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Elevated CO_2 along with inoculation of cyanobacterial biofilm or its partners differentially modulates C–N metabolism and quality of tomato beneficially

Venkatesh Kokila [a], Radha Prasanna [a,*], Arun Kumar [b], Sekar Nishanth [a], Bhupinder Singh [c], Shalini Gaur Rudra [d], Priya Pal [d], Madan Pal [e], Yashbir Singh Shivay [f], Awani Kumar Singh [g]

[a] Division of Microbiology, ICAR-Indian Agricultural Research Institute, New Delhi, 110012, India
[b] National Phytotron Facility, ICAR-Indian Agricultural Research Institute, New Delhi, 110012, India
[c] Division of Environment Science, ICAR-Indian Agricultural Research Institute (IARI), New Delhi, 110 012, India
[d] Division of Food Science and Post Harvest Technology, ICAR-Indian Agricultural Research Institute, New Delhi, 110012, India
[e] Division of Plant Physiology, ICAR-Indian Agricultural Research Institute, New Delhi 110012, India
[f] Division of Agronomy, ICAR-Indian Agricultural Research Institute, New Delhi, 110012, India
[g] Centre for Protected Cultivation Technology (CPCT), ICAR-Indian Agricultural Research Institute, New Delhi, 110012, India

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ABSTRACT

Diazotrophic cyanobacteria are known to influence nutrient availability in soil, however, their benefits under elevated CO_2 environment, particularly on fruit quality attributes, is a less investigated aspect. Laboratory developed cyanobacterium-fungal biofilm (An-Tr), composed of *Anabaena torulosa* (An) as the matrix with the partner as *Trichoderma viride* (Tr), along with the individual partners were evaluated under ambient (aCO_2-400 $\pm$ 50 ppm) and elevated (eCO_2-700 $\pm$ 50 ppm) conditions, with and without tomato plants. An-Tr inoculation exhibited distinct and significantly higher values for most of the soil microbiological parameters, plant growth attributes and antioxidant/defense enzyme activities measured at 30 and 60 DAI (days after inoculation). Significant enhancement in soil nutrient availability, leaf chlorophyll, with 45–50% increase in the enzyme activities related to carbon and nitrogen assimilation, higher yields and better-quality parameters of tomato, with An-Tr biofilm or An inoculation, were recorded, particularly under eCO_2 conditions. The fruits from An-Tr treatments under eCO_2 exhibited a higher titrable acidity, along with more ascorbic acid, carotenoids and lycopene content, highlighting the superiority of this inoculant. Multivariate analyses revealed significant ($p \leq 0.05$) interactions among cultures, DAI, and CO_2 levels, illustrating that cyanobacterial inoculation can be advocated as a strategy to gainfully sequester eCO_2. Significant improvement in yield and fruit quality along with 50% N savings, further attest to the promise of cyanobacterial inoculants for tomato crop in the climate change scenario.

* Corresponding author. Division of Microbiology, ICAR-Indian Agricultural Research Institute, New Delhi, 110012, India.
E-mail address: radhapr@gmail.com (R. Prasanna).

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1. Introduction

Globally, elevated CO_2 (eCO_2) represents one of the major drivers of climate change [1], and biological fixation through photosynthesis and recycling CO_2 into useful products are promising and eco-sustainable options, particularly in terms of downstream benefits [2,3]. However, whether it really benefits the climate is still questionable, as it seems to only delay carbon's journey into the atmosphere. Enriching soil organic carbon reserves seems a more promising long-term proposition, as it influences the soil biota and their carbon sequestration dynamics [4]. In agriculture, this can be facilitated through the practice of intercropping and mulching, which can reduce the soil loss by 35%, increasing soil microstructure, water stable aggregation and infiltration rate, thereby, reducing run-off [5]. Photosynthetic fixation of carbon dioxide (CO_2) into carbohydrates (source) and its translocation from the site of synthesis to regions of utilization and/or storage (sink) is essential for plant growth and development of plants and other phototrophic organisms.

In this context, photosynthetic microbes with the ability to grow autotrophically using sunlight and CO_2 are gaining importance as agents of CO_2 sequestration, among which diazotrophs may prove more beneficial to C–N dynamics. Several cyanobacteria release metabolites rich in carbon, which stimulate the growth of plants and improve the C–N status of soil, as documented in tomatoes and other crops [6,7]. Cyanobacteria are recognized for their role as bioinoculants which lead to savings of nitrogenous fertilizers, in addition to improvement in soil nutrient availability, plant growth and yields in cereals, cotton, chrysanthemum, and solanaceous vegetables including tomato [6,8–10]. Cyanobacterial inoculation can also be effective in biocontrol of soil-borne phytopathogenic fungi [11], and elicit plant defense machinery and immunity towards abiotic and biotic stresses. Inoculation of cyanobacteria in soil is known to moderate the changes brought about by abiotic stress, particularly environmental stressors such as acidity or salinity, by buffering the pH and increase the efficiency of fertilizer use in crop plants aiding the plants to proliferate even under the unfavourable conditions [12].

Cyanobacteria are often an important component of phototrophic biofilms, found as a dominant constituent of biological soil crusts, which help in greening deserts and preventing soil erosion [13]. Laboratory developed biofilms comprising cyanobacterium as a matrix with agriculturally important bacteria such as *Rhizobium*, or fungus *Trichoderma viride* have shown promise as inoculants which can stimulate plant growth and sustain nutrient availability, up to harvest stage, much better than individual cultures in various crops, including under eCO_2 environment [11,14]. However, effects on fruit quality is less investigated.

In the present investigation, tomato, an important vegetable valued globally for its taste and color was chosen, as it is a rich source of minerals, vitamins, and antioxidants essential for human health. Several strategies, such as the use of microbial inoculants or protected cultivation or eCO_2, have been evaluated to improve its productivity [15]. In leguminous crops, the cyanobacterium *Anabaena torulosa* (An) based biofilms prepared using different rhizobial strains as partner(s) performed well under aCO_2 and eCO_2 environments [14,16]. In a previous study cyanobacterial inoculation led to significant C- enrichment and enhanced plant growth, and the performance of biofilm of *Anabaena torulosa -Trichoderma viride* (An-Tr) was superior, as compared to the other two cyanobacterial cultures evaluated [17]. In the present investigation, the hypothesis being explored is whether the synergy of metabolism among the partners in the cyanobacterial biofilm- *Anabaena torulosa -Trichoderma viride* (An-Tr) under aCO_2 or eCO_2 conditions, is more beneficial to plant, vis a vis the inoculation effects of only the partners - *Anabaena torulosa* or *Trichoderma viride* individually. Also, whether the contributions towards enhanced C sequestration in soil can lead to better yields and fruit quality of tomato crop.

2. Materials and methods

2.1. Cultures and their maintenance

The cyanobacterial culture *Anabaena torulosa,* deposited as NAIMCC-C-00345 (National Agriculturally Important Culture Collection -NAIMCC, ICAR-National Bureau of Agriculturally Microorganisms-NBAIM, India), and available in the Division of Microbiology, ICAR-IARI, New Delhi and fungal culture *Trichoderma viride* (Tr) ITCC 2211 obtained from Indian Type Culture Collection, Division of Pathology, IARI, New Delhi, India) were used in the present investigation. Both An and An-Tr were grown in N-free BG-11 medium, with temperature of 27 ± 2 °C, along with 16:8 h light and dark cycles (white light; 50–55 μmol photons m^{-2} s^{-1}). The biofilm *Anabaena torulosa -Trichoderma viride* (An-Tr) was prepared, as given earlier [16,18]. Tr was grown in Potato dextrose broth at 30 °C for 48 h, till the population reached 4×10^{12} CFU ml^{-1} and the mycelial suspension was inoculated into to a week-old *Anabaena torulosa* culture, maintaining 10^4 CFU ml^{-1} of Tr. After two weeks of incubation under optimized conditions, harvesting was done. The chlorophyll content of the cyanobacterial culture and An-Tr biofilm were measured, while for Tr alone, CFU of 48 h old culture was enumerated before mixing with the carrier (1:1 - Paddy straw compost and vermiculite), The formulations of An/An-Tr had 5 μg chlorophyll g^{-1} carrier, and CFU of $10^5/10^4$ g^{-1} in the *T. viride*/An-Tr g^{-1} carrier, were maintained, as optimized earlier [9,19].

2.2. Experimental set-up

The experiment was undertaken in the National Phytotron Facility (NPF, IARI, New Delhi, India) with a wall-mount type growth chambers, model PGW36, Conviron, and a Horiba APBA-250E Indoor CO_2 Monitoring system, in which aCO_2 (400 ± 50 ppm) and eCO_2 (700 ± 50 ppm) conditions are maintained. The chamber had the dimensions of height x length x width of as 200 × 255 × 135 cms, with a total floor area of 36 square feet. Controlled environmental conditions of photoperiod and light intensity (10 h; 400–450 μmol photons m^{-2} s^{-1}, using cool white, fluorescent lamps), maximum and minimum temperature (22/18 °C), humidity (65–75%), and CO_2 concentrations was maintained to facilitate uniform growth conditions in the pots with soil, inoculum and tomato plants. To

evaluate the impact of elevated CO_2, the unit was connected to an external CO_2 cylinder, kept adjacent to the chamber and compared with ambient CO_2.

Soil samples were collected in triplicate, from a depth of 15 cm from IARI field, pooled together and used for nutrient analysis before using for experiment, which was alluvium-derived sandy clay loam with 51.7%, 21.9% and 26.4% sand, silt and clay respectively, having nutrient status of the soil at the time of setting up the experiment as 3.1 g kg^{-1} soil organic carbon (SOC), and 98.0 kg ha^{-1} available N and pH of 7.6. The soil was sterilized (intermittent wet sterilization using autoclave) and 10 kg soil was filled in fifteen-inch pots, and recommended doses of fertilizers- RDNPK (60:50:65) added as basal dose in the form of urea (only 50%), SSP (Single Super Phosphate), and MOP (Muriate of Potash). After 60 days, the remaining 50% N was applied only to the control and Tr inoculated pots. This was done as *Anabaena torulosa* (An), and biofilm *Anabaena torulosa- Trichoderma viride* (An-Tr), are diazotrophic and provide fixed N to the plants but *Trichoderma viride* (Tr) cannot fix atmospheric N. 30 days-old tomato (variety *Avinash*) seedlings were obtained from the nursery maintained in the Centre for Protected Cultivation (CPCT), IARI, New Delhi, India. Two plants (+P) were transplanted into each pot, immediately after inoculation with formulations, and the treatment details are given in Supplementary Table 1. All treatments were taken in triplicate and destructive sampling of three pots at all stages (30 and 60 DAI) as well as harvest stage was done. As both cyanobacterial inoculation and transplanting of tomato plants were done on the same day, the term DAI is used throughout for soil and plant analyses. Control and Carrier treatments (+P, −P) were included to understand the role of the aCO_2 and eCO_2 environments on soil and plant, without cyanobacterial inoculation, and with/without carrier used in the formulations.

