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RESEARCH ARTICLE

Effects of short-term exposure to elevated atmospheric CO_2 on yield, nutritional profile, genetic regulatory pathways, and rhizosphere microbial community of common bean (*Phaseolus vulgaris*)

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Abstract

Aim Legumes are vital to agroecosystems and human nutrition, yet climate change is compromising their nutritional value. This study aims to assess how a one-month exposure to elevated CO_2 (eCO_2) impacts biomass yield, mineral profile, gene expression, and the soil microbiome of common bean plants (*Phaseolus vulgaris* L.).

Methods *Phaseolus vulgaris* L. was grown in field conditions under ambient CO_2 (control, aCO_2, 400 ppm) or eCO_2 (600 pm) from the start of pod filling until plant maturity and analyzed for several morphophysiological and nutritional parameters.

Results Compared with aCO_2, eCO_2 exposure significantly increased plant and grain biomass, with fluctuations in mineral accumulation. Notably, it decreased grain iron and zinc concentrations, two essential microelements related to food security, by 59% and 49%, respectively. Additionally, grain phenolic content decreased by up to 41%. Genes involved in mineral uptake (such as *FER1*, *ZIP1*, and *ZIP16*), plant response to stress (*TCR1*, *TCR2*, and *HLH54*) and symbiosis with soil microorganisms (*NRMAP7* and *RAM2*) seemed to regulate effects. Microbiome analysis supported these findings, with an increase in the relative abundance of Pseudomonadota by 10%, suggesting eCO_2-induced alterations in microbial community structure.

Conclusions This research demonstrates how eCO_2 impacts the nutritional quality of common beans regarding micronutrients and phenolic content, while also affecting soil microbiome composition. Highlighting the value of shorter term eCO_2 treatments, the findings provide early insights into immediate plant responses. This underscores the need for crop improvement strategies to address nutrient deficiencies that may arise under future eCO_2 conditions.

Keywords Legumes · Climate change · Elevated CO_2 (eCO_2) · Soil microbiome · Microelements · Phenolic content · Gene expression

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Introduction

With the global population projected to reach ~ 9.7 billion in 2050 and 11.1 billion by 2100 (Lee 2011;

Sadigov 2022), sustainable food production is increasingly critical. However, climate change, driven by rising atmospheric carbon dioxide (CO_2) levels, temperature fluctuations, and erratic rainfall, threatens this goal (Solomon et al. 2009; Mirzabaev et al. 2023). By 2050, CO_2 levels are expected to reach 600 ppm (Vanaja et al. 2024), leading to physiological and biochemical changes that affect protein content and cause nutrient imbalances in crops (Thompson et al. 2017). This effect is particularly concerning for C3 plants, such as legumes, which have less efficient carbon fixation compared to C4 plants (Bräutigam and Gowik 2016). Elevated CO_2 influence further extends to belowground, affecting root growth, nodulation, and the rhizosphere in legumes like soybean and common bean, which play a critical role in nutrient cycling and ecosystem functioning (Palit et al. 2020). For instance, recent studies have highlighted the role of rhizobia, such as *Rhizobium leguminosarum*, in enhancing plant resilience to eCO_2 through improved nitrogen uptake and antioxidant activity (Bellido et al. 2023). Beyond *Rhizobium* species, other microbial groups such as *Pseudomonadota* and *Actinobacteria* also play key roles in mediating soil carbon (C) and nutrient cycles under elevated CO_2 conditions, impacting microbial diversity and activity in the rhizosphere (Joshi et al. 2023; Wu et al. 2024). While most research has focused on long-term exposures (Byeon et al. 2021; Rosado-Porto et al. 2023; Wang et al. 2023), short-term studies can provide early insights into microbial communities shifts, serving as predictors for long-term microbiome alterations (Österreicher-Cunha et al. 2015; Silva et al. 2022). Such findings are especially important in under-resourced regions where nutrient imbalances caused by eCO_2 cannot be mitigated through the application of fertilizers (Nyamasoka-Magonziwa et al. 2023), justifying the need for closer examination of these early microbial changes.

Under-resourced regions are also heavily reliant on legumes to maintain food security, as they serve as essential crops due to their role in providing protein, micronutrients (e.g., iron and zinc) (Ferreira et al. 2021) and phytochemicals (e.g., polyphenols) (Duarte et al. 2024). Nevertheless, these C3 plants are especially susceptible to eCO_2 levels, which affect their carbon metabolism (Ainsworth and Long 2005), root nodulation, and nutrient uptake (Soares et al. 2019a). The pod-filling period is of particular concern, as it involves significant nutrient transfer from vegetative tissues to the developing seeds, determining the crop's final nutrient composition. Elevated CO_2 can alter this process, leading to fluctuations in micronutrient concentrations (Loladze 2014; Soares et al. 2019b) and antioxidant balances (Dong et al. 2018) within the seeds. Understanding how eCO_2 affects plant physiology during this key developmental stage is essential for ensuring the continued productivity and nutritional quality of legume crops under changing environmental conditions (Tulchinsky 2010). Furthermore, considering the diverse strategies that legumes employ to cope with environmental stresses (Duarte et al. 2020), identifying genotypes with favourable responses to eCO_2 will be key to sustaining their critical role in global food security.

Common beans (*Phaseolus vulgaris*) are a model legume species with prior work detailing their ability to tolerate eCO_2.*P. vulgaris* cv. Logan, in particular, has previously shown an ability to cope with long-term eCO_2 without significant nutritional losses in field conditions (in a free air CO_2 enriched – FACE – platform) under aCO_2 (400 ppm) or eCO_2 (600 pm) (Soares et al. 2019a). Nonetheless, we hypothesize that a shorter-term exposure of *P. vulgaris* to eCO_2, namely one month, could affect its nutritional quality and ecological interactions with the rhizosphere microbiome, although some of these microbiome changes might be transient. Demonstrating that this artificial eCO_2 exposure can induce measurable changes offers a practical advantage, as these responses could be detected and analysed without the need for longer-term experiments. This approach can facilitate the improvement of agricultural practices and the selection of germplasm for breeding more adapted genotypes. Thus, this work aimed to explore the physiological, biochemical, and molecular responses of *P. vulgaris* cv. Logan to rapid changes in CO_2 levels, providing insights into the immediate impacts of eCO_2 on biomass accumulation in different plant tissues, grain mineral accumulation, the genetic mechanisms driving observed phenotypic changes, and alterations in microbiome structure and its potential effects on plant growth.

