stress alleviation in soybean grown under elevated CO_2 and temperature. Cyanobacteria such as *Anabaena laxa* (RPAN8) and *Calothrix elenkinii* (RPC1) have been evaluated for their plant-growth-promoting and biocontrol potential and found promising in several legumes [12,27]. Earlier reports of increased yield and biomass accumulation in mungbean crops at elevated CO_2 levels, using cyanobacterial inoculation [28,29] support our hypothesis that similar beneficial changes may be observed in soybean. Cyanobacterial inoculation and nitrogen fixation can help in improving soil N status under changing climatic conditions. However, the response to cyanobacterial inoculation to both high temperature and elevated CO_2 conditions, and their effect on crops has not been investigated. Overall, very limited information is available on the interactive effect of elevated CO_2 and temperature on soil nitrogen [30]. Hence, this study was undertaken to investigate the interactive effect of elevated CO_2 and temperature, on plant and soil nitrogen (N) in soybean receiving cyanobacterial inoculation.

2 | MATERIALS AND METHODS

2.1 | Experimental setup

An experiment was conducted at the Genetics-H field of ICAR-Indian Agricultural Research Institute (IARI), New Delhi situated at 28°35′ N and 77°12′ E with an altitude of 228 m above mean sea level. The climate of the experimental site was semi-arid and subtropical. The mean annual maximum and minimum temperatures were 35°C and

18°C, respectively, while annual rainfall was around 750 mm. The seasonal mean temperature during the crop growth period was 28°C. Soybean variety Pusa 9712 was grown in pots during the *Kharif* season (July to October) of the year 2018 inside the Open Top Chambers (OTC) located in the field (Supporting Information: Figure S1a). The OTCs were cylindrical in shape and fabricated with galvanized iron (GI) pipe (Supporting Information: Figure S1b). The sidewalls of the OTCs were made up of polyvinyl chloride (PVC) sheets having 90% transparency. The top of the OTCs was kept open to provide natural air exchange, as well as to maintain ambient humidity and temperature [31]. Separate OTCs were there for each treatment, that is, chamber control, elevated CO_2, elevated temperature, and both elevated CO_2 plus temperature. Two levels of carbon dioxide concentrations were maintained inside the OTCs, that is, ambient concentration of around 400 ppm and elevated CO_2 concentration (550 ± 25 ppm). Since plants use CO_2 for photosynthesis during the daytime, the elevated CO_2 level was maintained only during the daytime. Carbon dioxide gas was supplied into the OTCs from CO_2 gas cylinders (commercial grade) of 30 kg capacity through 6 mm polyurethane tubes to maintain elevated CO_2 concentration inside the OTCs. Desired CO_2 concentration inside the OTCs was maintained using solenoid valves and rotameters [31]. Air inside the OTCs was pumped and analyzed using Infrared Gas Analyser (IRGA) (Supporting Information: Figure S1c). Besides CO_2, the elevated temperature was also maintained inside the OTCs by partially covering the upper portion of the OTCs (Supporting Information: Figure S1d). Two temperature levels, that is, chamber control and elevated temperatures were maintained. Daily maximum temperature and minimum temperatures were recorded in each OTC, using a digital thermometer kept within the OTCs. From the daily temperature data, daily mean and seasonal mean temperature were calculated for all the OTCs. The seasonal mean temperature in the elevated temperature treatment was 2.5°C higher than chamber control, while the temperature in the elevated CO_2 plus temperature treatment was 2.8°C higher than the chamber control treatment. Soil temperature was measured at weekly intervals using a soil thermometer and it was observed that it was 0.8°C–1°C higher in elevated CO_2 plus temperature treatment than in chamber control.

2.2 | Crop management

Soybean variety, Pusa 9712 was grown in pots inside the OTCs. The bulk soil was collected from the field and each pot was filled with 15 kg of soil. The soil was slightly alkaline (pH 7.8) in reaction with nonsaline nature (EC 0.47 dS m^{-1}). It contained 0.5% organic C and 200.4 kg ha^{-1} available N. Soybean seeds for all treatments were inoculated with *Bradyrhizobium japonicum* obtained from the Division of Microbiology, ICAR-IARI New Delhi and coated using carboxymethyl cellulose (1%) as adherent, based on the recommended rate. During sowing, diammonium phosphate (DAP) was applied to supply a recommended dose of nitrogen (20 kg N ha^{-1}) and phosphorus (60 kg P ha^{-1}) to the crop while muriate of potash (MOP; potassium chloride) was applied to supply potassium (40 kg K ha^{-1}). Four different treatments were used for the *B. japonicum* coated seeds (i) no cyanobacterial inoculation, cyanobacterial inoculation with (ii) *Calothrix elenkinii (C. elenkinii),* (iii) *Anabaena laxa* (*A. laxa*; RPAN8), and (iv) *Anabaena torulosa—Bradyrhizobium japonicum* biofilm. Treatment details are given in Supporting Information: Table S1. The cyanobacterial cultures obtained from the Division of Microbiology, ICAR-IARI, New Delhi, were grown in Haffkine flasks with N-free BG-11 medium at 27 ± 2°C, maintained at 16:8 h light and dark cycles under white light (50–55 μmol photons m^{-2} s^{-1}) for a period of 21 days [32], harvested and kept for drying. Dried samples were powdered, and formulations were prepared by mixing with a carrier material (Paddy straw and vermiculite 1:1 ratio). For biofilm preparation, *A. torulosa* and *B. japonicum* were grown under their respective optimized conditions, with the former used as the matrix for developing biofilms [23,33]. Harvested biofilms were mixed with the carrier vermiculite: compost (1:1), keeping the cyanobacterial and rhizobial colony forming units (CFU) of inoculants as $10^4$and 10^8, respectively (based on plate count) and 10 μg chlorophyll *a* g^{-1} carrier, as optimized in earlier studies [20]. Aliquots of formulations (1 g) were added to each hole, before sowing the *B. japonicum* coated seeds, as optimized earlier [11,34]. For each treatment, there were six pots. The crop was sown in the last week of June and three plants were maintained in each pot.