2.3. Soil analyses

For the analyses of soil parameters, cores (30g soil for each core) from 0 to 15 cm depth were collected in triplicate, at 30 and 60 DAI from all the treatments, using a steel auger, placed in zip lock bags and stored for further analyses at 4 °C. The protocol optimized by Nayak et al. (2004) was followed to estimate soil chlorophyll [19], which is used as an indicator of photosynthetic biomass and cyanobacterial enrichment in soil. Aliquots of solvent mixture (containing acetone and dimethyl sulphoxide (DMSO) in the ratio of 1:1) was added (4 ml of solvent mix g^{-1} soil) to each soil core, and vortexed. The glass tubes were covered and incubated in the dark for 48–96 h at room temperature. The contents were filtered using Whatman No. 1 filter paper, and the filtrate was analyzed spectrophotometrically at 630, 645, and 663 nm. The values were expressed as mg chl g^{-1} soil, using the equation

$$\text{Soil chlorophyll (mg g}^{-1}\text{ soil)} = (11.64*\text{A663}) - (2.16*\text{A645}) + (10.10*\text{A630})$$

Dehydrogenase activity was measured using the standard method, involving the use of triphenyl tetrazolium chloride (TTC, 3%) [20]. Rhizospheric soil (6 g) samples were taken in screw-capped glass tubes, 1–2 ml of distilled water, 1 ml of 3% TTC was added along with a pinch of calcium carbonate and mixed. Tubes were then capped and incubated at 30 °C for 24 h in the dark. After incubation, 10 ml absolute methanol was added, vortexed, and incubated for 30 min in dark. The solution containing the extracted triphenyl tetrazolium formazan (TPF) was filtered using Whatman No. 1 filter paper. The absorbance of the clear solution was measured at 485 nm; the activity is expressed as μg TPF g^{-1} soil d^{-1}.

Soil Microbial biomass C (SMBC) was determined by the chloroform fumigation method [21]. Two sets of tubes were prepared, both with fresh rhizospheric soil (3.5 g) but one was amended with chloroform (200 μl) and the other without chloroform. The tubes were incubated for 24 h in the dark condition. The addition of 14 ml Potassium sulfate (0.5 M), shaking at 28 °C for 30 min was followed by filtering using Whatman No. 1 filter paper, and the filtrate was used to measure the OD at 280 nm in a spectrophotometer. SMBC content was derived from the following formula:

$$\text{SMBC } (\mu\text{g / g dry soil}) = (\text{ECF} - \text{NECF} / \text{kEC})$$

here: ECF/NF refers to extractable carbon in fumigated (ECF) and non-fumigated soil samples (ECNF) and kEC = 0.45.

The protocol given by Lowe was used to estimate polysaccharide content [22], in which soil samples (1 g) were placed in Erlenmeyer flasks; 4 ml sulfuric acid (12 M) was added and incubated for 2 h. The contents were then diluted to 0.5 M by addition of 92 ml distilled water and autoclaved for 60 min, followed by filtration using Whatman No. 1 filter paper. To 1 ml of filtrate, 5% aqueous phenol was added (1 ml), along with 1 ml of concentrated H_2SO_4. Tubes were then incubated for 30 min at 30 °C in a water bath. The intensity of the developed color was measured spectrophotometrically at 490 nm; values are expressed as mg g^{-1} soil.

For soil nutrient analysis, rhizospheric soil was air dried, ground and sieved for determination of available N by the Kjeldahl method and Soil organic carbon (SOC) by the Walkley Black method [23].

2.4. Plant growth-related parameters

For the analyses of leaf parameters, leaves were collected in zip lock bags at 30 and 60 DAI in triplicate. To estimate the pigments, 0.1 g leaf sample was added to a test tube with 10 ml DMSO and incubated for 30 min at 70 °C [24]. After cooling, extracted pigments were diluted with DMSO in the 3:1 ratio and estimated spectrophotometrically by measuring absorbance at 480, 510, 645, and 663 nm using a UV-VIS spectrophotometer (Model Evolution 300, Thermo Scientific). The pigment content was calculated based on equations and values expressed as mg g^{-1} leaf [25].

The concentration of proteins was determined with bovine serum albumin (BSA) as standard [26]. Chromogenic changes were measured at 590 nm, using 1 ml Folin-Ciocalteu reagent with 100 μl leaf extractant. The total leaf sugars were estimated by the method given by Dubois and co-workers [27]. One g of leaf tissue was used to grind with 80% ethanol (10 ml), and the contents were

centrifuged at 8000×*g* for 15 min. The supernatant was transferred into another glass tube and kept for ethanol evaporation in the water bath at 80 °C. One 'ml of aqueous phenol (5%) and 1 ml of concentrated sulfuric acid was added to the 1 ml residual sample after cooling, the tubes were incubated at 30 °C in the water bath for 30 min to develop the color, it was measured spectrophotometrically at 490 nm and the sugar concentration was expressed in terms of mg g^{-1} tissue, using glucose as standard.

IAA (Indole acetic acid) estimation in fresh leaf samples, was done as given by Gordon and Paleg [28]. Fresh plant tissue (0.5 g) was macerated in chilled methanol (2 ml), then centrifuged at 9000×*g* (10 min, 4 °C). The supernatant was collected (1 ml) and acidified with 2–3 drops of orthophosphoric acid, then 2 ml of reagent containing perchloric acid (35%) and ferric chloride (0.5 M) was added and incubated for 1 h at 30 °C in dark. The intensity of color was measured spectrophotometrically at 535 nm. The standard curve of IAA was used to express the values in terms of μg g^{-1} plant material.

2.5. Carbon assimilation and nitrogen fixation related enzymes

The activity of the major CO_2 fixing enzyme RuBisCO was measured by ribulose-1,5-biphosphate (RuBP) dependent incorporation of radio labelled $NaH^{14}CO_3$ into an acid-stable product and estimated using a liquid scintillation cocktail in a scintillation vial [29]. The enzyme extract was prepared by grinding the leaf sample (0.5 g) in 5 ml ice-cold extraction medium at 4 ºC containing 15 mM Tris (hydroxymethyl) amino methane hydrochloride (Tris-HCl) buffer (pH 8), 1 mM ethylene diamine tetra acetic acid (EDTA), 1 mM phenyl methyl sulfonyl fluoride (PMSF) and 0.1% bovine serum albumin (BSA). Polyvinyl pyrrolidone (PVP) was added during grinding to facilitate the process. The homogenate was centrifuged at 18,000 g for 15 min at 4 ºC, the obtained supernatant was used as a crude enzyme extract. Reaction mixture contained: $NaH^{14}CO_3$ (specific activity 0.5 μ Ci/1.85 *10^5 Bq) and 20 μ M $NaHCO_3$ along with 0.1 μM EDTA, 30 μM (pH 8) Tris HCl buffer, 3.03 μM $MgCl_2$, 0.2 μM ribulose-1,5-biphosphate (RUBP). The reaction was initiated by adding 50 μl of crude enzyme extract, incubated for 5 min at 30 ºC and the reaction terminated by adding 0.1 ml of 6 M acetic acid. The samples were oven-dried in the scintillation vials at 65 ºC and 14 C-stable product counts were measured using the liquid scintillation counter (1900 TR, Tri-Carb Liquid Scintillation Analyzer, Packard Bioscience, Boston, MA, USA) after adding 5 ml of the scintillation cocktail to the vials. The RuBisCO enzyme activity was expressed in μmoles CO_2 fixed g^{-1} dry weight (dry wt.) h^{-1}.

Leaf Carbonic anhydrase (CA) activity was measured by the method devised by Badger and Price [30]. Fresh leaves (0.5 g) were macerated in 2 ml of ice-cold extraction buffer (0.1 M Tris HCl and 0.2 mM Na-EDTA (pH-8.0), with a pinch of 1% PVP). The sample mixture was centrifuged at 12000 rpm for 15 min at 4 °C. To the assay mixture containing 1 ml Veronal buffer (25 mM, with pH 8.2, 100 μl et-BTB (ethanolic bromothymol blue; 0.2%), 200 μl enzyme extract was added, followed by saturated CO_2 (2.5 ml) the time taken for color change from blue to greenish-yellow recorded. The enzyme activity was expressed in terms of EU (Enzyme Units) mg^{-1} proteins.

The assay devised by Wu and Wedding was used for phosphoenol pyruvate (PEP) carboxylase activity [31]. Fresh plant tissue (0.5g) was macerated with extraction buffer (2 ml) containing Tris-HCl (0.1 M, pH 7.8), sucrose (0.5 M), $MgCl_2$ (0.01 M), dithiothreitol (0.05 M) and PVP (1 g), followed by centrifugation at 9000×*g* for 15 min at 4 °C. The supernatant (100 μl) and 1 ml incubation buffer [containing Tris-HCl (0.1 M pH, 7.8), $MgCl_2$ (0.01 M), $NaHCO_3$ (0.05 M), PEP-Na_3 (1 mM), and NADH (0.1 mM)] were taken in a quartz cuvette and OD measured at 340 nm. The change in enzyme activity was expressed as μM oxidized NADH min^{-1} mg^{-1} chl.

The activity of glutamine synthetase (GS) was measured by following the protocol outlined by Shapiro and Stadtman [32]. The plant tissue (0.5 g) was macerated with 1.5 ml extraction buffer containing 2-Imidazole HCl (50 mM, pH 7.0), sodium glutamine (10 mM) and $MgCl_2$ (5 mM). The contents were centrifuged at 10,000×*g* for 15 min at 4 °C. Clear extract (100 μl) was incubated with assay mixture (pH 7.0) containing $MnCl_2$ (0.1 M), glutamate (0.1 M), disodium hydrogen arsenate (1.0 M), ADP (0.01 M), hydroxylamine HCl (2 M), sodium hydroxide (2 M), and imidazole HCl buffer (50 mM) at 37 °C for 30 min. Absorbance was measured at 540 nm and activity was expressed in μmoles γ-glutamyl hydroxamate g^{-1} f. wt. min^{-1}.

2.6. Hydrolytic enzymes

The activity of β-1,4 endoglucanase was determined using the method outlined using DNSA reagent [33]. Fresh plant tissue (1 g) was macerated using 0.1 M phosphate buffer (pH 7.0), followed by centrifugation at 8000×*g* for 15 min at 4 °C. The activity was assayed by taking 0.5 ml of filtrate broth, 0.5 ml of 1% CMC (carboxy methyl cellulose), and 0.5 ml of citrate buffer (pH 5.5), followed by incubation at 50 °C for 30 min. Tubes were taken out and 3 ml of DNSA reagent was added and incubated at 100 °C for 15 min, absorbance was taken at 575 nm after cooling of tubes.