Materials and methods

Study area and experimental conditions

The study area was located at Campus Klein Altendorf of the University of Bonn, at 15 km in South-West direction from Bonn, Germany (50°37′29.39"N, 6°59′11.33"E), where FACE facilities were installed to recreate an eCO_2 environment (Supplementary Material—Fig. 1). The CO_2 was contained in a capacity tank at ~ 150 m away from the FACE ring. The ring was an octahedron of ~ 7.25 m steel pipes (~ 254 m^2) with small openings each 20–30 cm for the ejection of CO_2 in the opposite direction of the wind. Two small cabinets are placed close to the ring, one to distribute the CO_2 into the eight pipes through conduction lines, and the second for power supply. In the centre of the ring, sensors of wind speed, wind direction and CO_2 concentration were installed. Intended to detect which pipes have to be activated, and to monitor the amount of CO_2 that was reaching the furthest point from the pipes. Additionally, four CO_2 sensors were placed at ~ 4 m inner ring.

The seeds of *Phaseolus vulgaris* cv. Logan were obtained from the International Centre for Tropical Agriculture, CIAT (Cali, Colombia). The experiment followed a split-plot design, with two main plots: one exposed to ambient CO_2 levels (aCO_2, 400 ppm) and one fumigated with eCO_2 (600 ppm) using the FACE system (Supplementary Material—Fig. 1). Subplots within each main plot measured 1.20 × 2.60 m, with a sowing density of 25 plants/m^2. The soil was a loamy-clay silt soil (luvisol) and its properties were as follows: pH 6.8, electrical conductivity 0.066 dS m^{-1}, organic matter 1.77%, total N 1.09 mg kg^{-1}, P 60.04 mg kg^{-1}, K 152.72 mg kg^{-1}, B 0.679 mg kg^{-1}, Zn 3.23 mg kg^{-1}, Cu 4.64 mg kg^{-1}, Mn 21.7 mg kg^{-1}, Fe 36.8 mg kg^{-1}, Ca^{2+} 9.68 cmolc kg^{-1}, K^+ 0.362 cmolc kg^{-1}, Na^+ 0.083 cmolc kg^{-1}. Average precipitation and daytime temperatures during the 2019 growing season were 47.3 and 64 mm, and 19.1°C and 18.9°C in July and August, respectively. No irrigation or fertilization was applied; plants received water solely through rainfall.

Three subplots within each main plot were randomly selected, with plants exposed to the two CO_2 conditions (aCO_2 at 400 ppm and eCO_2 at 600 ppm) for one month, from pod filling (R8, Supplementary Material—Fig. 2) until harvest (R). The fixed infrastructure required for CO_2 delivery and monitoring in FACE systems inherently poses challenges, preventing a fully randomized design. To mitigate the potential impact of surrounding conditions and control for external influences, plots were strategically distributed within the experimental area, and buffer zones consisting of two additional rows of *P. vulgaris* were established around each plot perimeter (Supplementary Material—Fig. 1). Despite some limitations, the setup provides valuable insights into the effects of elevated CO_2 on legume crops under controlled conditions.

Plant sampling, biomass estimation and soil sampling chemical analysis

Plant sampling was performed after 1 month of exposure to aCO_2 or eCO_2, when grain maturity was achieved (29 August 2019). A total of 10 representative plants were randomly selected per plot and uprooted, and separated into roots, shoots, and pods. Grains were collected and weighted, and the average seed size was determined. All plant tissues were carefully rinsed with deionized water and dried at 60 °C for 5 days for dry weight estimation, mineral analysis, phenolic content, and antioxidant capacity. An additional set of three plants per treatment was collected as described, flash-frozen in liquid nitrogen and stored at −80 ℃ for gene expression analysis. Rhizospheric soil samples were obtained from individual experimental plots, where each sample constituted a composite of 5 subsamples collected at random within the respective plot. These subsamples were then pooled, homogenized and subsequently sieved (2 mm) for later analysis. Soil material for chemical and microbiome analysis was stored at 4, and −80 °C, respectively. For chemical analysis, soil samples underwent analysis at A2 Análises Químicas, Lda (Guimarães, Portugal) for a wide array of parameters, including pH, electrical conductivity, organic matter content, total nitrogen, phosphorus (P), potassium (K), calcium (Ca^{2+}), magnesium (Mg^{2+}), potassium ions (K^+), sodium ions (Na^+), boron (B), zinc (Zn), copper (Cu), manganese (Mn), and iron (Fe).

Mineral composition

Mineral analysis of shoots and grains was performed as previously described (Santos et al. 2015). Briefly, three replicates of 200 mg of dried sample (previously homogenized into a fine powder using a kitchen mill) were digested with 6 mL of 65% HNO_3(v/v) and 1 mL of H_2O_2 30% (v/v) in a Teflon reaction vessel and heated in a SpeedwaveTM MWS-3 + (Berghof, Germany) microwave system with stepwise heating at 130 °C (10 min), 160 °C (15 min), 170 °C (12 min), 100 °C (7 min), and 100 °C (3 min). The resulting clear solutions of the digestion procedure were diluted to 50 mL with deionized water for further analysis. Mineral concentration of phosphorous (P), potassium (K), magnesium (Mg), calcium (Ca), zinc (Zn), manganese (Mn), iron (Fe) and boron (B) was assessed through inductively coupled plasma optical emission spectrometry (ICP-OES) Optima 7000 DV (PerkinElmer, USA) with radial configuration, through comparison with a multi-element standard solution (TraceCERT®, in 10% nitric acid, Sigma-Aldrich, Missouri, USA) and the standard reference material NIST1573A (tomato leaves, NIST® SRM®, Gaithersburg, USA).