2.3 | Crop growth and yield parameters

Photosynthesis rate was recorded using Infrared Gas Analyzer (LI-6400XT, LiCOR) during the vegetative stage of the crop. Observations were recorded between 9:00 and 11:00 a.m. in the morning on physiologically matured leaves. Plant samples were collected at maturity and seed weight per plant was recorded.

2.4 | Collection and processing of plant roots

The roots of three plants were collected with the help of a small shovel (*khurpi*), during the flowering stage (last week of August). The adhering soil was removed by placing it in flowing water. The weight of roots along with the nodules was recorded using a weighing balance.

2.4.1 | Nitrogenase activity

Acetylene reduction assay (ARA), an index of nitrogenase activity was estimated in the root nodules with the help of a gas chromatograph (GC), Model Bruker 450. Roots along with the nodules collected at the flowering stage were kept in test tubes (55 ml) and sealed by stoppers. The tubes were incubated for 1 h after replacing 10% of the air space (v/v) inside the tubes with acetylene following the methodology described by Prasanna et al. [35]. After 1 h of incubation, gas samples were analyzed in GC and the amount of ethylene produced was calculated and expressed as nmoles of ethylene per gram fresh weight of nodules per hour [12].

2.5 | Collection, preparation, and processing of soil samples

At the flowering stage (50 days after sowing), soil samples were collected from each pot. Soil samples were stored in the refrigerator at 4°C.

2.5.1 | Soil available nitrogen

Soil samples were collected from three pots and analyzed for their available nitrogen content using the method given by Subbiah and Asija [36]. Soil samples were distilled with alkaline potassium permanganate ($KMnO_4$) and sodium hydroxide (NaOH). The released ammonia was estimated by titrating it with standard sulfuric acid (H_2SO_4).

2.5.2 | Microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN)

The MBC in soil samples collected at the flowering stage was analyzed as per the method given by Jenkinson and Powlson [37]. Both fumigated and unfumigated soil samples were extracted with 0.5 M potassium sulfate (K_2SO_4). The extracts were treated and digested under refluxing conditions. Cooled samples were then titrated with ferrous ammonium sulfate solution. The same solution was used to extract MBN in soil by fumigation extraction method [37].

2.5.3 | Dehydrogenase activity

Soil samples collected at the flowering stage were saturated with triphenyl tetrazolium chloride (TTC) solution. The dehydrogenase activity in soils was estimated following the method given by Klein et al. [38] and expressed as μg TPF formed g^{-1} soil h^{-1}.

2.6 | Plant nitrogen

The crop was harvested during the third week of October. After harvest, soybean seeds were collected from each treatment and were dried in an oven at 65 ± 2°C for 72 h. Dried samples were ground in a Wiley mill and stored in an airtight container for further analysis. Nitrogen concentration in soybean seeds was analyzed by the method given by Jackson [39].

2.6.1 | Seed N uptake

N uptake in seed was calculated using the formula:

$$\text{Seed N uptake}(\text{mg plant}^{-1}) = \text{Seed weight}(\text{mg plant}^{-1}) x \text{ Seed N concentration}(\%)/100. \quad (1)$$

2.7 | Statistical analysis

The experiment was set up as a factorial completely randomized design. There were 16 treatments, with 6 pots for each treatment. (Supporting Information: Table S1). Statistical analysis of the data was done with the help of SAS software (ver. 9.3; SAS Institute Inc.).

3 | RESULTS

3.1 | Interactive effect of elevated CO_2 and temperature on growth and yield of soybean

The photosynthesis rate of soybean ranged from 15.8 to 24.6 μmol m^{-2} s^{-1} in different treatments (Figure 1a). The photosynthesis rate significantly increased under

elevated CO_2 conditions. Soybean plants grown with cyanobacterial biofilm inoculation showed a higher photosynthesis rate than others. Elevated CO_2 levels significantly increased the seed weight per plant while elevated temperature reduced seed yield (Figure 1b and Table 1). Application of cyanobacterial biofilm increased seed weight in soybean as compared with other treatments. The highest seed yield (10.9 g plant^{-1}) was recorded in cyanobacterial biofilm applied treatment under elevated CO_2 and chamber with control temperature conditions. Photosynthesis rate was found to be positively correlated with seed weight ($r = 0.84$) of the crop (Table 2).

3.2 | Interactive effect of elevated CO_2 and temperature on nodulation and nitrogen fixation in soybean

3.2.1 | Root and nodule weight

Elevated CO_2 levels significantly increased root with nodule weight in soybean plants at the flowering stage of the crop (Figure 2a). All the three cyanobacterial inoculations significantly increased root and nodule weight per plant as compared with no cyanobacteria treatment. Root with nodule weight was highest (5 g plant^{-1}) in cyanobacterial biofilm treatment under elevated CO_2 conditions.

3.2.2 | Nitrogenase activity

ARA is an index of nitrogenase activity of the nodules of legume crops. ARA was significantly higher in elevated CO_2 treatment than in ambient treatment (Figure 2b). Elevated CO_2 level increased nitrogenase activity by 47.8% over ambient conditions at the flowering stage of the crop. However, high temperature decreased the nitrogenase activity by 28.8%, as compared with chamber control treatment. Cyanobacterial inoculation as *A. laxa* and biofilm significantly increased the nitrogenase activity in soybean nodules by 55% and 72%, respectively, over treatment with no inoculation. Positive interactions of elevated CO_2 and cyanobacterial inoculation led to an overall enhancement in nitrogenase activity at the flowering stage in soybean crops. Nitrogenase activity was found to be positively correlated with the weight of root with nodules ($r = 0.78$). This indicated that more nodules weight under elevated CO_2 conditions and with cyanobacterial inoculation led to more N_2 fixation in soybean (Table 2).