Chitosanase activity was performed using the protocol optimized earlier [6]. Fresh leaf samples (1 g) were finely grounded using phosphate buffer (0.1 M, pH 7.0), followed by centrifugation at 9000×*g* for 10 min at 4 °C. To the supernatant (0.5 ml), 0.1 ml glycol chitosan (1%) was added and incubated at 37 °C for 10 min followed by stopping the reaction by boiling for 4 min. Further 0.5 ml deionized water and 0.5 ml acetyl acetone reagent were added and boiled for 20 min. After cooling at room temperature, ethanol (2.5 ml) and Ehrlich's reagent (0.5 ml) were added to the cocktail and made up to 5 ml using ethanol. Followed by incubation at 70 °C for 10 min and after cooling the absorbance was recorded at 530 nm and enzyme activity was expressed as micromoles of glucose liberated min^{-1} ml^{-1} filtrate under standard assay conditions.

2.7. Antioxidative and defense enzymes

Fresh leaf samples were taken (2 g) in a chilled mortar and pestle, macerated using 0.1 M sodium phosphate buffer (5 ml, pH 7.2), centrifuged at 9000×*g* for 15 min at 4 °C and the clear supernatant transferred into another tube and used for assaying the four

enzymes, as optimized earlier [6]. The activity of Phenylalanine ammonia-lyase **(**PAL) was carried out using the supernatant (100 μl) and 0.2% L-phenylalanine (600 μl) in a glass vial, incubated at 30 °C for 60 min, followed by the addition of 2 N HCl to stop the reaction. To the reaction mixture, 1.5 ml toluene was added followed by vortexing for 20 s and centrifugation at 10,000×*g* for 5 min. Absorbance was measured using the upper phase at 290 nm, and activity expressed as n moles of *trans*-cinnamic acid g^{-1} f. wt. min^{-1}. For measuring peroxidase activity, in a glass cuvette, leaf extract (100 μl) was taken, 1% guaiacol (0.5 ml) and Tris-HCl buffer (1.5 ml) were added. The reaction initiated with the addition of 1% H_2O_2 (0.5 ml). The absorbance was measured spectrophotometrically at 470 nm, based on a time-span of 0–180 s at an interval of 60 s, and enzyme activity expressed as IU g^{-1} f. wt.

Catalase (CAT) activity was assayed using the supernatant (100 μl) taken in a quartz cuvette, distilled water (900 μl), and 0.1 M phosphate buffer (1.5 ml) was added. The reaction was started by the addition of H_2O_2 (50 μl). The absorbance was measured at 240 nm with a time-span of 0–60 s at an interval of 15 s and enzyme activity expressed as Units g^{-1} f. wt. Polyphenol oxidase (PPO) activity was measured using catechol, which serves as the substrate. The assay was carried in 3 ml glass cuvettes, into which 0.5 ml of 0.02 M citrate phosphate buffer (pH 6.0), 0.5 ml proline (5 mg ml^{-1}), 0.5 ml catechol (2 mg ml^{-1}), and 0.5 ml of enzyme extract were added. Before addition of catechol, the mixture was aerated. The change in absorbance at 546 nm was recorded at 30 s intervals for 3 min. One unit of enzyme is defined as the change in absorbance of 0.01 IU g^{-1} f. wt.min^{-1}.

Superoxide dismutase (SOD) activity was measured using fresh leaf samples, macerating with 2 ml sodium phosphate buffer (0.1 N, pH 6.8), followed by centrifugation at 9000×*g* for 15 min at 4 °C. The cocktail for the assay contains 1 ml enzyme extract and 2 ml reaction mixture (NBT, nitroblue tetrazolium; 75 μM), methionine (13 mM), EDTA (0.1 mM), and riboflavin (0.002 mM). The activity was carried out in two sets, with one set being illuminated using fluorescent lamps (20 W), the other set kept in dark at room temperature. The tubes without enzyme, in light, were regarded as blank, while dark reaction tubes with enzyme were considered as the control. The activity is calculated in terms of the difference in NBT reduction recorded at 560 nm in with and without enzyme extract. One unit of SOD activity was taken as that amount of enzyme required to inhibit 50% initial reduction of NBT in light.

The number of flowers and number of fruits per plant were recorded at three days intervals from the first flowering day (30 DAI).

2.8. Fruit quality parameters

For quality parameters, three replicates per treatment were taken, with each replicate comprising ten fruits. Ripe fruits were harvested after 90 DAI (first harvest stage) and number of fruits per plant, and the weight of the fruit(s) were recorded. Firm fruits with red color were chosen for quality measurements. Fruit firmness (FM) was measured by piercing on the middle of the pericarp, using a 2 mm cylindrical probe attached to a TAXT *plusC* Stable Micro Systems (Surrey, UK) and readings recorded for each fruit. The percent Titrable acidity (TA) was measured by the method devised by Ranganna [34]. Fruit samples were converted to a paste, using pestle and mortar, and 10 ml juice was taken. After addition of 100 ml distilled water, it was filtered using Whatman no.1 filter paper, 10 ml filtrate taken into conical flask, 2–3 drops of Phenolphthalein indicator added and titrated against 0.05 N NaOH solution, until light pink color is observed (end point).

Ascorbic acid was estimated by the method devised by Ranganna [34]. Five g fruit samples were macerated, added to a 100 ml volumetric flask and volume made up to 100 ml using 3% HPO_3, followed by filtration using Whatman no-1 filter paper. Aliquots of 10 ml filtrate were taken in a conical flask and titrated against dye solution (50 mg 2,6 dichlorophenol indophenol in 150 ml hot water and 42 mg sodium bicarbonate; volume made up to 200 ml using 3% metaphosphoric acid).

Lycopene content was estimated by standard protocols [34]. The fruit sample (5 g) was macerated using 5 ml acetone in pestle and mortar and the extract was decanted into a conical flask and again the same step was repeated, till the residue was colorless and extracts were pooled and transferred into a separate funnel. Ten ml of petroleum ether and 20 ml of 5% sodium sulfate were added, 5 ml distilled water followed by gentle shaking to separate the top yellow-colored petroleum ether layer. Extraction was further separated using petroleum ether and water until all the pigments were extracted. The extracts were pooled and transferred into a 50 ml volumetric flask and the volume was made up with petroleum ether. The absorbance of combined layers was measured at 502 nm on a UV-VIS spectrophotometer, using petroleum ether as blank. The purity of the extracted standard lycopene was checked using its extinction coefficient (3150) and its standard curve.

2.9. Statistical analyses

Analyses of the relationships among and between the treatments, aCO_2 and eCO_2, with or without plant and two different time intervals (30 and 60 DAI) was done using the software package OPSTAT (factorial analysis). Pearson correlation coefficients were computed and Principal Component Analysis (PCA) and Multivariate analysis undertaken using software package, JMP 16.1, Copyright SAS Institute Inc. Analyses of soil and leaf samples, and fruit samples was done in triplicates. Plant-related parameters are expressed in terms of the fresh weight of tissues. Duncan's Multiple Range Test (DMRT) was used and standard error determined using statistical package WASP 2.0 (Web Agri Stat Package, Indian Council of Agricultural Research, India). Critical differences (CD) were calculated using factorial CRD with analysis of variance (ANOVA), and the least significant difference (LSD) values of $P \leq 0.05$ are depicted in graphs as error bars.

3. Results

3.1. Soil biochemical parameters

Inoculation with biofilm *Anabaena torulosa-Trichoderma viride* (An-Tr + P) led to significantly higher soil chlorophyll (1.60 mg g^{-1}) in treatments with plant, followed by no plant at 30 DAI in eCO_2 condition. Significantly increase in eCO_2 conditions, as compared to aCO_2, at both 30 and 60 DAI was recorded. At 60 DAI, An-Tr treatment (+P) showed the highest value of 1.4 mg g^{-1}, followed by *Anabaena torulosa (*An + P) with 1.22 mg g^{-1} in eCO_2 condition (Table 1). The interaction effect was significant between treatments, CO_2 levels and DAI, and treatments x CO_2 level, with and without plants, but non-significant effect between treatments, CO_2 levels and presence/absence of plants, and between treatments, CO_2 level, DAI and with and without plants.

Soil microbial biomass carbon (SMBC) was significantly higher in eCO_2 than aCO_2 environment, in all the treatments, with distinctly higher values at 60 DAI, than 30 DAI. The An-Tr (+P) treatment showed the highest values of SMBC (606.21 μg g^{-1} soil at 30 DAI, and 688.8 μg g^{-1} soil at 60 DAI, followed by 557.53 μg g^{-1} soil, at 30 DAI with An-Tr (−P) and 660.78 μg g^{-1} soil at 60 DAI (Table 1). An enhancement of several folds was recorded in An-Tr treatment, compared to uninoculated controls under both aCO_2 and eCO_2 conditions. A significant interactive effect was found between treatments, CO_2 level and DAI, however, interactions with plant, was non-significant.

Soil polysaccharides were estimated at 30 and 60 DAI, which significantly increased in all the treatments at 60 DAI and showed a distinct increment under eCO_2 than aCO_2 environment. An-Tr inoculation (+P)_led to the highest polysaccharide content under both aCO_2 (24.08 mg g^{-1} soil) and eCO_2 (23.71 mg g^{-1} soil) conditions, followed by An-Tr (−P) under eCO_2 condition (21.93 mg g^{-1} soil) at

Table 1
Soil biological parameters, as influenced by cyanobacterial inoculants under ambient CO_2 (aCO_2) and elevated CO_2 (eCO_2) conditions at 30 and 60 DAI (days after inoculation), in the absence or presence of tomato plants (-P, +P).

Factors	Soil chlorophyll (mg g^{-1})		Soil microbial biomass (μg g^{-1})		Soil polysaccharides (mg g^{-1})	
	30 DAI	60 DAI	30 DAI	60 DAI	30 DAI	60 DAI
CO_2 conditions						
aCO_2	0.79	0.91	276.40	404.57	16.96	20.13
eCO_2	1.07	0.96	443.82	559.16	17.93	22.81
CD ($P \leq 0.05$)	0.02	0.03	13.38	8.52	0.22	0.33
Treatments						
Control	0.55	0.66	249.27	362.09	13.52	18.05
Carrier	0.70	0.81	306.06	426.26	15.35	20.86
An	1.10	1.06	412.24	533.57	17.68	23.44
Tr	1.01	1.00	347.72	481.20	18.52	22.24
An-Tr	1.28	1.16	485.27	606.22	22.19	25.27
CD ($P \leq 0.05$)	0.04	0.04	21.16	13.48	0.35	0.52
+/−P						
−P	0.88	0.87	347.51	471.84	16.67	20.03
+P	0.98	1.00	372.71	491.90	18.22	23.91
CD ($P \leq 0.05$)	0.02	0.03	13.38	8.52	0.22	0.33
CO_2 conditions/Treatments/ ± P						
aCO_2						
Control (−P)	0.53	0.59	162.24	269.92	12.53	14.21
Control (+P)	0.56	0.70	207.97	290.57	13.47	21.61
Carrier(−P)	0.62	0.81	234.52	311.21	14.63	17.41
Carrier (+P)	0.64	0.90	271.39	318.59	15.68	23.66
An (−P)	0.77	0.94	297.94	464.61	17.04	18.98
An(+P)	0.98	1.04	308.27	491.16	18.46	26.28
Tr (−P)	0.85	0.95	244.99	405.61	16.88	18.83
Tr (+P)	0.88	0.96	259.44	418.89	17.93	23.71
An-Tr (−P)	0.96	0.98	367.26	528.03	18.88	20.45
An-Tr (+P)	1.07	1.21	410.04	547.21	24.08	26.18
eCO_2						
Control (−P)	0.53	0.64	306.79	435.11	13.41	17.41
Control (+P)	0.57	0.70	320.06	452.81	14.66	18.98
Carrier (−P)	0.72	0.73	355.46	528.03	14.75	20.45
Carrier (+P)	0.83	0.79	362.84	547.21	16.36	21.93
An (−P)	1.25	1.05	510.33	572.28	17.83	23.71
An (+P)	1.43	1.22	532.46	606.21	17.41	24.81
Tr (−P)	1.12	0.96	438.06	542.78	18.83	22.71
Tr (+P)	1.20	1.11	448.39	557.53	20.45	23.71
An-Tr (−P)	1.48	1.05	557.53	660.78	21.93	26.18
An-Tr (+P)	1.60	1.40	606.21	688.80	23.71	28.28
CD ($P \leq 0.05$)	NS	NS	NS	NS	0.70	NS
CO_2 conditions x Treatments x± P						