Phenolic content and antioxidant capacity

For the extraction of total phenolic compounds, *ca.* 500 mg of grains were macerated (using a kitchen mill) and mixed with 10 mL acetone/water/acetic acid (70:29.5:0.5, v:v:v) overnight in an orbital shaker in the dark. After centrifugation at 1600 g for 10 min, the extracts were stored at 4 °C in the dark until further use (Xu and Chang 2007). Total phenolic content (TPC) was determined by the Folin–Ciocalteu method (Ramos et al. 2019), with some modifications. Briefly, in a 96-well plate, 80 µL of Folin–Ciocalteu reagent, previously diluted 1:10 (v/v), and 100 µL of sodium carbonate (7.5%, m/v) were added to 20 µL of extract and allowed to react in the dark at room temperature for 1 h. Afterwards, absorbance was measured at 750 nm in a Microplate Spectrophotometer (Multiskan GO, Thermo FisherScientific Inc., MA, USA). The concentration of total phenolics was determined as gallic acid equivalents (GAE) using the linear regression equation obtained from a standard curve of gallic acid (0.010–0.125 mg mL^{-1}). Results were expressed as milligrams equivalent of gallic acid per 100 g of dry extract (mg GAE 100 g^{-1} DW).

To evaluate grain antioxidant capacity, the ABTS (2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid)) assay was performed by measuring the relative ability of antioxidants to scavenge the radical cation 2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonate) ($ABTS^{\bullet+}$), adapted from (Gonçalves et al. 2009). The $ABTS^{\bullet+}$ solution was generated through a chemical oxidation reaction with potassium persulfate and its concentration adjusted with methanol to an initial absorbance of 0.700 (±0.020) at 734 nm. To 180 µL of this solution was added 20 µL of sample or Trolox or solvent in a 96 micro-well plate. The mixture was incubated for 5 min at 30 °C, and the absorbance at 734 nm was measured in a microplate reader. The ABTS scavenging percentage of each sample was compared with a 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) standard calibration curve (0.025–0.175 mg mL^{-1}), and the results expressed as Trolox equivalent (µmol Trolox g^{-1} DW).

Gene expression

RNA was extracted from leaves and roots (previously ground into a fine powder with liquid nitrogen) using the RNeasy Plant Mini Kit (Qiagen, Hilden, Germany), and complementary deoxyribonucleic acid (DNA) was synthesized using iScript™ cDNA Synthesis Kit (Bio-Rad Laboratories, Inc, California, USA), according to the manufacturer's instructions. Candidate genes were selected according to their established or possible role related to plant response to stress (*TCR1*, *TRC2* and *HLH54*), mineral metabolism (*FRO2*, *FER1*, *ZIP1* and *ZIP16*), and symbiosis with rhizobia and arbuscular mycorrhiza (*NRAMP7*, *ERN1*, and *RAM2*). Tubulin beta-9 and actin11 genes were used as housekeeping genes. The primers for the housekeeping genes (*TUB9* and *ACT11*), *FRO2*, *FER1*, *NRAMP7*, *ERN1*, *RAM2* and *HLH54* were retrieved from (Kavas et al. 2016; Santos et al. 2016; Nanjareddy et al. 2017; Ishida et al. 2018), whereas for the remaining a query search was performed to identify homologous sequences in *P. vulgaris*. Utilizing the primer design-tool from National Centre for Biotechnology Information (NCBI) (Primer – Basic Local Alignment Search Tool (BLAST)), *ZIP1*

primers were design based on the blast of a known sequence of *Arabidopsis thaliana* with the most homologous sequence (E-value < 10–20) specifying an expected polymerase chain reaction (PCR) product of 100 – 200 base pairs (bp) and primer annealing temperatures between 58 °C and 60 °C (Santos et al. 2013), with sequences for all the genes being detailed in the Supplementary Material—Table 1. Quantitative Real-Time PCR (qPCR) reactions were performed on a Chromo4 thermocycler (Bio-Rad Laboratories, USA) with six replicates per gene, as well as the controls and two technical replicates for each plate. Amplifications were carried out using 2 µL of the specific forward (1 µL) and reverse (1 µL) primers and mixed to 10 µL of 2xPCR iQ SYBR Green Supermix (Biorad) and 8 µL of cDNA in the final volume of 20 µL. Gene amplification was performed according to (Han et al. 2013). Briefly, samples were heated at 95 ºC for 3 min, followed by 38 cycles of 95 ºC for 15 s, primmer specific annealing temperature for 30 s and 72 ºC for 30 s. Melt curve profiles were analysed for each gene tested. The comparative CT method (ΔΔCT) (Livak and Schmittgen 2001) was utilized for the relative quantification of gene expression value of target genes using the housekeeping genes *tubulin beta-9* (*TUB9*) and *actin11* (*ACT11*) (CFX Manager™ Software, Bio-Rad Laboratories, USA).

Soil microbiome

To gain early insights into the dynamic changes occurring within soil microbial communities, microbiome analysis of the rhizosphere was performed at 15 days (T1) and 30 days (T2) of exposure to aCO_2 or eCO_2. Total DNA was extracted from 250 mg of rhizosphere soil, following the previously described sampling method, and we used a DNEasy PowerSoil Kit (Qiagen Laboratories, the Netherlands). DNA concentration was measured with the QubitTM fluorometer (ThermoFisher Scientific, USA) and nanophotometer (Implen, Isaza, Portugal), and its integrity was confirmed by gel electrophoresis (1% agarose in tris acetate EDTA buffer) before being sent for 16S rRNA gene-based microbial community analysis with high throughput sequencing (STAB VIDA, Lda., Portugal). Pooled DNA extracts for each sampling time were prepared and analysed based on the V3/V4 hypervariable region of the 16S rRNA gene by paired-end Illumina Miseq. Library construction was performed using the Illumina 16S Metagenomic Sequencing Library preparation protocol. The generated DNA fragments (DNA libraries) were sequenced with MiSeq Reagent Kit v3 in the lllumina MiSeq platform 19 considering the 300 bp paired-end sequencing reads, as sequences shorter than 300 bp or with a score lower than 25 were eliminated. The good quality reads were analysed and processed using QIIME2 (version 2019.7; http://qiime2.org/). Sequences were filtered, merged and, chimeric reads removed by the DADA2 software package enclosed in QIIME2 (Callahan et al. 2016). Through ARB SILVA taxonomic database (Yilmaz et al. 2014), taxonomy was assigned to the amplicon sequence variants (ASVs) with QIIME2 being used to calculate for each sample the reads abundance. With STAMP v2.1.3. graphical software package, hypothetical tests and exploratory plots on taxonomic and functional profiles were explored for possible identification of biologically relevant differences (Parks et al. 2014).