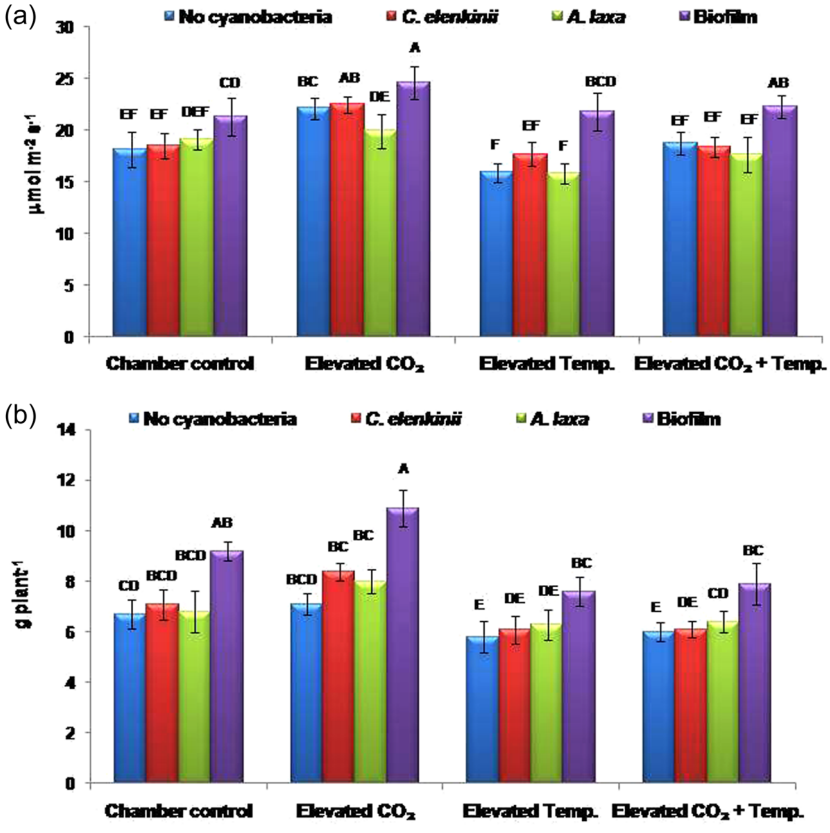

FIGURE 1 (a) Photosynthesis rate and (b) seed weight in soybean under elevated CO_2, high temperature, and cyanobacterial inoculation treatment at flowering stage. Column with different alphabets is significantly different ($p \leq 0.05$).

TABLE 1 Effect of elevated CO_2, high temperature, and cyanobacterial inoculation on seed and soil nitrogen in soybean

	Seed *N* (%)				Seed *N* uptake (mg plant^{-1})				Soil available N (kg ha^{-1})			
	Ambient CO_2		Elevated CO_2		Ambient CO_2		Elevated CO_2		Ambient CO_2		Elevated CO_2	
Treatment	CC Temp.	Elevated Temp.	CC Temp.	Elevated Temp.	CC Temp.	Elevated Temp.	CC Temp.	Elevated Temp.	CC Temp.	Elevated Temp.	CC Temp.	Elevated Temp.
Control	4.5	4.3	4.9	4.3	303	246	346	261	158	146	188	164
C. elenkinii	4.7	4.7	5.0	4.3	330	288	420	264	181	161	203	183
A. laxa	5.3	5.3	6.2	5.3	360	339	499	342	218	183	235	202
Biofilm[a]	5.8	5.8	6.9	5.9	531	440	758	471	296	280	323	299
LSD ($p \leq 0.05$)	CO_2: 0.2				CO_2: 31				CO_2: 12			
	Temp: 0.2				Temp: 31				Temp: 12			
	Cyan: 0.3				Cyan: 45				Cyan: 18			
	$CO_2 \times$ Temp:0.3				$CO_2 \times$ Temp: 45				$CO_2 \times$ Temp: NS			
	$CO_2 \times$ Cyan: NS				$CO_2 \times$ Cyan: NS				$CO_2 \times$ Cyan: NS			
	Temp $\times$ Cyan: NS				Temp $\times$ Cyan: NS				Temp $\times$ Cyan: NS			
	$CO_2 \times$ Temp $\times$ Cyan: NS				$CO_2 \times$ Temp $\times$ Cyan: NS				$CO_2 \times$ Temp $\times$ Cyan: NS			

Abbreviations: Control, no cyanobacteria; CC, chamber control; CO2, carbon dioxide; Cyan, cyanobacteria; LSD, least significant difference; NS, nonsignificant; Temp., temperature.

[a]A.*torulosa*–B. *japonicum* biofil.

TABLE 2 Correlation matrix of variables related to plant and soil N in soybean crop.

	Photosynthesis rate	Seed wt.	Root plus nodule wt.	Nitrogenase activity	Seed N (%)	Seed N uptake	MBC	MBN	Dehydrogenase activity	Soil available N
Photosynthesis rate	1.00									
Seed wt.	0.84**	1.00								
Root plus nodule wt.	0.66**	0.64**	1.00							
Nitrogenase activity	0.72**	0.80**	0.78**	1.00						
Seed N (%)	0.65*	0.82	0.70	0.78*	1.00					
Seed N uptake	0.78**	0.97**	0.69*	0.83	0.93**	1.00				
MBC	0.78**	0.80	0.78	0.84	0.66**	0.76	1.00			
MBN	0.89	0.86*	0.57	0.67	0.73	0.85**	0.65	1.00		
Dehydrogenase activity	0.69**	0.79	0.88	0.88	0.80**	0.81	0.91**	0.60	1.00	
Soil available N	0.77**	0.84**	0.66	0.77	0.89**	0.89	0.76**	0.79*	0.82	1.00

Note: * and ** Significant at 0.05 and 0.01 level (2-tailed), respectively.