F and p-values illustrated in Supplementary Table 3; Treatments: Control, No inoculation; Carrier, Paddy straw compost and vermiculite at 1:1; An, *Anabaena torulosa*; Tr, T*richoderma viride*; and An-Tr, biofilm *Anabaena torulosa- Trichoderma viride.*

30 DAI. At 60 DAI, An-Tr (+P) samples recorded higher polysaccharides (28.28 mg g^{-1} soil), followed by without plant (26.18 mg g^{-1} soil) (Table 1). An increase of 88 and 69% were recorded in An-Tr treatment (+P) compared to controls under aCO_2 and eCO_2 conditions respectively. The interaction effect between treatments, CO_2 level, DAI, and with and without plants was significant.

Significant increase in soil dehydrogenase activity was recorded in all the treatments under eCO_2 conditions. Highest values were recorded in An-Tr (+P) treatment at both 30 DAI (37.31 μg TPF g^{-1} soil d^{-1}) and 60 DAI (29.54 μg TPF g^{-1} soil d^{-1}), followed by An-Tr (−P) 35.32 μg TPF g^{-1} soil d^{-1} at 30 DAI and 26.39 μg TPF g^{-1} soil d^{-1} at 60 DAI under eCO_2 condition (Table 2). The values were higher by 53 and 62% in An-Tr treatment (+P), compared to control, under CO_2 and eCO_2 conditions respectively. The activity was much higher at 30 DAI, in eCO_2 environment. The interaction effect between treatments and CO_2 levels, treatments and DAI, CO_2 level and DAI was significant, however, the tripartite effect was non-significant among the different treatments used, CO_2 level, DAI, and with and without plants.

Soil organic carbon (SOC) content showed a significant increase in all the treatments under eCO_2, as compared to aCO_2. The values at 60 DAI were significantly higher compared to 30 DAI. Treatments receiving An-Tr (+P) showed the highest SOC at both 30 DAI (0.75%) and 60 DAI (0.87%), followed by An-Tr (−P) (0.73%) at 30 DAI and Tr (+P) with 0.74% at 60 DAI, in eCO_2 condition

Table 2
Soil microbial activity and nutrient-related parameters, as influenced by cyanobacterial inoculants and CO_2 conditions (ambient and elevated CO_2- aCO_2, eCO_2) at 30 and 60 DAI (days after inoculation), in the absence and presence of tomato plants (−P, +P).

Factors	Soil dehydrogenase activity (μg TPF g^{-1} soil d^{-1})		SOC (%)		Available N (mg kg^{-1})	
	30 DAI	60 DAI	30 DAI	60 DAI	30 DAI	60 DAI
CO_2 conditions						
aCO_2	19.12	21.21	0.39	0.50	99.48	129.59
eCO_2	29.60	23.80	0.65	0.74	137.33	165.45
CD ($P \leq 0.05$)	0.22	1.43	0.02	0.02	1.01	1.00
Treatments						
Control	18.98	18.73	0.44	0.53	87.75	115.35
Carrier	22.01	21.13	0.48	0.56	106.24	133.73
An	27.32	24.51	0.56	0.67	134.80	160.75
Tr	24.04	22.30	0.52	0.62	116.66	152.20
An-Tr	29.42	25.81	0.61	0.72	146.58	175.56
CD ($P \leq 0.05$)	0.36	2.27	0.03	0.03	1.60	1.54
+/−P						
−P	23.52	21.49	0.51	0.66	116.12	144.23
+P	25.20	23.51	0.53	0.63	120.70	150.80
CD ($P \leq 0.05$)	0.22	1.43	NS	NS	1.01	1.00
CO_2 conditions/Treatments/ ± P						
aCO_2						
Control (−P)	14.89	16.67	0.32	0.40	70.68	99.76
Control (+P)	15.05	18.67	0.33	0.45	73.80	105.42
Carrier(−P)	17.09	19.67	0.33	0.48	82.73	119.93
Carrier (+P)	18.72	20.72	0.34	0.49	87.20	123.21
An (−P)	19.56	22.19	0.42	0.53	113.75	131.84
An(+P)	22.08	25.23	0.44	0.55	118.66	140.62
Tr (−P)	18.30	20.46	0.39	0.51	101.84	125.89
Tr (+P)	20.46	21.14	0.40	0.51	92.32	135.71
An-Tr (−P)	21.56	22.40	0.47	0.57	122.91	155.65
An-Tr (+P)	23.50	24.92	0.49	0.57	130.95	157.88
eCO_2						
Control (−P)	22.40	18.88	0.56	0.64	100.80	124.40
Control (+P)	23.61	20.72	0.57	0.65	105.71	131.84
Carrier (−P)	25.34	21.56	0.61	0.66	124.40	140.62
Carrier (+P)	26.91	22.61	0.65	0.68	130.65	151.18
An (−P)	32.85	24.29	0.68	0.79	151.18	180.82
An (+P)	34.79	26.34	0.69	0.82	155.65	189.74
Tr (−P)	27.91	22.40	0.64	0.72	131.84	171.44
Tr (+P)	29.49	25.23	0.65	0.74	140.62	175.75
An-Tr (−P)	35.32	26.39	0.73	0.87	161.02	191.98
An-Tr (+P)	37.31	29.54	0.75	0.87	171.44	196.74
CD ($P \leq 0.05$)	NS	NS	NS	NS	3.20	3.10
CO_2 conditions x Treatments x± P						

F and p-values illustrated in Supplementary Table 3.
Treatments: Control, No inoculation; Carrier, Paddy straw compost and vermiculite at 1:1; An, *Anabaena torulosa*; Tr, T*richoderma viride*; and An-Tr, biofilm *Anabaena torulosa- Trichoderma viride.*

(Table 2). The SOC increased by 48 and 35% in soil treated with An-Tr (+P), compared to control, under aCO_2 and eCO_2 conditions respectively. A significant interaction effect was found among cultures, CO_2 levels, with and without plants, and DAI and the tripartite interplay among treatments, CO_2 levels, and DAI. The interaction effect was non-significant, with and without plants.

The available nitrogen content significantly increased under eCO_2 compared to aCO_2 and with period of incubation (30–60 DAI) in all the treatments. An-Tr inoculated treatment, with plant showed the highest available N (171.4 mg kg^{-1}) at 30 DAI and 196.7 mg kg^{-1} at 60 DAI, followed by An-Tr inoculation-without plant treatment (161.02 mg kg^{-1}) at 30 DAI and 192.0 mg kg^{-1} at 60 DAI (Table 2). There was an increase of 71% and 64.07% in the soil available N content in An-Tr (+P), compared to control, under aCO_2 and eCO_2 conditions respectively. The interaction effects between treatments, CO_2 level, DAI and with and without plants, as well as the tripartite interactions, were significant.

3.2. *Interrelationships among soil parameters and cyanobacterial inoculants ambient and elevated CO_2 conditions*

Correlation matrices were generated using the correlation coefficients at 5% level of significance, and interactions among various soil biological and nutrient-related parameters. Soil chlorophyll, dehydrogenase activity, SMBC, soil polysaccharides, SOC, available N, and soil polysaccharides exhibited a positive correlation with one another under both aCO_2 and eCO_2 conditions (Supplementary

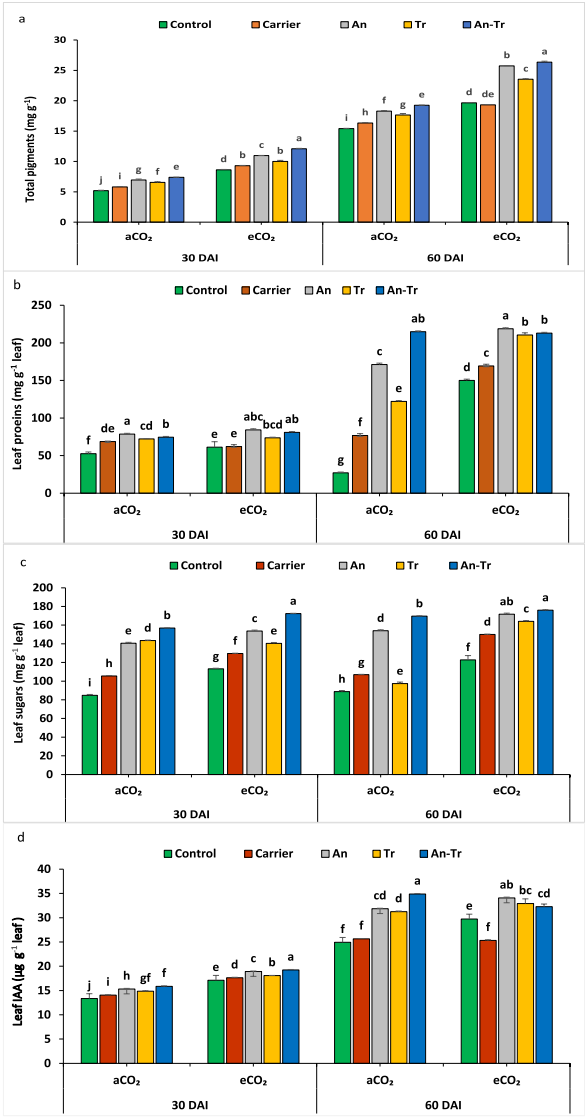

Fig. 1. Leaf parameters as influenced by cyanobacterial inoculants ambient CO_2 (aCO_2) and elevated CO_2 (eCO_2) conditions at 30 and 60 DAI (days after inoculation); a. Leaf chlorophyll; b. Leaf proteins; c. Leaf sugars; d. Leaf IAA; Treatments: Control, no inoculation; Carrier, Paddy straw compost and vermiculite at 1:1; *Anabaena torulosa* (An), *Trichoderma viride* (Tr), and biofilm *Anabaena torulosa- Trichoderma viride* (An-Tr). Details of F and p-values are given in Supplementary Tables 4 and 5