Statistical analysis

Mean comparison between aCO_2 and eCO_2 regarding biomass, mineral accumulation, total phenolic content, and antioxidant activity was performed through analysis of variance (ANOVA) followed by Fisher's LSD test ($p < 0.05$) in GraphPad Prism version 9.0 (GraphPad Software, Inc., California, USA). Heatmap representation of relative gene expression was produced in Multiple Experiment Viewer version 4.9.0 (Dana-Farber Cancer Institute, Boston, USA). ANOVA (post hoc Tuckey HSD and Bonferroni, $p < 0.05$) was also applied to define the statistical difference among the diversity indexes measured in the soil. To evaluate the statistical difference between the beta diversity metrics in the different soil conditions was calculated the permutational analysis of variance (PERMANOVA, 999 permutations, $p < 0.05$) using QIIME2 functions. Relative abundance of bacterial groups at phylum, class and order levels was analysed using the statistical software STAMP v2.1.3 (Parks et al. 2014).

Results

Plant biomass and soil chemical properties

No phenological differences were observed between *P. vulgaris* grown under aCO_2 and eCO_2, but plant exposure to eCO_2 led to a significant increase of pod biomass (250%) as compared with aCO_2 (Fig. 1, Supplementary Material – Fig. 2). Though not significant, there was a clear tendency of increased weight for shoot biomass. Similarly, for grain weight and size, we saw a significantly increase under eCO_2 (by 30% and 20%, respectively) (Fig. 1).

The CO_2 treatment did not affect soil chemical properties including electrical conductivity, organic matter content and nutrient levels (Supplementary Material—Table 2).

Shoot and grain mineral composition

Elevated CO_2 did not alter the accumulation of any macronutrients in the shoot or grains (Fig. 2A). This resilience was not observed in micronutrient concentrations, as we detected that in shoots grown under eCO_2 there is a significant increase of Fe (200%), as compared with aCO_2 (Fig. 2B). Contrastingly, eCO_2 did not significantly decreased or increased any of the other minerals accumulation. Grain Zn and Fe concentrations significantly decreased in plants grown under eCO_2 (up to 51% and 41%, respectively).

Grain phenolic content and antioxidant capacity

Comparing with aCO_2, plant growth under eCO_2 led to a significant decrease in total phenolics concentration (by 77%) (Fig. 3A). Grain antioxidant capacity on the other hand did not follow the same trend, as no significant differences were found (Fig. 3B).

Gene expression

Elevated CO_2 resulted in the upregulation of genes *ZIP1, ERN1, RAM2* and *HLH54* in leaves and roots (from 1.5- to 2.7-fold); *NRAMP7* and *TCR1/TCR2* in leaves (1.8- and 1.7-fold, respectively). *ZIP16* and *FER1* were also upregulated in roots (6.9- and 2.4-fold, respectively) (Fig. 4).

Contrastingly, leaf transcript levels of *FER1* and *ZIP16* were found to be downregulated, 0.1- and 0.3-fold respectively, while root transcript levels were, from 0.4- to 0.6-fold downregulated regarding TCR1 and TCR2. The expression of gene *FRO2* was not found to be affected by CO_2 conditions.

Soil microbiome

Evaluation of soil microbial diversity revealed that, among the 32 phyla detected, the most abundant were *Pseudomonadota*, *Acidobacteriota*, *Actinomycetota* and *Bacteroidota*, which accounted for more than 60% of all phyla detected (Supplementary Material 3). Plant growth under eCO_2 for 15 days (T1) was

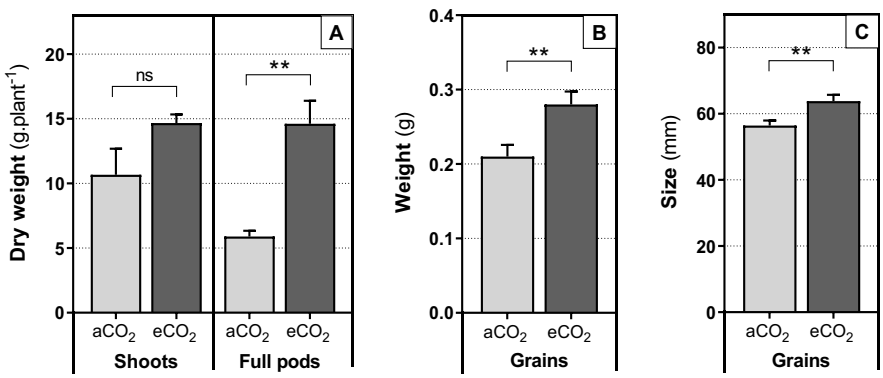

Fig. 1 Shoot and full pods (**A**) biomass, seed weight (**B**) and size (**C**) of Phaseolus vulgaris cv. Logan, grown under ambient (400 ppm, aCO_2) or elevated atmospheric CO_2 concentration (600 ppm, eCO_2) from pod filing to harvest (1 month). Each value represents the average of 3 sub-plots composed of ten plants each ($N=3$) ± SEM and asterisks indicate significant differences between aCO_2 and eCO_2. **$=p<0.01$; ns = not significant

associated with a significant increase of the relative abundance of *Pseudomonadota* (by 10%) and simultaneous decrease of the groups BRC1 and WPS-2 (by 69% and 44% respectively) (Supplementary Material – Fig. 3). *Verrucomicrobiota* significantly declined at T1 (18%), increasing again after 30 days (T2) of exposure to eCO_2. *Euryarchaeota* was only recovered at T1 (regardless of the CO_2 conditions) and FCPU426 and WS4 bacteria were only detected under aCO_2 at T1, while *Halanaerobiaeota* was only recovered at T2 (under both aCO_2 and eCO_2) (Supplementary Material 3).

A total of 47 classes were identified, among which *Alphaproteobacteria*, *Bacteroidia*, *Verrucomicrobiae* and *Gammaproteobacteria* presented the highest relative abundance (up to 50.6%) (Supplementary Material 3). The relative abundance of 10 of these significantly varied after exposure to eCO_2 (Fig. 5). At T1, the relative abundance of *Alphaproteobacteria* and *Acidimicrobiia* significantly increased (by 10% and 20%, respectively), whereas *Acidobacteriota* Sg 5, *Fimbriimonadia* and *Chlamydiae* decreased (up to 36%). At T2, no significant changes were observed in these classes, but a suppression of TK10, *Thermoleophilia*, MB-A2-108 and *Armatimonadia* was observed (from 78 to 56%). *Verrucomicrobiae* significantly declined at T1 (82%), increasing again after 30 days (T2) of exposure to eCO_2.