Abbreviations: MBC, microbial biomass carbon; MBN, microbial biomass nitrogen.

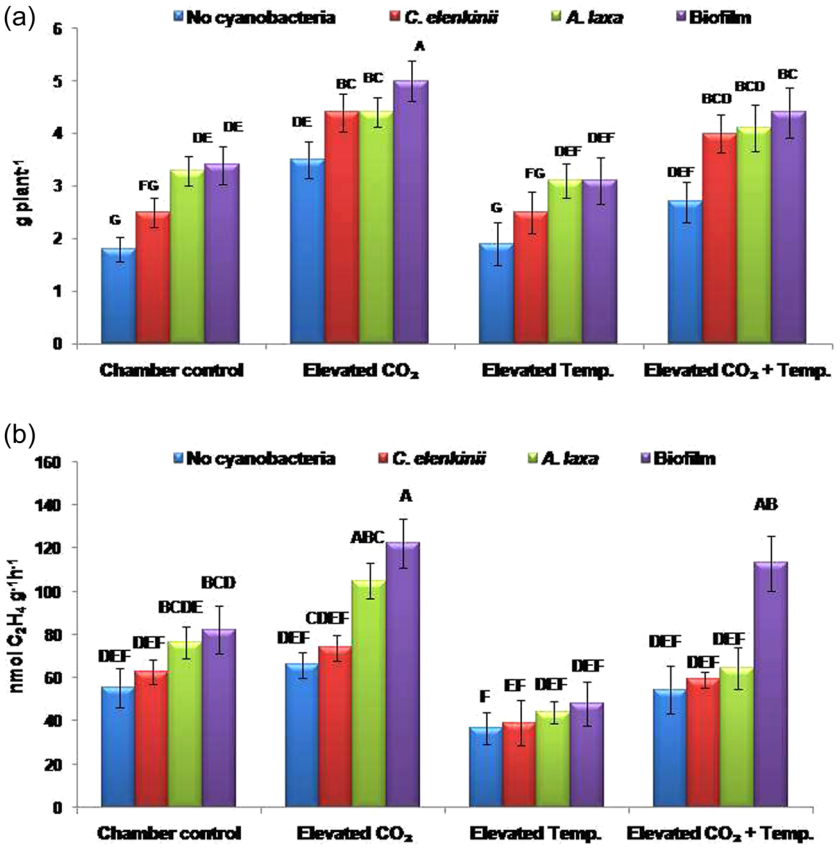

FIGURE 2 (a) Root with nodule weight and (b) acetylene reduction activity (ARA) in soybean roots under elevated CO_2, high temperature, and cyanobacterial inoculation treatment at the flowering stage. Column with different alphabets is significantly different ($p \leq 0.05$)

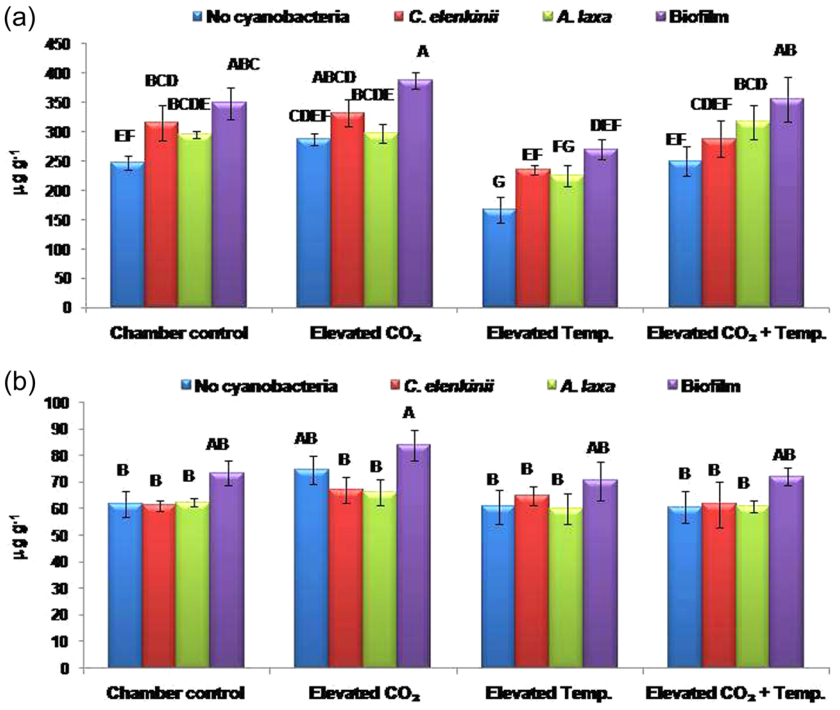

FIGURE 3 (a) Microbial biomass carbon (MBC) and (b) microbial biomass nitrogen (MBN) of soil under soybean grown in elevated CO_2, high temperature, and cyanobacterial inoculation treatment at flowering stage. Column with different alphabets is significantly different ($p \leq 0.05$)

3.3 | Interactive effect of elevated CO_2 and temperature on soil biological properties

3.3.1 | MBC

MBC of soil was significantly more under elevated CO_2 concentration than ambient, at the flowering stage of the crop (Figure 3a). The value of soil MBC was 314.6 $\mu g\, g^{-1}$ in elevated CO_2 treatment, while in ambient treatment it was 263.0 $\mu g\, g^{-1}$. MBC significantly reduced from 314.2 to 263.4 $\mu g\, g^{-1}$ in high-temperature treatment. Although high temperature reduced the MBC of soil, in elevated CO_2 plus high-temperature treatment, MBC was similar to chamber control. Elevated CO_2 levels might have increased the microbial activity as well as microbial biomass leading to higher MBC in soils under elevated CO_2 plus high-temperature treatment. The MBC of soil in the different treatments (*C. elenkinii, A. laxa*, and cyanobacterial biofilm) was 292.8, 283.8, and 340.7 $\mu g\, g^{-1}$, respectively.