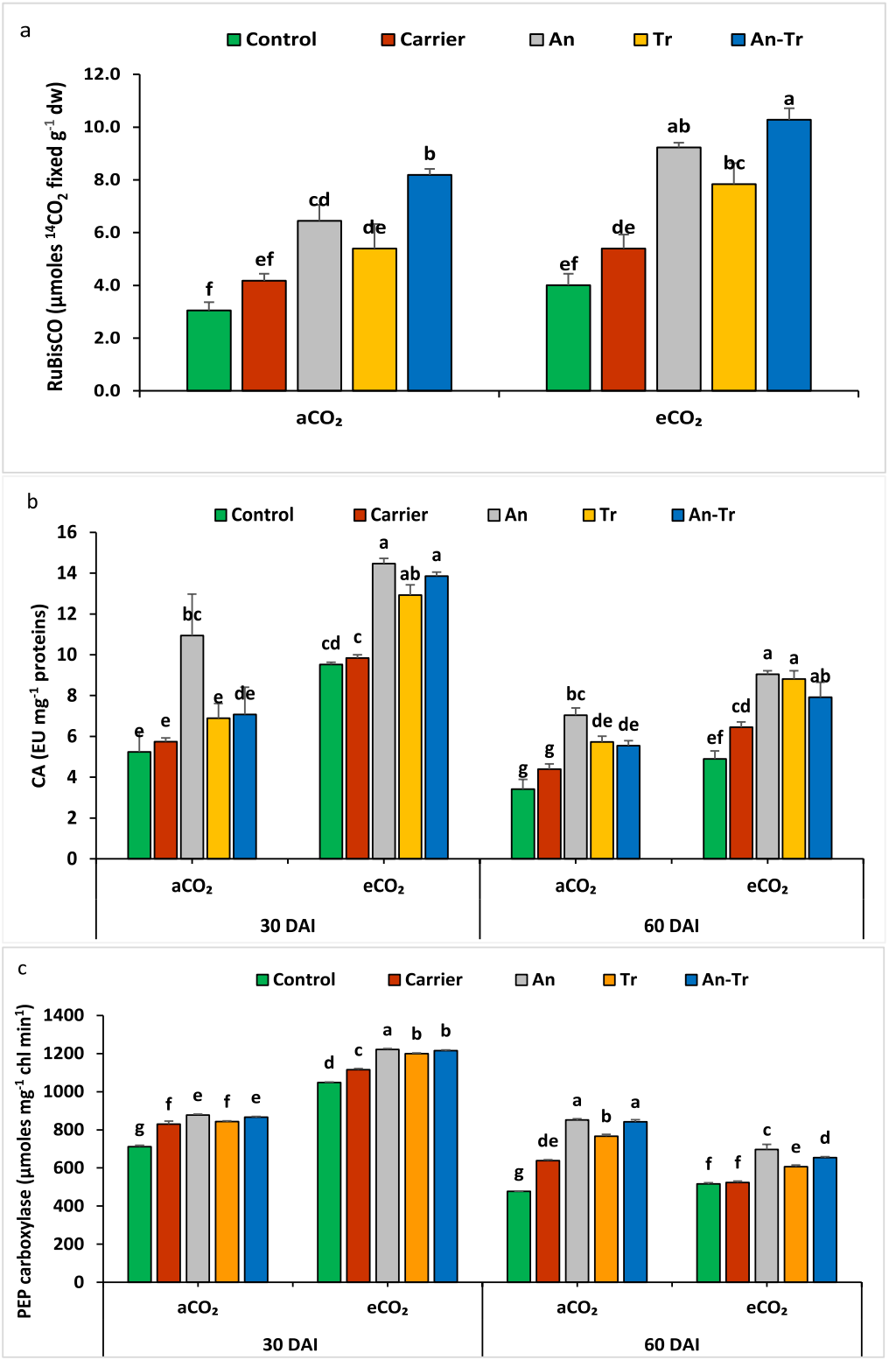

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Fig. 2. Enzyme activity related to N assimilation, in tomato leaves, as influenced by cyanobacterial inoculants under ambient CO_2 (aCO_2) and elevated CO_2 (eCO_2) conditions at 30 and 60 DAI (days after inoculation); a. Ribulose 1,5- bisphosphate carboxylase/oxygenase (RuBisCO); b. Carbonic anhydrase; (CA); c. Phosphoenolpyruvate carboxylase (PEPCase); Treatments: Control, No inoculation; Carrier, Paddy straw compost and vermiculite in 1:1; *Anabaena torulosa* (An), *Trichoderma viride* (Tr), and biofilm *Anabaena torulosa- Trichoderma viride* (An-Tr); F and p-values were illustrated in Supplementary Table 5.

Figs. 1a and b).

3.3. Leaf growth related parameters

Leaf chlorophyll estimated at 30 and 60 DAI, in all the treatments showed a significant increase under eCO_2 than aCO_2 conditions. The plants inoculated with biofilm An-Tr showed the highest chlorophyll content (12.09 mg g^{-1}) at 30 DAI and 26.35 (mg g^{-1}) at 60 DAI, followed by An (10.97 mg g^{-1}) at 30 DAI and 25.75 mg g^{-1}) at 60 DAI under eCO_2 conditions (Fig. 1a). An increase of 51.25% and 37% in chlorophyll content in An-Tr compared to control, under aCO_2 and eCO_2 conditions respectively. The interaction effect between culture, CO_2 level, and days was significant. Under both aCO_2 and eCO_2 condition, the values of leaf proteins significantly increased with time, from 30 to 60 DAI in all the treatments. The biofilm An-Tr inoculation showed the highest values of leaf proteins (218 mg g^{-1}) under eCO_2 level at 60 DAI (Fig. 1b). Significant interactions between treatments, CO_2 levels, and DAI were also recorded.

Total leaf sugars significantly increased under eCO_2 condition in samples from An-Tr treatment showing the highest total sugars (172.30 mg g^{-1}) at 30 DAI and 176.0 mg g^{-1}at 60 DAI under eCO_2 condition, followed by An (171.78 mg g^{-1}) under eCO_2 condition at 60 DAI (Fig. 1c). Leaf sugar content showed an increase of 87 and 47% in An-Tr treated plants compared to control, under aCO_2 and eCO_2 conditions respectively. A significant interaction effect between treatments, CO_2 level, and DAI was recorded.

Leaf IAA significantly increased from 30 to 60 DAI under both aCO_2 and eCO_2 conditions in all the treatments. The plants inoculated with An-Tr recorded the highest values, with 19.24 μg g^{-1} at 30 DAI under aCO_2 condition and 34.89 μg g^{-1} at 60 DAI under eCO_2 condition, followed by An under both conditions (Fig. 1d). There was an increase of 28% in leaf IAA content in An-Tr treated

Table 3
Evaluation of the activities related to N assimilation, antioxidative and defense enzymes in tomato leaves, as influenced by cyanobacterial inoculants under two different CO_2 conditions (ambient and elevated CO_2 conditions-aCO_2, eCO_2) at 30 and 60 DAI.

Factors	GS (μmoles γ-glutamyl hydroxamate g f. wt.$^{-1}$ min^{-1})		PAL (nmoles transcinnamic acid g f. wt.$^{-1}$ min^{-1})		SOD (IU g f. wt.$^{-1}$ min^{-1})		Peroxidase (IU g f, wt.$^{-1}$)	
	30 DAI	60 DAI	30 DAI	60 DAI	30 DAI	60 DAI	30 DAI	60 DAI
CO_2 conditions								
aCO_2	3.67	3.70	26.29	52.27	0.014	0.031	32.98	55.03
eCO_2	5.00	5.48	60.25	67.88	0.021	0.038	38.97	73.16
CD ($P \leq 0.05$)	0.07	0.14	0.26	0.27	0.004	0.002	0.67	1.10
Treatments								
Control	3.30	3.66	36.57	49.15	0.014	0.020	29.71	57.07
Carrier	3.94	4.15	38.27	52.26	0.016	0.028	31.40	61.21
An	4.77	4.96	47.15	74.71	0.018	0.041	39.52	67.99
Tr	4.32	4.81	43.58	57.22	0.018	0.034	38.24	64.29
An-Tr	5.34	5.38	50.78	67.03	0.021	0.050	41.02	69.90
CD ($P \leq 0.05$)	0.19	0.22	0.41	0.43	0.006	0.003	1.06	1.73
CO_2 conditions/Treatments								
aCO_2								
Control	2.82	2.49	21.98	42.23	0.011	0.018	26.61	48.10
Carrier	3.29	3.12	23.02	46.21	0.012	0.028	28.43	53.47
An	4.18	4.38	27.45	60.05	0.014	0.033	36.31	57.50
Tr	3.71	3.97	25.94	49.54	0.016	0.032	35.66	54.95
An-Tr	4.36	4.56	33.05	63.31	0.018	0.042	37.87	61.10
eCO_2								
Control	3.79	4.84	51.16	56.07	0.017	0.022	32.80	66.04
Carrier	4.59	5.18	53.52	58.32	0.019	0.029	34.36	68.94
An	5.37	5.53	66.84	89.36	0.022	0.049	42.73	78.48
Tr	4.92	5.65	61.22	64.91	0.020	0.036	40.82	73.62
An-Tr	6.33	6.19	68.51	70.76	0.025	0.057	44.16	78.69
CD ($P \leq 0.05$) CO_2conditions x Treatments	0.17	0.31	0.59	0.60	0.009	0.004	1.50	2.45

F and p-values illustrated in Supplementary Table 4.
Treatments: Control, No inoculation; Carrier, Paddy straw compost and vermiculite at 1:1; An, *Anabaena torulosa*; Tr, T*richoderma viride*; and An-Tr, biofilm *Anabaena torulosa - Trichoderma viride.*

plants compared to control, under both the CO_2 levels. The interaction effect between treatments, CO_2 level, and DAI was significant.

3.4. Leaf enzyme activities related to C and N metabolism

RuBisCO activity assayed at 30 DAI was significantly higher under eCO_2 compared to aCO_2 incubation. The plants inoculated with the biofilm An-Tr showed the highest enzyme activity (10.28 μmoles $^{14}CO_2$ fixed g^{-1} dw), followed by An inoculated plants (9.23 μmoles $^{14}CO_2$ fixed g^{-1} d. w.) (Fig. 2a). A several-fold increase in enzyme activity under both the CO_2 conditions, was recorded, in plants treated with An-Tr, as compared to control.

A significant increase in the carbonic anhydrase (CA) activity in all the treatments under aCO_2, as compared to eCO_2 condition was documented. The plants treated with An (14.47 EU mg^{-1} proteins) and An-Tr (13.86 EU mg^{-1} protein) showed the highest CA activity, followed by Tr (12.93 EU mg^{-1} protein) at 30 DAI. At 60 DAI, the treatments An (9.05 EU mg^{-1} proteins), followed by Tr (8.81 EU mg^{-1} proteins) showed the highest enzyme activity (Fig. 2b). An increase of 87% in the activity was observed, in plants treated with An, compared to control. The interaction effect between treatments and CO_2 levels, and CO_2 levels and DAI was non-significant, but significant for the tripartite interactions among treatments, CO_2 levels, and DAI.