From the 219 orders identified, the ones with the highest relative abundance were *Tepidisphaerales*, *Chitinophagales*, *Chthoniobacterales*, *Rhizobiales*, *Betaproteobacteriales* and *Sphingomonadales*, reaching up to 41% (Supplementary Material 3). Exposure to eCO_2 was associated with significantly variations in the relative abundance of 26 orders, most of them belonging to the phyla *Acidobacteriota* and *Pseudomonadota* (Table 1). At T1, eCO_2 led to a significant increase in the relative abundance of *Acetobacterales*, *Azospirillales*, *Corynebacteriales*, *Microtrichales*, *Tistrellales*, *Anaeromyxobacter* and *Geothrix* sp. (from 120 to 270%). Contrastingly, the relative abundance of *Babeliales*, *Candidatus* Yanofskybacteria, *Chlamydiales*, *Fimbriimonadales*, *Latescibacterales*, *Planctomycetales* and *Gemmatimonadales* decreased (from 29 to 87%). In addition, GWA2-38-13b was only detected in plants grown under aCO_2, whereas *Acidimicrobiales* was only observed in plants exposed to eCO_2. At T2, most classes were observed to have reduced relative abundance under eCO_2 conditions, including *Armatimonadales*, *Gaiellales*, *Gammaproteobacteria*, *Glycomycetales*, *Isosphaerales*, *Rhodospirillales* among other, which decreased from 27 to 81%. Also, the class *Erysipelotrichales* was not detected at eCO_2. In contrast, the relative abundance of *Enterobacteriales* was significantly increased under eCO_2 (150%).

Discussion

Implications of eCO_2 in *P. vulgaris* yield and quality

In this study, eCO_2 did not promote plant biomass (shoot) accumulation. This lack of biomass response could be attributed to the treatment starting at the pod-filling stage when plants may have already reached peak biomass. Conversely, it improved seed productivity (weight and size) (Fig. 1), which might indicate that increased photosynthetic efficiency (Shimono and Bunce 2008) contributed to higher grain yields during the late growth stages. This indicates that even short-term eCO_2 exposure, applied from the pod-filling stage, can stimulate seed production without affecting biomass, potentially improving productivity without the usual trade-offs of long-term exposure, such as biomass dilution.

Macronutrient concentrations were largely unaffected by eCO_2 (Fig. 2), except for a pattern of decreased shoot K accumulation after eCO_2 exposure. These results suggest a tissue-specific response, with K levels declining in shoots but remaining stable in grains, suggesting that the plants might have compensated for the lower K accumulation in the vegetative parts by prioritizing the reproductive tissues (Soares et al. 2021). Similar studies have observed species-specific K uptake responses to eCO_2 (Shinano et al. 2007; Zhuang et al. 2018; Shi et al. 2021), but in this case, *P. vulgaris* managed to maintain nutrient balance under short-term exposure.

For micronutrients, however, eCO_2 significantly impaired Zn and Fe accumulation in grains while increasing Fe levels in shoots. This tissue-specific response points to an impaired remobilization of these nutrients from shoots to grains, although no significant differences were observed for specific mineral partitioning between seed and shoots (Supplementary

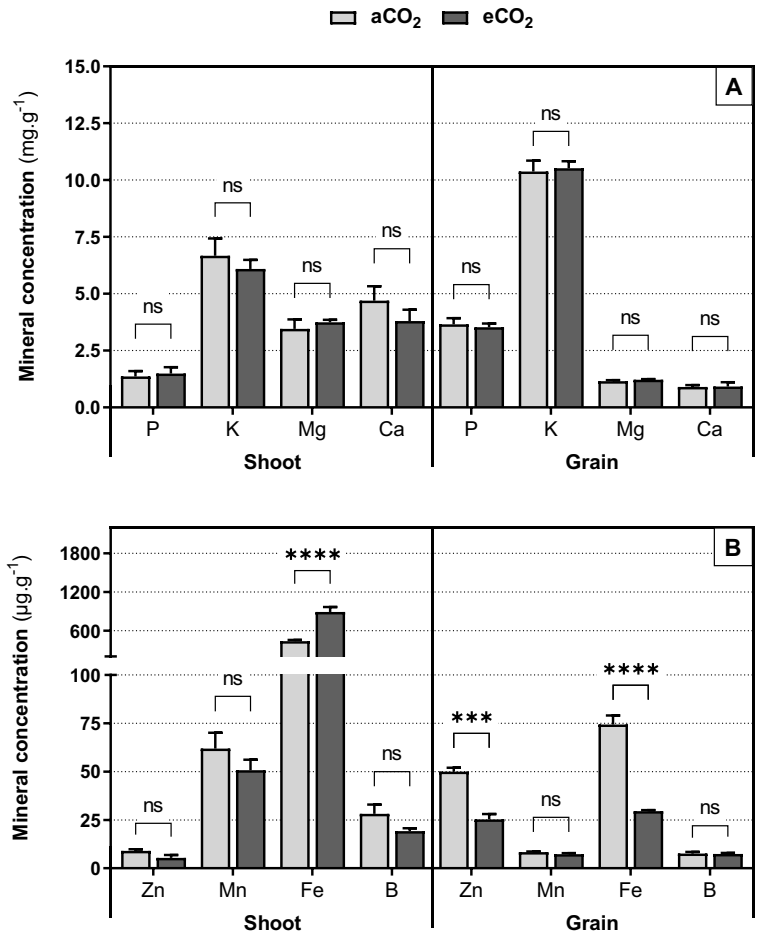

Fig. 2 The concentrations of macronutrients (**A**) and micronutrients (**B**) in shoots and grain of *Phaseolus vulgaris* cv. Logan, grown under ambient (400 ppm, aCO_2) or elevated atmospheric CO_2 concentration (600 ppm, eCO_2) from pod filing to harvest (1 month). Each value represents the average of 3 sub-plots composed of ten plants each ($N=3$) ± SEM and asterisks indicate significant differences between aCO_2 and eCO_2. ****$=p<0.0001$; ***$=p<0.001$; ns = not significant

Material – Fig. 4). The inability to efficiently translocate Fe and Zn during grain filling, a critical period for micronutrient deposition in edible tissues, suggests that eCO_2 disrupts remobilization mechanisms, which could have implications for nutritional quality. Notably, Fe accumulated in vegetative tissues but not in grains, mirrors similar findings in other crops under eCO_2 (Loladze 2014; Jin et al. 2019). Our data supports these conclusions by showing that Fe translocation was hindered, while Zn accumulation in grain tissues was similarly reduced, indicating a broader issue with micronutrient remobilization possibly due to limitations in the translocation pathways or the differential activation of transporters like ZIPs (Astudillo et al. 2013).