3.3.2 | MBN

Elevated CO_2 concentration significantly increased MBN of soil at the flowering stage of the crop (Figure 3b). The highest value of soil MBN (84.1 $\mu g\, g^{-1}$) was recorded under elevated CO_2 conditions and chamber control temperature with cyanobacterial biofilm inoculation. Cyanobacterial inoculation with biofilm significantly increased soil MBN than other treatments by 16.3%–20.2%. Under ambient CO_2 and temperature control conditions, MBN was 73.5 $\mu g\, g^{-1}$ in cyanobacterial biofilm applied treatment while it was 61.9 $\mu g\, g^{-1}$ in treatment without cyanobacterial inoculation.

3.3.3 | Dehydrogenase activity

Dehydrogenase activity of soil at the flowering stage ranged from 1.3 to 5.6 $\mu g\, TPF\, g^{-1}\, h^{-1}$ in different treatments (Figure 4). Elevated CO_2 treatment showed significantly higher values of dehydrogenase activity (3.8 $TPF\, g^{-1}\, h^{-1}$) than ambient (2.9 $TPF\, g^{-1}\, h^{-1}$). Elevated temperature significantly reduced dehydrogenase activity (2.7 $TPF\, g^{-1}\, h^{-1}$) than chamber control treatment (4.0 $TPF\, g^{-1}\, h^{-1}$). Cyanobacterial inoculation as *A. laxa*, *C. elenkinii*, and cyanobacterial biofilm significantly increased dehydrogenase activity with values of 3.2, 3.7, and 4.5 $TPF\, g^{-1}\, h^{-1}$. In all the treatments, the dehydrogenase activity of soil showed a close relationship with soil available N (Figure 4, Supporting Information: Table S2).

3.4 | Interactive effect of elevated CO_2 and temperature on plant and soil nitrogen in soybean

3.4.1 | Seed N

Soybean seeds had higher nitrogen concentration under elevated CO_2 concentration, while the N concentration in seeds was lower under elevated temperature conditions. Seed N concentration was 5.4% in elevated CO_2 conditions, while it was 5.1% in ambient conditions (Table 1). High temperature reduced seed N to 5.0%, as compared with 5.4% in the chamber control treatment. Cyanobacterial inoculation with *A. laxa* and biofilm brought about a significant increase in seed N concentration. The highest seed nitrogen concentration (6.9%) was recorded under elevated CO_2 and chamber control temperature treatment with cyanobacterial biofilm inoculation. Multiple linear regression analysis revealed that seed N uptake was significantly related to nitrogenase activity in chamber control, elevated CO_2, and elevated CO_2 plus temperature treatment (Supporting Information: Table S2). But in elevated temperature treatments, seed N uptake was not significantly related to nitrogenase activity.

Higher seed N concentration along with higher seed weight increased seed N uptake under elevated CO_2 concentration. Seed N increased by 18.6% in elevated

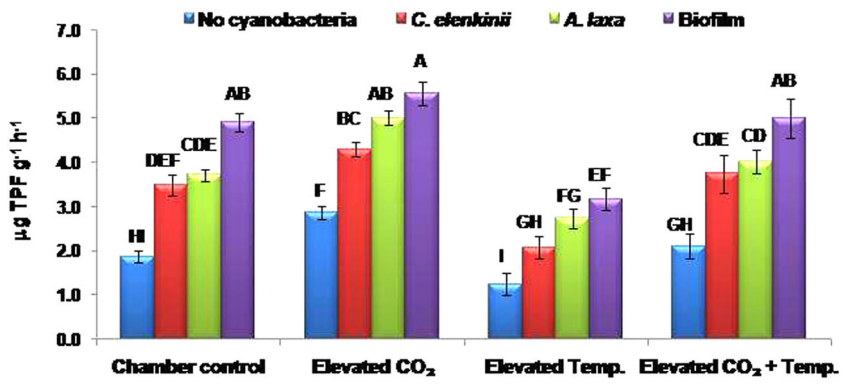

FIGURE 4 Dehydrogenase activity of soil under soybean, grown in elevated CO_2, high temperature, and cyanobacterial inoculation treatment at flowering stage. Column with different alphabets is significantly different ($p \leq 0.05$)

CO_2 treatment than that of ambient treatment (Table 1). On the other hand, the elevated temperature decreased seed N uptake by 25.3% more than chamber control. Cyanobacterial inoculation with *A. laxa* and biofilm significantly increased seed N uptake to other treatments. The highest seed N uptake (758 mg plant^{-1}) was found in biofilm inoculated treatment under elevated CO_2 and temperature. Nitrogenase activity in soybean nodules was positively correlated with seed N concentration ($r = 0.78$) (Table 2).