The leaf PEP carboxylase activity significantly increased under aCO_2 in all the treatments, compared to eCO_2 condition, with significant higher values at 30 DAI. The plants inoculated with An exhibited the highest values (1221.4 μmoles mg^{-1} chl min^{-1}), followed by An-Tr (1215.9 μmoles mg^{-1} chl min^{-1}) at 30 DAI under aCO_2 condition (Fig. 2c). At 60 DAI, the plants treated with An (851.9 μmol mg^{-1} chl min^{-1}) and An-Tr (841.9 μmoles mg^{-1} chl min^{-1}) showed the highest enzyme activity under eCO_2 condition. The interaction effect between treatments, CO_2 level, and DAI was significant.

The activity of GS assayed at 30 and 60 DAI, revealed a gradual increase in the enzyme activity under eCO_2 than aCO_2 condition, in all the treatments. Inoculation with An-Tr showed the highest enzyme activity (6.32 μmoles **γ**-glutamyl hydroxamate g^{-1} f. wt. min^{-1}) at 30 DAI and 6.19 μmoles **γ**-glutamyl hydroxamate g^{-1} f. wt. min^{-1} at 60 DAI under eCO_2 condition (Table 3). There was a significant increase of 57% in enzyme activity in An-Tr treated plants, compared to control plants. The tripartite interaction between treatments, CO_2 level, and DAI was significant.

3.5. Hydrolytic enzymes assays

The leaves from An-Tr treatment showed the highest β-1,4 endoglucanase enzyme activity- 2.03 IU g^{-1} and 1.89 IU g^{-1} under eCO_2 and aCO_2 levels respectively at 30 DAI. At 60 DAI, An inoculated plants showed greater enzyme activity (2.24 IU g^{-1}) under eCO_2 condition. There was an enhancement of 33% and 38% in enzyme activity, over control, in An-Tr treated plants under aCO_2 and eCO_2 condition respectively. The chitosanase activity significantly increased from 30 to 60 DAI with a gradual increase under eCO_2 than aCO_2 conditions (Suppl. Figs. 2a and 2b). The plants treated with An-Tr showed highest enzyme activity of 2.81 IU g^{-1} and 5.38 IU g^{-1} at 30 and at 60 DAI respectively under eCO_2 compared to aCO_2 condition and with respect to controls. The interaction effect was significant between treatments, CO_2 level, and DAI for these two hydrolytic enzyme activities.

3.6. Antioxidant enzyme assays

The activity of enzyme PAL significantly increased under eCO_2 compared to aCO_2 condition. An increment of 40% was recorded in An-Tr treated plants compared to control, when measured at 30 and 60 DAI. The plants inoculated with An-Tr showed the highest enzyme activity (68.51 n moles t-cinnamic acid min^{-1} g^{-1} f. wt.), at 30 DAI, while at 60 DAI, the activity was higher in An (89.36 n moles t-cinnamic acid min^{-1} g^{-1} f. wt.) closely followed by An-Tr (70.76 n moles t-cinnamic acid min^{-1} g^{-1} f. wt.) under eCO_2 condition (Table 3). The interaction effect was significant between treatments, CO_2 levels and DAI.

The SOD activity gradually increased from 30 to 60 DAI in all the treatments, under both environments. The plants treated with An-Tr showed the highest enzyme activity (0.025 IU g^{-1} f. wt. min^{-1}) at 30 DAI and 0.050 IU g^{-1} f. wt. min^{-1} at 60 DAI followed by An (0.022 IU g^{-1} f. wt. min^{-1}) at 30 DAI and 0.049 IU g^{-1} f. wt. min^{-1} at 60 DAI under eCO_2, as compared to aCO_2 conditions (Table 3). A significant increase of almost 91% in enzyme activity in An-Tr inoculated plants was recorded, as compared to controls, under both conditions. The interaction effect was significant between treatments and CO_2 level and treatments and DAI, but the effect was non-significant between CO_2 level and between DAI and treatments, CO_2 level and DAI.

The peroxidase activity showed a significant increase at 60 DAI, under eCO_2 condition. The plants inoculated with An-Tr was showed the highest enzyme activity (44.16 IU g^{-1} f. wt.) at 30 DAI and 78.69 IU g^{-1} f. wt. at 60 DAI under eCO_2 condition (Table 3). There was an increase of 30% in the enzyme activity in An-Tr treated plants, compared to controls under both conditions. The interaction effect was significant between treatments and DAI, and CO_2 level and DAI, but the effect was non-significant between treatments and CO_2 level, and between treatments, CO_2 levels, and DAI.

Catalase (CAT) activity showed a gradual increase from 30 to 60 DAI. The plants treated with An (0.17 IU g^{-1} f. wt.)) under aCO_2 and An-Tr (0.16 IU g^{-1} f. wt.)) under eCO_2 showed the highest enzyme activity at 30 DAI. At 60 DAI, the An-Tr treated plants showed the highest enzyme activity (0.19 IU g^{-1} f. wt.)) followed by An (0.17 IU g^{-1} f. wt.)) under eCO_2 conditions (Supplementary Table 2). The interaction effect was non-significant between treatments, CO_2 levels, and DAI.

Under both CO_2 conditions, PPO activity exhibited a gradual increase from 30 to 60 DAI in all the treatments. plants inoculated with An-Tr showed the highest enzyme activity (13.65 Units g^{-1} f. wt.)), followed by An (12.35 Units g^{-1} f. wt. min^{-1})) at 30 DAI. At 60 DAI, An-Tr (14.82 Units g^{-1} f. wt. min^{-1})) and An (14.26 Units $g^{-1}min^{-1}$) showed the highest activity followed by Tr (13.65 Units g^{-1} f. wt. min^{-1}) (Supplementary Table 2). An-Tr treated plants recorded 50% increase in enzyme activity, as compared to control

under both CO_2 conditions. The interaction was non-significant between treatments, CO_2 levels, and DAI.

3.7. *Interrelationships among leaf growth parameters, leaf enzyme activities and cyanobacterial inoculants under ambient and elevated CO_2 conditions*

The matrix generated using the correlation coefficients at 5% level of significance based on the interactions among various leaf growth parameters and enzyme activities illustrated that under aCO_2 condition, leaf chlorophyll positively correlated with leaf proteins, leaf sugars ($r > 0.97$), IAA ($r > 0.99$), and enzyme activities- RuBisCO ($r > 0.90$), PEP carboxylase ($r > 0.84$), CA ($r > 0.47$), GS ($r > 0.98$), β-1,4 endoglucanase ($r > 0.77$), chitosanase ($r > 0.99$), PAL ($r > 0.93$), SOD ($r > 0.69$), CAT ($r > 0.54$), peroxidase ($r > 0.95$) and PPO ($r > 0.92$). Similar correlations among all these parameters, on one-one basis was also recorded (Fig. 3a and Supplementary Fig. 3a).

A similar picture emerged under eCO_2 conditions, with leaf chlorophyll positively correlating with leaf proteins ($r > 0.80$), leaf sugars ($r > 0.99$), IAA ($r > 0.99$), RuBisCO ($r > 0.99$), PEP carboxylase ($r > 0.86$), CA ($r > 0.86$), GS ($r > 0.98$), β-1,4 endoglucanase ($r > 0.92$), chitosanase ($r > 0.91$), PAL ($r > 0.96$), and lower correlation with SOD ($r > 0.41$), and catalase ($r > 0.42$), peroxidase ($r > 093.$) and PPO ($r > 0.92$). Interrelationships among these parameters was also significant (Fig. 3b and Supplementary Fig. 3b).

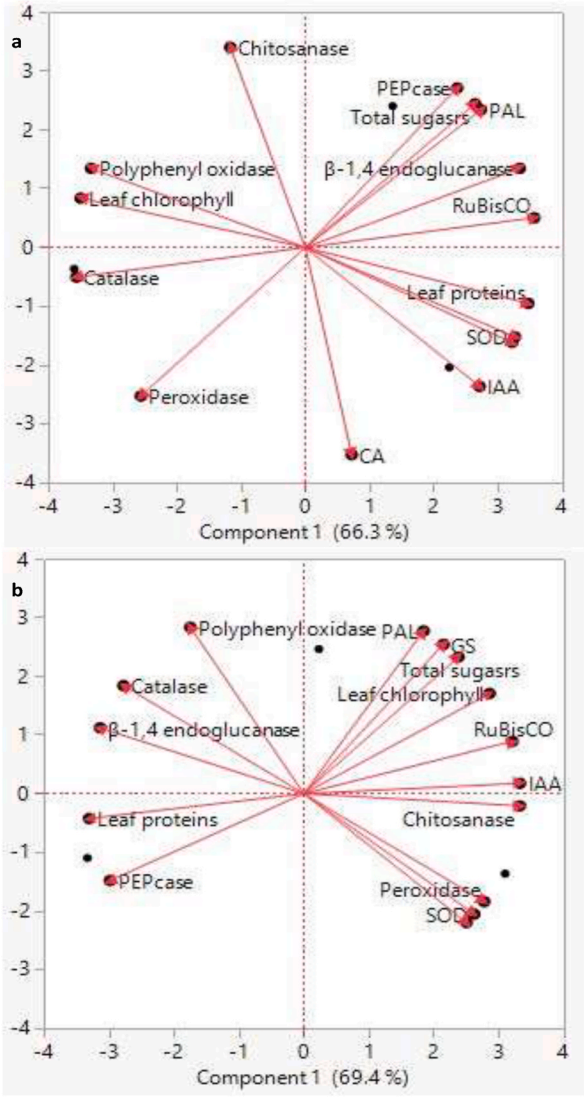

Fig. 3. Principal component analysis (PCA) biplot of tomato leaves parameters, as influenced by cyanobacterial inoculants under two different CO_2 conditions. a. ambient CO_2 and b. elevated CO_2 conditions-aCO_2, eCO_2) at 30 and 60 DAI (days after inoculation); Treatments: Control, No inoculation; Carrier, Paddy straw compost and vermiculite in 1:1; *Anabaena torulosa* (An), *Trichoderma viride* (Tr), and biofilm *Anabaena torulosa-Trichoderma viride* (An-Tr).