Grain total phenolic content was also negatively affected by eCO_2, in contrast with the non-significant differences observed in the ABTS assay results regarding the antioxidant activity. These results contrast with findings that eCO_2 promotes the accumulation of polyphenols and antioxidants in vegetables (Dong et al. 2018), due to increased synthesis and accumulation of soluble sugars that could be precursors to antioxidants (Wang and Bunce 2004; Becker and Kläring 2016). However, the literature reviewed primarily focused on long-term elevated CO_2 conditions during earlier plant phenology, a scenario not directly comparable to the conditions examined in the current study. Overall, the observed increases in pod biomass, grain weight, and size, coupled with stable macronutrient accumulation, suggest an overall positive influence of eCO_2 on the sink size of *P. vulgaris* cv. Logan. However, the contrasting responses in micronutrient concentrations, phenolic concentration, and antioxidant capacity highlight the complexity of plant responses to elevated CO_2 and the need for further investigation into these dynamics.

Table 1 Mean relative abundance (%) of the microbial orders significantly affected in the rhizosphere soil of the plants exposed to ambient (400 ppm, aCO_2) or elevated atmospheric CO_2 concentration (600 ppm, eCO_2) for 15 (T1) or 30 days (T2)

Order	aCO_2	eCO_2	*p*-value
15 days (T1)			
Acetobacterales	0.13 ± 0.03^{b}	0.22 ± 0.03^{a}	0.014
Acidimicrobiales	0 ± 0^{b}	$0.013 \pm 7 \times 10^{-3a}$	0.032
Azospirillales	0.34 ± 0.03^{b}	0.49 ± 0.04^{a}	0.007
Babeliales	0.040 ± 0.02^{a}	$5.0 \times 10^{-3} \pm 4 \times 10^{-3b}$	0.024
Candidatus Yanofskybacteria	$0.020 \pm 6 \times 10^{-3a}$	$3.0 \times 10^{-3} \pm 5 \times 10^{-3b}$	0.017
Chlamydiales	0.065 ± 0.01^{a}	$0.024 \pm 9 \times 10^{-3b}$	0.007
Corynebacteriales	0.51 ± 0.1^{b}	0.89 ± 0.1^{a}	0.017
Fimbriimonadales	$0.27 \pm 8 \times 10^{-3a}$	0.17 ± 0.04^{b}	0.016
GWA2-38-13b	$6.0 \times 10^{-3} \pm 3 \times 10^{-3a}$	0 ± 0^{b}	0.027
Latescibacterales	$0.073 \pm 6 \times 10^{-3a}$	0.045 ± 0.02^{b}	0.041
Microtrichales	0.94 ± 0.04^{b}	1.1 ± 0.05^{a}	0.007
Planctomycetales	0.51 ± 0.06^{a}	0.36 ± 0.04^{b}	0.021
Tistrellales	0.050 ± 0.02^{b}	0.11 ± 0.01^{a}	0.007
Uncultured Anaeromyxobacter sp.	0.12 ± 0.01^{b}	0.17 ± 0.02^{a}	0.013
Uncultured Gemmatimonadales bacterium	0.043 ± 0.01^{a}	0.012 ± 0.01^{b}	0.039
Uncultured Geothrix sp.	0.040 ± 0.03^{b}	0.11 ± 0.02^{a}	0.022
Uncultured prokaryote	0 ± 0^{b}	$4.0 \times 10^{-3} \pm 4 \times 10^{-3a}$	0.049
30 days (T2)			
Armatimonadales	0.17 ± 0.02^{a}	0.11 ± 0.03^{b}	0.048
Enterobacteriales	0.054 ± 0.04^{b}	0.15 ± 0.03^{a}	0.034
Erysipelotrichales	0.018 ± 0.01^{a}	0 ± 0^{b}	0.048
Gaiellales	1.7 ± 0.02^{a}	1.2 ± 0.1^{b}	0.002
Gammaproteobacteria Incertae Sedis	0.73 ± 0.06^{a}	0.50 ± 0.1^{b}	0.041
Glycomycetales	$0.018 \pm 3 \times 10^{-3a}$	$4.0 \times 10^{-3} \pm 5 \times 10^{-3b}$	0.010
Isosphaerales	0.088 ± 0.02^{a}	0.042 ± 0.01^{b}	0.034
Rhodospirillales	0.048 ± 0.01^{a}	$0.025 \pm 7 \times 10^{-3b}$	0.049
Uncultivated soil bacterium clone C112	0.098 ± 0.02^{b}	0.14 ± 0.01^{a}	0.021
Uncultured Kineosporiaceae bacterium	0.20 ± 0.02^{a}	$0.14 \pm 7 \times 10^{-3b}$	0.005

Each value represents the average of three plants ± SEM and different letters indicate significant differences between aCO_2 and eCO_2 within each class

Contribution of plant molecular mechanisms to *P. vulgaris* resilience to eCO_2

Here, we analysed the response of genes potentially involved in stress response (TCR1, TCR2, and HLH54), uptake and metabolism of Fe (FRO2 and FER1) and Zn (ZIP1 and ZIP16), and symbiosis with beneficial soil microbes (NRAMP6, NRAMP7, ERN1, RAM1, RAM2) (Fig. 4). Elevated CO2 downregulated the genes TCR1 and TCR2 in roots, relatively to aCO2, and upregulated them in leaves. Tunicamycin-induced CONSTANS-like-related genes, such as TCR1 and TCR2, mediate transcriptional regulation during endoplasmic reticulum stress, a process activated by misfolded proteins that accumulate in the endoplasmic reticulum under adverse environmental conditions (Iwata et al. 2008). Contrastingly, HLH54 was upregulated by eCO2 in both leaves and roots. Several transcription factors of the bHLH family have important roles in response to abiotic stress, contributing to the maintenance of Fe homeostasis and function at the root epidermis as positive regulators of FRO2 and IRT1 (Gao et al. 2019). Interestingly, the expression of FRO2, a membrane-bound ferric chelate reductase essential for Fe absorption by Fe3+ reduction to Fe2+ (Satbhai et al.