3.4.2 | Soil available nitrogen

Soil available N was significantly higher (225 kg ha^{-1}) in elevated CO_2 treatment than in ambient treatment (203 kg ha^{-1}) at the flowering stage of the crop. Elevated temperature significantly decreased soil available N to 202 kg ha^{-1} as compared with chamber control treatment (225 kg ha^{-1}) (Table 1). The highest value of soil available N was observed in cyanobacterial biofilm applied treatment under elevated CO_2 conditions and temperature control treatment (323 kg ha^{-1}). Cyanobacterial inoculation as *C. elenkinii, A. laxa*, or cyanobacterial biofilm significantly increased soil available N, to 182, 210, and 300 kg ha^{-1} over uninoculated treatments (164 kg ha^{-1}). Soil available N was positively correlated with MBC ($r = 0.76$) and MBN ($r = 0.79$) in soil (Table 2). From the multiple linear regression analysis, nitrogenase activity and soil available N were closely related in chamber control, elevated CO_2, and elevated CO_2 plus temperature treatment (Supporting Information: Table S2). On the other hand, the relationship between nitrogenase activity and soil available N was not significant in elevated temperature treatment. However, soil available N was found to be significantly related to seed N uptake in all the treatments (Supporting Information: Table 2).

4 | DISCUSSION

An increase in atmospheric CO_2 concentration is known to enhance the productivity of C3 crops like soybean, and in managed ecosystems, leguminous crops respond to elevated CO_2 levels more than nonlegumes [14]. Under elevated CO_2 conditions, legumes have an additional benefit over other crops due to their capacity to fix atmospheric N_2 [13]. But this benefit to legumes may be reduced, due to a rise in temperature, as it is one of the important limiting factors affecting the distribution and productivity of both crop species [40] and soil microbial communities [41]. In the present investigation, photosynthesis rate, seed weight, modulation, and nitrogen fixation in soybean significantly increased under elevated CO_2 concentration, with the increased weight of root with nodules and nitrogenase activity recorded. But the increase in temperature decreased seed weight, N_2 fixation, and seed N. Interactive effect of elevated CO_2 and cyanobacterial inoculation through *A. laxa* and biofilm further improved the growth, yield, nodulation, nitrogen fixation, and seed N in soybean crops. Contrasting reports on the effects of the increase in temperature on the growth and productivity of soybean are available; some researchers illustrate reduced growth [42,43], while several workers reported that under elevated CO_2 concentration, nitrogen fixation by legumes increases [12,44]. The positive effect of elevated CO_2 on nitrogen fixation is often limited by other nutrient deficiency [45], therefore, C and other macro and micronutrients in sufficient amounts are essential for improving productivity under these conditions. Increased CO_2 leads to greater carbon assimilation and more carbon partitioning to roots and nodules, thereby more nodulation and nitrogen fixation. However, high temperature significantly reduces total nitrogenase activity in soybean crops.

Land ecosystems are known to sequester carbon rapidly to counteract CO_2 emissions through stimulation in photosynthesis mediated carbon fixation by plants and photosynthetic microflora [46]. The role of photosynthetic microbes for CO_2 sequestration is of emerging interest as they are autotrophs and use sunlight and CO_2. Among them, diazotrophic cyanobacteria help in savings of nitrogenous fertilizers, and also improve plant growth and soil fertility [26,47]. As they utilize CO_2 for photosynthesis and evolve oxygen it can be a potential sink for atmospheric CO_2 [48]. The presence of mucilage or polysaccharide-rich sheath leads to the growth of microorganisms as biofilms, which are robust under extremes of environment and facilitate better nutrient translocation. Such biofilms may comprise single, or multiple organisms belonging to single genera or phyla [20,21] and can be interesting models of antagonism, synergism, and nutritional linkages. Cyanobacterial inoculation through biofilm was observed to significantly increase the belowground biomass of the crop, leading to the formation of more nodules, thereby fixing more amount of nitrogen. The study by Dey et al. [12] elaborated on the synergistic effect of elevated CO_2 and cyanobacteria in enhancing biological nitrogen fixation in cowpea crops. Enhancement of nitrogenase activity in chickpea was also recorded earlier in cyanobacteria inoculated treatments [21]. In this study, more N_2 fixation resulted in more N concentration and N uptake in soybean plants in elevated CO_2 and elevated CO_2 plus

temperature treatment, which was stimulated further by cyanobacteria application. Besides this, more biomass accumulation under elevated CO_2 conditions and cyanobacteria applied treatments led to increased seed N. More crop growth under elevated CO_2 conditions and cyanobacterial biofilm applied treatment led to the accumulation of more belowground biomass, and more N_2 fixation. Soil biological activity also increased under elevated CO_2 along with cyanobacterial inoculation. Higher N_2 fixation also increased soil available N in elevated CO_2 and elevated CO_2 plus temperature treatment which further improved seed N in the soybean plant. These organisms are promising as biofertilizing options and can improve crop yields as well as the nutrient status of soil [49]. It is well established that cyanobacteria fix atmospheric N_2 leading to enhancement of soil available N resulting in 25% N savings [21,34,50]. Hence, they can help in reducing the consumption of inorganic chemical fertilizers, providing both economic and environmental benefits.

The study illustrated that the performance of soybean in terms of N_2 fixation, as well as soil N, was superior to cyanobacterial biofilm inoculation under elevated CO_2 and high-temperature conditions. Hence, cyanobacterial inoculation, particularly, novel cyanobacteria-based biofilm developed using *Bradyrhizobium* sp. as a partner can be explored as a promising option to improve crop productivity, seed N content and soil N status in soybean cultivation under elevated temperature and CO_2 scenarios.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