3.8. Flower and fruit parameters

The highest number of flowers and fruits per plant was recorded in plants treated with An-Tr under eCO_2 as compared to aCO_2 (Fig. 4a and b), while the values recorded for fruit weight, fruit firmness, ascorbic acid, and lycopene contents were significantly higher in all the treatments under eCO_2 condition. The plants inoculated with An-Tr showed the highest fruit weight (44.99 g), followed by An inoculated plants (41.43 g) under eCO_2 condition (Table 4). The plants inoculated with An-Tr showed the highest values for fruit firmness (235.2 g), followed by An inoculated plants (193.4 g) under eCO_2 condition (Table 4). An-Tr inoculation led to highest titrable acidity (0.45%) under eCO2 condition, followed by An inoculated plants (0.41%) and Tr inoculated plants (0.39%) under eCO_2 and An-Tr inoculated plants (0.40%) under aCO_2 condition (Table 4). The plants inoculated with An-Tr also exhibited the highest ascorbic acid content (15.05 mg 100 g^{-1} f. wt.), followed by An inoculated plants (14.09 mg 100 g^{-1} f. wt.) under eCO_2 condition (Table 3). The plants inoculated with An-Tr exhibited the highest lycopene content (74.79 mg kg^{-1} f. wt.) under eCO_2 condition, followed by An-Tr inoculated plants (70.63 mg kg^{-1} f. wt.)) under aCO_2 condition (Table 4). The interaction effect was significant between treatments, CO_2 level, and DAI.

Using the correlation coefficients generated based on the interactions among various fruit quality parameters and leaf enzyme activities, a matrix was developed. Under aCO_2 condition, fruit weight exhibited a positive correlation with fruit firmness ($r > 0.76$), titrable acidity ($r > 0.86$), ascorbic acid ($r > 0.94$), and lycopene ($r > 0.95$). Fruit firmness showed a positive correlation with titrable acidity ($r > 0.76$), ascorbic acid ($r > 0.75$), and lycopene ($r > 0.79$). Titrable acidity was positively correlated with ascorbic acid ($r > 0.92$), and lycopene ($r > 0.92$). Ascorbic acid content positively correlated with lycopene content in fruits ($r > 0.98$) (Supplementary Fig. 4a). Under eCO_2 condition, a similar trend emerged, however, the correlation coefficients were significantly higher, particularly, fruit weight with fruit firmness ($r > 0.83$), TA ($r > 0.93$), ascorbic acid ($r > 0.94$), and lycopene ($r > 0.97$), and titrable acidity with ascorbic acid ($r > 0.90$), and lycopene ($r > 0.96$). (Supplementary Fig. 4b).

4. Discussion

Fertilization of crops with bio-inputs, to reduce the hazards of chemical fertilizers and enrich nutrient-poor habitats is an important

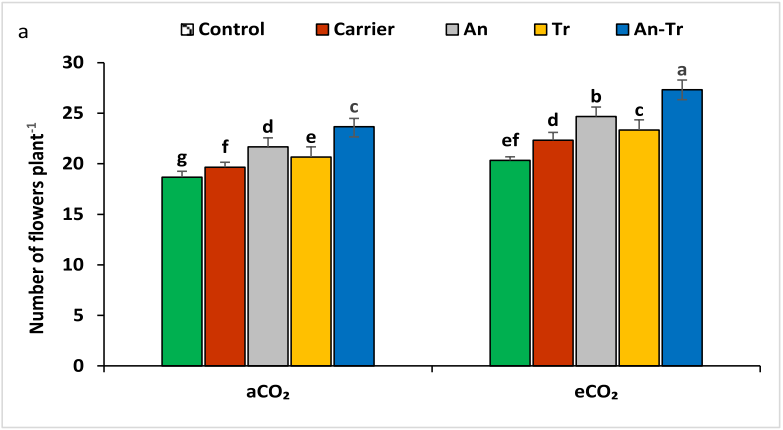

Fig. 4. Number of flowers and fruits in tomato crop, as influenced by cyanobacterial inoculants under ambient CO_2 (aCO_2) and elevated CO_2 (eCO_2) conditions at 30 and 60 DAI (days after inoculation); a. number of flowers; b. number of fruits; Treatments: Control, No inoculation; Carrier, Paddy straw compost and vermiculite in 1:1; *Anabaena torulosa* (An), *Trichoderma viride* (Tr), and biofilm *Anabaena torulosa- Trichoderma viride* (An-Tr).

Table 4
Fruit quality parameters in tomato as influenced by cyanobacterial inoculants under two different CO_2 conditions (ambient and elevated CO_2 conditions-aCO_2, eCO_2) at harvest stage.

Factors	Fruit weight (g)	Fruit firmness (g)	Titrable acidity (%)	Ascorbic acid (mg 100 g f. wt. $^{-1}$)	Lycopene (mg kg f. wt. $^{-1}$)
CO_2 conditions					
aCO_2	36.42	116.31	0.35	10.52	64.66
eCO_2	39.11	187.66	0.39	12.68	67.33
CD ($P \leq 0.05$)	0.57	6.25	0.006	0.13	0.14
Treatments					
Control	32.61	126.88	0.29	9.42	58.88
Carrier	35.42	139.80	0.35	10.24	62.62
An	40.27	162.84	0.39	12.76	69.36
Tr	37.55	146.68	0.37	11.75	66.42
An-Tr	42.99	183.73	0.43	13.84	72.72
CD ($P \leq 0.05$)	0.90	9.96	0.010	0.20	0.22
CO_2 conditions/Treatments					
aCO_2					
Control	31.92	92.07	0.28	8.46	58.05
Carrier	34.32	111.30	0.33	9.65	61.55
An	39.12	132.27	0.37	11.42	68.64
Tr	35.75	113.63	0.35	10.43	64.43
An-Tr	40.99	132.27	0.40	12.63	70.64
eCO_2					
Control	33.29	161.69	0.30	10.38	59.71
Carrier	36.51	168.30	0.37	10.82	63.69
An	41.43	193.40	0.41	14.09	70.07
Tr	39.35	179.73	0.40	13.07	68.40
An-Tr	44.99	235.19	0.45	15.05	74.80
CD ($P \leq 0.05$) CO_2 conditions x Treatments	1.28	14.00	0.014	0.29	0.30

F and p-values illustrated in Supplementary Table 6.
Treatments: Control, No inoculation; Carrier, Paddy straw compost and vermiculite at 1:1; An, *Anabaena torulosa*; Tr, *Trichoderma viride*; and An-Tr, biofilm *Anabaena torulosa- Trichoderma viride.*

area of research, and cyanobacterial inoculation is known to benefit, both as a biofertilizer and soil conditioner [35]. In agriculture, the role of cyanobacteria as biofertilizers, plant growth promoting and micro- and macronutrient-enriching options, has been documented across crops [9,36], however, information on deploying them as a biofilm to modulate plant C–N metabolism and effects on fruit quality under eCO_2 condition is limited. With the increasing awareness of increasing CO_2 levels in the environment leading to global warming-related climate change, scientists are looking towards biological options which can recycle this excess CO_2, towards value addition. Carbon storage in soil, can be a promising long-term alternative and crop residues and organic inputs has shown promising results in terms of increasing SOC [37].

Cyanobacteria-mediated restoration of degraded land in diverse ecosystems is one of the means through which carbon sequestration improve soil physicochemical properties, including neutralization of the pH and boost ecological services [38,39], or through exopolysaccharides (EPS) which represent an important component of biota and C and associated polymers improve soil aggregation, and movement of water and air in the soil column [40]. A much less investigated aspect is chanelling this towards enhancing the nutritional quality of produce.

Synergistic interactions of cyanobacteria with agriculturally important bacteria and fungi observed in consortia inspired the development of biofilm biofertilizers [16,41,42]. Such biofilms lead to enhanced nutrient availability, growth, crop yield, plant attributes besides exhibiting a positive correlation with microbiological activities such as nitrogen fixation, soil chlorophyll, and soil available nitrogen across crops [6,42]. Biofilm inoculationaids in better colonization of roots and rhizosphere, and enhances the polysaccharide content and through its interactions with plant/microbial exudates leads to enhanced soil fertility, aggregate formation and provision for carbon [11], as also recorded in this study. Previous investigations provided useful preliminary information regarding the effect of cyanobacterial inoculation in terms of C, N enrichment under both aCO_2 and eCO_2 conditions [17]. In the present study, in-depth analyses into the influence of cyanobacterial inoculum as an individual culture and biofilm illustrated a significant enhancement in the nutrient availability, microbiological, and plant biometric parameters in tomato plants, besides better quality of product and yields under elevated CO_2 condition.

4.1. Effect on soil microbiological activity and nutrient-availability

In the present study, a significant increase was recorded in C–N mobilizing enzyme activities, along with higher nitrogen fixation rate, increase in available nitrogen, and higher yield with An-Tr biofilm inoculation, supporting previous observations of cyanobacterial inoculation under eCO_2 conditions. Our earlier reports under ambient conditions showed that cyanobacterial biofilm inoculation elicited the plant metabolic machinery, resulting in better nutrient availability, including Zn and growth, and yields, as recorded in maize [43,44], however, the effect of eCO_2 as an elicitor was not investigated. In legumes, *Anabaena-T. viride* biofilm formulations proved to be the most promising, recording 12–25% enhanced yield and dehydrogenase activity [45]. An increase in dehydrogenase activity is considered a suitable index for evaluating microbial activity in soil [46]. Earlier studies have shown that *Trichoderma viride-Bradyrhizobium* biofilm exhibited 20–45% enhancement in fresh/dry weight of plants while the *T viride–Azotobacter* biofilm exhibited the highest dehydrogenase activity in the soil and nitrogen fixation [45,47]. The addition of cyanobacterial formulation composed of *Tolypothrix tenuis* and *Microchaete tenera* to the soil led to improved soil quality, which could be correlated with exopolysaccharides produced by these organisms [48]. This supports our observations on the higher values of organic carbon (OC) and available nitrogen content (than uninoculated soil) under eCO_2 condition, with inoculation of *Anabaena torulosa* (An), and An-Tr, both under aCO_2 and eCO_2 conditions. It could be inferred from the data that eCO_2 had a more significant effect on growth of tomato crop, while the presence of plant was more influential towards modulating the soil polysaccharide content and available N. The use of dried cyanobacteria was found to enhance the N status of crops over the control, which may also positively influence the mobility and root uptake of Zn and Fe from the soil [49].

Overall, the use of cyanobacterial inoculants significantly enriched the availability of N, besides savings of almost 50% N; this can be a significant economical option for the grower.

4.2. Plant metabolism and growth-linked activities

Plants are known to produce a diverse range of metabolites in response to environmental perturbations [50,51], linked to growth or secondary metabolism, which may promote the earliness in reproductive phase. In soybean, the photosynthetic rate was positively influenced by every incremental increase in CO_2 concentration, regardless of the stage of the crop. eCO_2 conditions proved conducive for cyanobacterial proliferation, which in turn facilitated healthy growth of plants, as result of nutrient-rich inputs provided to the plant [17]. Although the plants grown under eCO_2 were approximately 15 cm taller, aCO_2 grown plants exhibited higher values for leaf area, higher chlorophyll *a and b*. The plants grown under eCO_2 exhibited greater water-use efficiency (WUE) than those grown under aCO_2. This illustrated the beneficial effects of increased biomass and yield because of increasing CO_2 supply on photosynthesis and WUE, rather than on transpiration, as reported earlier [52].