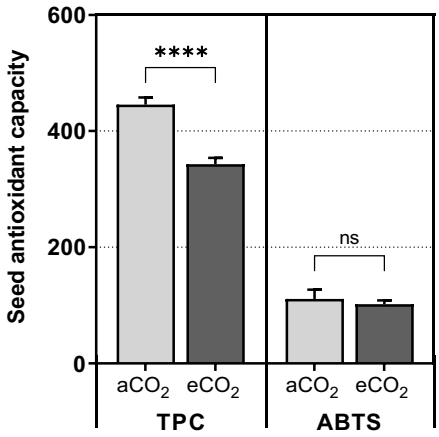

Fig. 3 Total phenolic content (TPC) and antioxidant capacity (ABTS equivalent) in grains of *Phaseolus vulgaris* cv. Logan, grown under ambient (400 ppm, aCO_2) or elevated atmospheric CO_2 concentration (600 ppm, eCO_2) from pod filing to harvest (1 month). Each value represents the average of 3 subplots composed of ten plants each ($N=3$) ± SEM and asterisks indicate significant differences between aCO_2 and eCO_2. **** = $p < 0.0001$; ns = not significant

2017), showed no significant changes with eCO2 exposure. On the other hand, reduced Fe is transported into root epidermal cells by the Iron-regulated Transporter 1 (IRT1) and further accumulated in shoot tissues through regulation by ferritin (FER1) (Santos et al. 2015). Here we see a clear upregulation of FER1 in root tissue, normally associated with increased Fe accumulation. Therefore, the accumulation of Fe in the leaves underpins a possible remobilization of Fe to the aerial parts as part of Logan's response mechanism to eCO2 (Fig. 2).

Elevated CO2 also increased the fold of expression of ZIP1 and ZIP16 in leaves and roots (with an exception for ZIP16 leaf expression levels). These genes are involved in Zn regulation in plant tissues, acting as a coping mechanism of eCO2 effects on Zn accumulation. Also, previous studies suggest that ZIP genes could be directly related to Zn remobilization to seeds and are usually highly expressed at flowering (Astudillo et al. 2013). Contrastingly, eCO2 did not affect the expression of NRAMP, which is usually involved in mineral uptake and mobilization in nodule tissues, suggesting that mineral-related nodulation mechanisms were not affected by one month exposure to eCO2. However, eCO2 upregulated RAM2, demonstrating the activation of a broad group of mechanisms involved in metal homeostasis, including symbiosis with beneficial microorganisms in the rhizosphere. In P. vulgaris, NRAMP7 is involved in mineral uptake and mobilization in nodule tissues (Ishida et al. 2018), while RAM2 has been implicated in root colonization by arbuscular mycorrhiza (Nanjareddy et al. 2017). It is likely that the negative impacts of eCO2 extend beyond yield and nutritional impairments, also affecting P. vulgaris regulatory mechanisms with symbiotic microbes in the roots.

Contribution of rhizosphere microbiome to *P. vulgaris* resilience to eCO_2

In terms of rhizosphere microbial community composition, we found considerable heterogeneity, with *Pseudomonadota* being the predominant phylum, followed by *Acidobacteriota*, *Actinomycetota*, *Bacteroidota*, and *Planctomycetes* (Supplementary Material – Fig. 4). Members of these phyla usually make up an average of over 80% of soil libraries (Janssen 2006). Soil microbial communities play a vital role in plant development and ecosystem functioning (Jacoby et al. 2017), and the increase in atmospheric CO_2 is known to impact microbial properties,

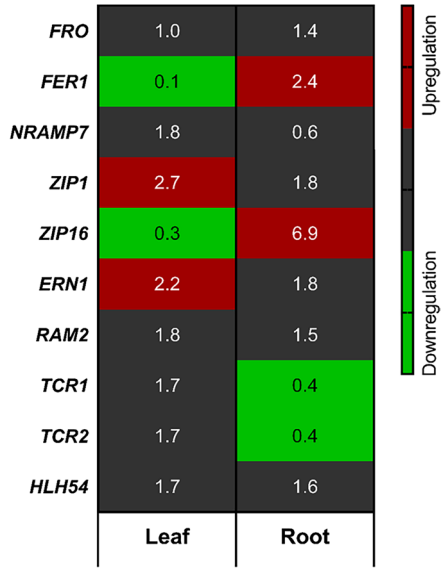

Fig. 4 Heatmap of relative fold of expression of genes *FRO2*, *FER1*, *NRAMP7*, *ZIP1*, *ZIP16*, *ERN1*, *RAM2*, *TCR1*, *TCR2*, and *HLH54* in shoots and roots of *Phaseolus vulgaris* cv. Logan, grown under ambient (400 ppm, aCO_2) or elevated atmospheric CO_2 concentration (600 ppm, eCO_2) from pod filing to harvest (1 month). Values represent the fold of expression relative to the housekeeping genes *TUB9* and *ACT11*

rhizosphere behaviour and overall soil health (Williams et al. 2018). In line with this, our findings revealed that eCO_2 exposure significantly increased the relative abundance of *Pseudomonadota*, which is involved in carbon, nitrogen, and sulfur cycling (Supplementary Material – Fig. 4). This microbial shift correlates with the observed increase in plant productivity under eCO_2, suggesting that enhanced microbial activity may have played a role in maintaining shoot biomass and enhancing seed productivity, linking microbial shifts to plant performance.

Plant growth under eCO_2 did not affect phylum diversity, as observed in other studies (Jin et al. 2022), but significantly affected the mean relative frequency of *Pseudomonadota*, *Verrucomicrobiota*, *BRC1*, and *WPS-2* (Supplementary Material – Fig. 4). Similarly, the few reports available with FACE experiments have also reported an increase of *Pseudomonadota* phylum (Bei et al. 2019; Rosado-Porto et al. 2022), a key contributor to carbon, nitrogen, and sulfur cycling (Rampelotto et al. 2013; Rosado-Porto et al. 2022) namely in the degradation of inorganic compounds and nitrogen fixation. This may lead to more carbon availability which is positively correlated with plant health by alleviating the adaption to harsher environments, therefore enhancing plant growth, as we observed in the present study with a significant increase of plant biomass (Fig. 1) (Babalola et al. 2020). On the other hand, phyla like *Verrucomicrobiota* and their ecological roles are still poorly understood, even though they appear ubiquitous in numerous soil bacterial communities and may be related to soil factors linked to soil fertility (Navarrete et al. 2015; Li et al. 2024).