[1] Dlugokencky E, Pieter T. Trends in atmospheric carbon dioxide. Boulder, CO: National Oceanic & Atmosphere Administration, Earth System Research Laboratory (NOAA-ESRL); 2015.
[2] IPCC Summary for policymakers. Climate change, mitigation of climate change, contribution of working group III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge: Cambridge University Press; 2014. p. 1–31
[3] Chakrabarti B, Singh SD, Kumar SN, Aggarwal PK, Pathak H, Nagarajan S. Low-cost facility for assessing impact of carbon dioxide on crops. Curr Sci. 2012;102:1035–40.
[4] Chakrabarti B, Singh SD, Kumar V, Harit RC, Misra S. Growth and yield response of wheat and chickpea crops under high temperature. Indian J Plant Physiol. 2013;18:7–14.
[5] Chakrabarti B, Bhatia A, Pramanik P, Singh SD, Jatav RS, Das Saha N, et al. Changes in thermal requirements, growth and yield of wheat under the elevated temperature. Indian J Agric Sci. 2021;91:435–9.
[6] Singh SD, Chakrabarti B, Muralikrishna KS, Chaturvedi AK, Kumar V, Mishra S, et al. Yield response of important field crops to elevated air temperature and CO_2 levels. Indian J Agric Sci. 2013;83:1009–12.
[7] Melillo JM, Butler S, Johnson J, Mohan J, Steudler P, Lux H, et al. Soil warming, carbon-nitrogen interactions, and forest carbon budgets. PNAS. 2011;108:9508–12.
[8] Raj A, Chakrabarti B, Pathak H, Singh SD, Mina U, Purakayastha TJGrowth. yield and nitrogen uptake in rice crop grown under elevated carbon dioxide and different doses of nitrogen fertilizer. Indian J Exp Biol. 2019;57:181–7.
[9] Lenka NK, Lenka S, Yashona DS, Shukla AK, Elanchezhian R, Dey P, et al. Carbon dioxide and/or temperature elevation effect on yield response, nutrient partitioning and use efficiency of applied nitrogen in wheat crop in central India. Field Crops Res. 2021;264:108084.
[10] Chakrabarti B, Singh SD, Bhatia A, Kumar V, Harit RC. Yield and nitrogen uptake in wheat and chickpea grown under elevated carbon dioxide level. Natl Acad Sci Lett. 2020;43:109–13.
[11] Myers SS, Zanobetti A, Kloog I, Huybers P, Leakey AD, Bloom AJ, et al. Increasing CO_2 threatens human nutrition. Nature. 2014;510:139–42.
[12] Dey SK, Chakrabarti B, Prasanna R, Pratap D, Singh SD, Purakayastha TJ, et al. Elevated carbon dioxide level along with phosphorus application and cyanobacterial inoculation enhances nitrogen fixation and uptake in cowpea crop. Arch Agron Soil Sci. 2017;63:1927–37.
[13] Rogers A, Gibon Y, Stitt M, Morgan PB, Bernacchi CJ, Ort DR, et al. Increased C availability at elevated carbon dioxide concentration improves N assimilation in a legume. Plant Cell Environ. 2006;29:1651–8.
[14] Ainsworth EA, Long SP. What have we learned from 15 years of free air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. New Phytol. 2005;165:351–72.
[15] Rogers A, Allen DJ, Davey PA, Morgan PB, Ainsworth EA, Bernacchi CJ, et al. Leaf photosynthesis and carbohydrate

dynamics of soybeans grown throughout their life cycle under free-air carbon dioxide enrichment. Plant Cell Environ. 2004;27:449–58.

[16] Lenka NK, Lenka S, Thakur JK, Yashona DS, Shukla AK, Elanchezhian R, et al. Carbon dioxide and temperature elevation effects on crop evapotranspiration and water use efficiency in soybean as affected by different nitrogen levels. Agric Water Manag. 2020;230:105936.

[17] Lenka NK, Lenka S, Singh KK, Kumar A, Aher SB, Yashona DS, et al. Effect of elevated carbon dioxide on growth, nutrient partitioning, and uptake of major nutrients by soybean under varied nitrogen application levels. J Plant Nutr Soil Sci. 2019;182:1–6.

[18] Hernandez-Armenta R, Wien HC, Eaglesham ARJ. Carbohydrate partitioning and nodule function in common bean after heat stress. Crop Sci. 1989;29:1292–7.

[19] Vejan P, Abdullah R, Khadiran T, Ismail S, Boyce AN. Role of plant growth promoting rhizobacteria in agricultural sustainability—a review. Molecules. 2016;21:573.

[20] Prasanna R, Triveni S, Bidyarani N, Babu S, Yadav K, Adak A, et al. Evaluating the efficacy of cyanobacterial formulations and biofilmed inoculants for leguminous crops. Arch Agron Soil Sci. 2014;60:349–66.

[21] Prasanna R, Ramakrishnan B, Simranjit K, Ranjan K, Kanchan A, Hossain F, et al. Cyanobacterial and rhizobial inoculation modulate the plant physiological attributes and nodule microbial communities of chickpea. Arch Microbiol. 2017;199:1311–23.

[22] Velmourougane K, Prasanna R, Singh S, Chawla G, Kumar A, Saxena AK. Modulating rhizosphere colonisation, plant growth, soil nutrient availability and plant defense enzyme activity through *Trichoderma viride—Azotobacter chroococcum* biofilm inoculation in chickpea. Plant Soil. 2017;421:157–74.

[23] Chaparro JM, Sheflin AM, Manter DK, Vivanco JM. Manipulating the soil microbiome to increase soil health and plant fertility. Biol Fertil Soils. 2012;48:489–99.

[24] Prasanna R, Pattnaik S, Sugitha TCK, Nain L, Saxena AK. Development of cyanobacterium-based biofilms and their in vitro evaluation for agriculturally useful traits. Folia Microbiol. 2011;56:49–58.

[25] Triveni S, Prasanna R, Saxena AK. Optimization of conditions for in vitro development of *Trichoderma viride*-based biofilms as potential inoculants. Folia Microbiol. 2012;57:431–7.

[26] Renuka N, Guldhe A, Prasanna R, Singh P, Bux F. Microalgae as multi-functional options in modern agriculture: current trends, prospects and challenges. Biotechnol Adv. 2018;36:1255–73.