In the present study, total leaf chlorophyll content was much higher in An-Tr treated plants than control, followed by An. The effect of eCO_2 on growing plants and during flowering time is highly variable among species, and often influences phenology or quality [53]. Plants grown under eCO_2 with cyanobacterial biofilm treated in soil exhibited early flowering, by almost 6–8 days, than plants grown with only the individual culture, under aCO_2 condition. Similar observations were reported by Ref. [54], who showed that tomato growth under eCO_2 gives early flowering than plants grown under aCO_2.

Several cyanobacteria have evolved a novel environmental adaptation, known as a carbon concentrating-mechanism (CCM), that vastly improves photosynthetic performance and survival under limiting CO_2 concentrations [30]. Application of cyanobacterial formulations in C3 crops provides a potential means of elevating CO_2 at the vicinity of RuBisCO, thereby decreasing photorespiration and increasing photosynthetic efficiency and yield. This is mainly attributed to the cyanobacterial carbon concentrating-mechanism (CCM) which influences leaf light-saturated CO_2 uptake (*Asat*). From these findings, we can say that an artificial rise in CO_2 will increase 60% *Asat*, resulting in a 36–60% increase in yield. In our research study, significantly higher yields were observed with C3 tomato plants grown under eCO_2 levels, which can be the net result of plants showing higher RuBisCO activity under eCO_2 condition. After an initial increase with CO_2 enrichment, net photosynthesis often declines which is mediated by a decline in RuBisCO activity; this decline results due to decreased RuBisCO protein, activation state, or feedback inhibition of the RuBP. However, for field-grown soybeans and salt marsh species, a reduction in net photosynthesis and RuBisCO activity was not an inevitable response under CO_2 enrichment, because strong sinks or rapid translocation may serve as acclimation responses [55]. The shoot biomass was relatively unaffected when plants grown under a combination of O_3 and eCO_2 environment, but plants grown under in combination of O_3 and aCO_2 showed decreased photosynthetic pigments, soluble proteins, and RuBisCO protein, which results in enhanced oxidative damage to proteins, but these effects were not observed in plants exposed to O_3 under eCO_2 [56]. In plants grown under eCO_2, a decrease in the photorespiration rate by reducing the oxygenase activity of RuBisCO occurs, which in turn, leads to an increase in the net photosynthesis rate [50]. The distinct effect of the inoculant An, in relation to leaf characteristics such as leaf proteins and C-related enzyme activities was clearly brought about, although An-Tr inoculation was the most superior in terms of both soil and plant-related traits. In an earlier study, elevated CO_2 elicited a positive impact on N-fixation, C-assimilation, and biomass production in cyanobacteria, which, in turn can influence plant metabolism; this highlights their beneficial role as a biofertilizer [56].

One critical plant carbon assimilation enzyme is PEP carboxylase, which is involved in photo respired CO_2 being recycled to improve C-capture and its assimilation. In our study, a significant enhancement in leaves from cyanobacteria-inoculated plants under aCO_2 condition at 30 DAI was recorded, but the activity reduced at 60 DAI i.e., during fruit maturity/ripening stage. This metabolic stimulation at flowering stage and the early fruit forming stage significantly correlate with the eCO_2 levels of antioxidant compounds and innate defense enzymes, which are known to orchestrate and mutually function, thereby delaying the release of senescence-inducing reactive oxygen species [57]. It is known that the regulation of the fruit-specific PEP carboxylase *SlPPC2* promoter occurs

at early stages of tomato fruit formation, which is known to respond to hormones (ethylene) and metabolites (sugars) and regulate leaf and fruit growth and metabolism [58], as also recorded in the present investigation. with beneficial effects on plant development. As a result of the regulation of the fruit-specific PEP carboxylase *SlPPC2* promoter at early stages of tomato fruit formation, a 1966 bp DNA fragment located upstream of the ATG codon of the *SlPPC2* gene was identified, which confers the appropriate fruit-specificity in tomato [58]. Also, the *SlPPC2* promoter region is known to respond to hormones (ethylene) and metabolites (sugars), both are enhanced in cyanobacteria, as shown in earlier studies under elevated CO_2 [56], which in turn, regulate leaf and fruit growth and metabolism, and confirmed by transient expression assays [57]. The activity of PEP carboxylase was 2.36-fold higher in the plants inoculated with *A. laxa* and with 100% N application, compared to control plants grown only with fertilizers in an earlier study in chrysanthemum [6].

CA activity was also significantly evaluated under aCO_2 condition, in leaves from plants inoculated with cyanobacterial biofilm at 30 DAI during the flowering stage, but the activity reduced at 60 DAI, as for PEP carboxylase activity. This can be attributed to the beneficial effect of cyanobacterial inoculation on plant growth, thereby making plants more robust and metabolically efficient at an early stage of growth.

Elevated CO_2 condition also helps to reduce the damaging effects of oxygen free radicals during vegetative stages of tomato plants as compared to O_3 grown plants under aCO_2 conditions [51]. In our study, the activity of antioxidative and defense enzymes in tomato leaves after 30 and 60 DAI, revealed significant differences in plants grown under eCO_2 condition with inoculants. The plants grown under O_3 exposure enhanced the specific activities of superoxide dismutase, peroxidase, glutathione reductase, and ascorbate peroxidase initially, but the specific activities decreased in plants with prolonged exposure to O_3 under aCO_2. But eCO_2 led to preferential changes in the isoform composition of these antioxidant enzymes of plants grown under a combination of O_3 and eCO_2, with the plant performing all the metabolic activities efficiently under abiotic stress [59]. The metabolic stimulation at flowering stage and the early fruit forming stage significantly correlate with the eCO_2 levels of antioxidant compounds and innate defense enzymes, which are known to orchestrate and mutually function, thereby delaying the release of senescence-inducing reactive oxygen species [57], with beneficial effects on plant development, as also recorded in the present investigation.

The enzyme activity of PAL, peroxidase, and total phenolic content were fairly high in rice leaves inoculated with *Oscillatoria acuta* and *Plectonema boryanum* after 30 days, as illustrated by Abuye and Achamo [60]. This was attributed to the differential systemic accumulation of phenylpropanoids, flavonoids, and plant growth regulators in plant leaves, which enhances the antioxidant and defense enzyme activity in presence of cyanobacteria. In the present experiments, the activities of SOD, PPO, and PAL were also significantly higher in the plants inoculated with *A. torulosa* and with full dose of N application, compared to control plants grown only with fertilizers.

The constitutive activities of SOD, dehydroascorbate reductase (DHAR), and CAT were similar in both wheat (C3) and maize (C4) plants, while higher values were recorded in the leaves of sunflower (C3) than in those of maize (C4). This shows that C3 plants may show higher antioxidant and defense mechanisms than C4 plants under abiotic stress; the enzyme activity largely depended on the plant organ and nitrogen source. Similar results were reported in terms of antioxidative enzyme activities in chrysanthemum receiving inoculation of *Anabaena torulosa* and different doses of N [6].

4.3. Elicitation of metabolic machinery towards higher yields and better fruit quality

The interactive effects of eCO_2, N fertilization, and reduced irrigation on fruit yield and quality in tomato was investigated [61]. who showed higher yield and good fruit quality attributes such as fruit firmness, fruit sugars, organic acids, and various ionic concentrations in fruit juice. The significance of microbial inoculation in improving crop growth, yields and quality of produce has been demonstrated in several crops, including tomato. Inoculation of root endophytic fungus *Piriformospora indica* can enhance tomato plant growth and yields, both under normal and salt stress conditions, as demonstrated by the promise of *Anabaena* sp. as a biofertilizer in tomato crop [60]. Improved soil fertility, along with increase in nutrient content of plant (including fruit β-carotene, total nitrogen (TN), phosphorus, zinc, iron) were the other beneficial attributes recorded.

eCO_2 also enhances the yield of vegetables and which could also affect the nutritional quality of vegetables [62]. Based on meta-analysis using 57 articles, it was illustrated that vegetables grown under eCO_2 exhibit an increase in the concentrations of glucose, fructose, total soluble sugars, ascorbic acid, total antioxidant activity, total flavonoids, total phenols, and calcium in the edible part. A similar response to inoculation and eCO_2 was recorded in our study. The effects of eCO_2 on the overall physiology, growth, yield and quality parameters of tomato fruit (*Arka Ashish*) were investigated [63]. Plants grown at eCO_2 (700 ppm) in open-top chambers (OTC) showed increased assimilation rate by decreased stomatal conductance and transpiration rate, reduced leaf osmotic potential by increased water potential during flowering and fruiting setting stages. As recorded in our study, plant height was significantly higher, and more number of branches, however, they recorded lower total chlorophyll content and leaf area, although specific leaf mass was found to be higher in their study, due to higher leaf and root dry mass. An increased number of flowers and fruits resulted in the highest yield, which supports our observations. The fruits showed a higher content of ascorbic acid, carotenoids, and lycopene content, which in the present investigation was further stimulated by inoculation, particularly, An-Tr and An compared to control plants grown under aCO_2.

Plants grown under eCO_2 exhibit higher values of fruit yield than those grown under aCO_2 condition, as reported earlier by other researchers who used microbial inoculation and modulation of environmental conditions, including eCO_2 [51,64]. The significance of cultivar selection for such experiments was also illustrated; in an earlier study [17], an indeterminate cultivar of tomato (NS4266) was tested, which elicited significant interactions with the cyanobacterium inoculated as well as CO_2 conditions for all soil-nutrient related attributes. In the present study, a determinate cultivar of tomato *Avinash* was used, which exhibited significant interaction for

availability of N, but SOC, interactions were non-significant, particularly in relation to + P/-P. In terms of MBC, the different inoculants showed a positive interaction with CO_2 levels, but neither DAI nor plant were influential. This highlights the need to define selection criteria which include details of cultivar, in terms of duration, days to flowering/fruiting, so that the generated data can be robust and provide a broader picture on plant-microbe interactions under eCO_2 conditions. Interaction effects were significant, illustrating that such tripartite interactions can gainfully use CO_2 for stimulating soil fertility and the quality of plant produce.

Comparing with our earlier publication [17], the promise of An-Tr, irrespective of being inoculated as fresh biomass or as dry biomass mixed with carrier, in terms of beneficial influence, even under eCO_2 was highlighted as a novel and reliable option. The investigation also clearly illustrates the synergistic interactions of the partners in the biofilm, as against the partners used alone, and their interplay with soil, plant and soil. eCO_2 facilitated better plant growth by elicitation of plant machinery towards enhanced photosynthetic activity. This in turn led to early flowering, soil nutrient availability, better N assimilation, improved fruit quality, and yields, along with 50% N savings in tomato.

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Ethics approval

Not relevant/applicable to the study.

Author contribution statement

Venkatesh Kokila: Performed the experiments; Analyzed and interpreted the data and Wrote the paper. RADHA PRASANNA: Conceived and designed the experiments; Analyzed and interpreted the data and Wrote the paper. Arun Kumar, Sekar Nishanth, Bhupinder Singh, Shalini Gaur-Rudra, Madan Pal, Awani Kumar Singh, Yashbir Singh Shivay and Priya Pal: Contributed to data and analysis toolsand Wrote the paper.

Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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