[27] Dey SK, Chakrabarti B, Purakayastha TJ, Prasanna R, Mittal R, Singh SD, et al. Interplay of phosphorus doses, cyanobacterial inoculation, and elevated carbon dioxide on yield and phosphorus dynamics in cowpea. Environ Monit Assess. 2019;191:223.

[28] Dey SK, Chakrabarti B, Prasanna R, Mittal R, Singh SD, Pathak H. Growth and biomass partitioning in mungbean with elevated carbon dioxide, phosphorus levels and cyanobacteria inoculation. J Agrometeorol. 2016;18:7–12.

[29] Dey SK, Chakrabarti B, Prasanna R, Singh SD, Purakayastha TJ, Datta A, et al. Productivity of mungbean (*Vigna radiata*) with elevated carbon dioxide at various phosphorus levels and cyanobacteria inoculation. Legum Res. 2017;40:497–505.

[30] Maity PP, Chakrabarti B, Purakayastha TJ, Bhatia A, Das Saha N, Jatav RS, et al. Do elevated CO_2 and temperature affect organic nitrogen fractions and enzyme activities in soil under rice crop? Soil Res. 2020;58:400–10.

[31] Kumar R, Mina U, Gogoi R, Bhatia A, Harit RC. Effect of elevated temperature and carbon dioxide levels on maydis leaf blight disease tolerance attributes in maize. Agric Ecosyst Environ. 2016;231:98–104.

[32] Stanier R, Kunisawa R, Mandel M, Cohen-Bazire G. Purification and properties of unicellular blue-green algae (order Chroococcales). Bacteriol Rev. 1971;35:171–205.

[33] Prasanna R, Babu S, Rana A, Kabi SR, Chaudhary V, Gupta V, et al. Evaluating the establishment and agronomic proficiency of cyanobacterial consortia as organic options in wheat–rice cropping sequence. Exp Agric. 2013;49:416–34.

[34] Prasanna R, Kanchan A, Ramakrishnan B, Ranjan K, Venkatachalam S, Hossain F, et al. Cyanobacteria-based bioinoculants influence growth and yields by modulating the microbial communities favourably in the rhizospheres of maize hybrids. Eur J Soil Biol. 2016;75:15–23.

[35] Prasanna R, Joshi M, Rana A, Shivay YS, Nain L. Influence of co inoculation of bacteria and cyanobacteria on crop yield and C-N sequestration in soil under rice crop. World J Microbiol Biotechnol. 2012;28:1223–35.

[36] Subbiah B, Asija GL. A rapid procedure for estimation of available nitrogen in soils. Curr Sci. 1956;25:259–60.

[37] Jenkinson DS, Powlson DS. The effects of biocidal treatments on metabolism in soil-V.A method for measuring soil biomass. Soil Biol Biochem. 1976;8:209–13.

[38] Klein DA, Loh TC, Goulding RL. A rapid procedure to evaluate the dehydrogenase activity of soils low in organic matter. Soil Biol Biochem. 1971;3:385–7.

[39] Jackson ML. Soil chemical analysis. In: Prentice Hall India Pvt. Ltd. 1973. New Delhi.

[40] Xu G, Singh SK, Reddy VR, Barnaby JY, Sicher RC, Li T. Soybean grown under elevated CO_2 benefits more under low temperature than high temperature stress: Varying response of photosynthetic limitations, leaf metabolites, growth, and seed yield. J Plant Physiol. 2016;205:20–32.

[41] Briones MJI, Ineson P, Piearce TG. Effects of climate change on soil fauna; responses of enchytraeids, Diptera larvae, and tardigards in a transplant experiment. Appl Soil Ecol. 1997;6:117–34.

[42] Lobell DB, Asner GP. Climate and management contributions to recent trends in US agricultural yields. Science. 2003;299:1032.

[43] Puteh AB, ThuZar M, Mondal MMA, Abdullah Nur APB, Halim MRA. Soybean (*Glycine max* (L.) Merrill) seed yield response to high temperature stress during reproductive growth stages. Aust J Crop Sci. 2013;7:1472–9.

[44] Bertrand A, Prévost D, Bigras FJ, Lalande R, Tremblay GF, Castonguay Y, et al. Alfalfa response to elevated atmospheric CO_2 varies with the symbiotic rhizobial strain. Plant Soil. 2007;301:173–87.

[45] van Groenigen KJ, Six J, Hungate BA, de Graaff MA, van Breemen N, van Kessel C. Element interactions limit soil carbon storage. Proc Natl Acad Sci USA. 2006;103: 6571–4.
[46] Tsai DD, Chen PH, Ramaraj R. The potential of carbon dioxide capture and sequestration with algae. Ecol Eng. 2017; 98:17–23.
[47] Karthikeyan N, Prasanna R, Nain L, Kaushik BD. Evaluating the potential of plant growth promoting cyanobacteria as inoculants for wheat. Eur J Soil Biol. 2007;43:23–30.
[48] Kumar K, Dasgupta CN, Nayak B, Lindblad P, Das D. Development of suitable photobioreactors for CO_2 sequestration addressing global warming using green algae and cyanobacteria. Bioresour Technol. 2011;102:4945–53.
[49] Nayak S, Prasanna R, Pabby A, Dominic TK, Singh PK. Effect of BGA-Azolla biofertilizers on nitrogen fixation and chlorophyll accumulation at different depths in soil cores. Biol Fertil Soils. 2004;40:67–72.
[50] Mandal B, Vlek PLG, Mandal LN. Beneficial effects of blue green algae and Azolla, excluding supplying nitrogen on wetland rice field: a review. Biol Fertil Soils. 1998;27: 329–42.

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