The bacterial orders with the highest relative abundance were *Tepidisphaerales*, *Chitinophagales*, *Chthoniobacterales*, *Rhizobiales*, *Betaproteobacteriales*, and *Sphingomonadales*, as previously observed in other species such as wheat

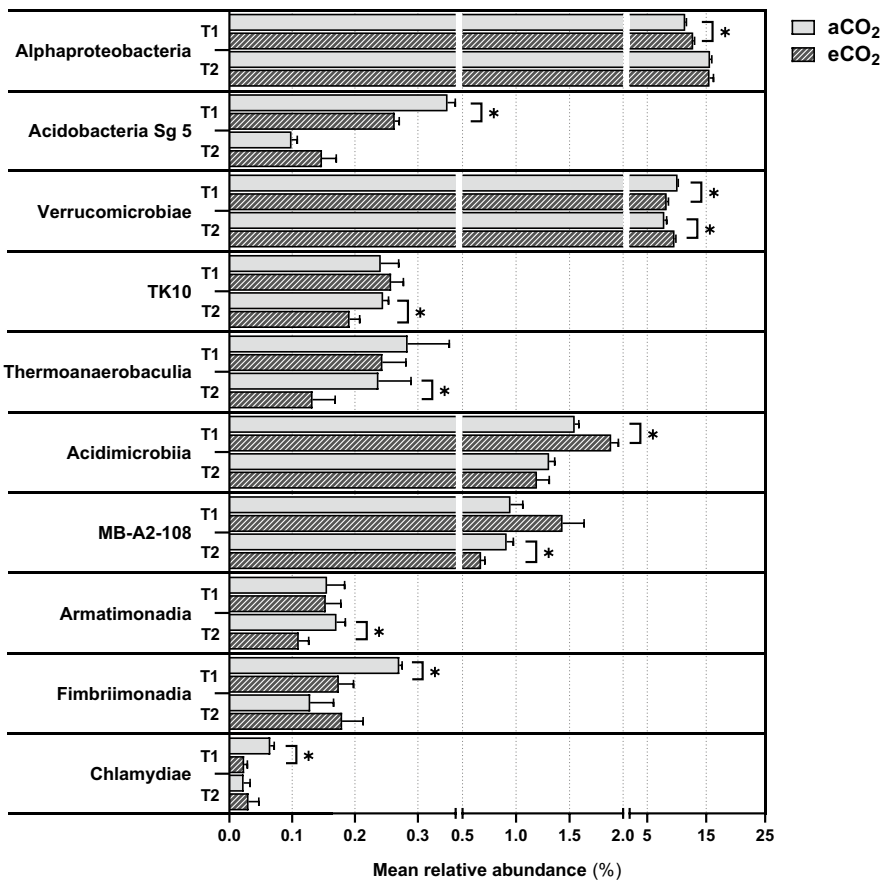

Fig. 5 Mean relative abundance of the microbial classes significantly affected in the rhizosphere soil of *Phaseolus vulgaris* cv. Logan, exposed to ambient (400 ppm, aCO_2) or elevated atmospheric CO_2 concentration (600 ppm, eCO_2), 15 days after pod filing (T1, 15 days of exposure to CO_2) and at harvest (T2, 30 days of exposure to CO_2). Each value the average of three plants ± SEM and asterisks indicate significant differences between aCO_2 and eCO_2 within each phylum and time-point of analysis ($p < 0.05$)

and *Brachypodium distachyon* (Usyskin-Tonne et al. 2020; Emmett et al. 2021). The relative abundance of *Alphaproteobacteria* and *Acidimicrobiia* increased, whereas *Acidobacteriota* Sg 5, *Fimbriimonadia*, *Chlamydiae*, *TK10*, *Thermoleophilia*, *MB-A2-108*, *Armatimonadia*, and *Verrucomicrobiae* often declined throughout the timing of exposure to eCO_2. Impairments in some of these classes are related to fluctuations in several nutrient levels. For example, *Fimbriimonadia* abundance is essential for predicting dissolved organic carbon levels in rice soils (Jiao et al. 2019). At the same time, it is even more relevant in soil nutrient cycling of maize fields as it correlates with dissolved organic carbon and available N and P (Jiao et al. 2019). Exposure to eCO_2 significantly affected 26 orders, most of them belonging to *Acidobacteriota* and *Pseudomonadota*, including *Acetobacterales* and *Azospirillales*, which correlate positively with the root secretion of organic acids that help cope with environmental changes, shaping the microbial community structure in the rhizosphere potentially leading to plant nutritional status and development (Chen et al. 2019; Mangeot-Peter et al. 2020).

Considering that plants rely on soil microbiota to obtain the soil nutrients they require, microbiome changes (for instance the increase in *Pseudomonadota*) are likely to contribute to plant growth alterations observed in *P. vulgaris* growth (Fig. 1), shedding light on the viability of future engineering of crop rhizosphere with plant growth promoting bacteria that could better cope with given abiotic stress. Nevertheless, plant–microbe nutritional interactions and the correlation of plant microbiome on plant nutritional status are highly complex and challenging to predict, cementing itself as a critical target of future research (Jacoby et al. 2017).

Conclusion

This study represents one of the first examinations of the nutritional resilience of *P. vulgaris* to future climate conditions in the short term, revealing important fluctuations in grain microelements and reduced phenolic content. Although less pronounced than in longer-term experiments, our findings suggest that a one-month exposure to eCO_2 still resulted in higher grain yield, at the expense of reduced levels of important grain nutrients, such as Fe and Zn. This underscores the importance of employing short-term eCO_2 treatments as a valuable tool for uncovering immediate plant responses and potential long-term implications. In addition to its direct effects on plant physiology and yield, microbial communities (critical for plant well-being and soil health) were also impacted. Shifts in the relative abundance of taxa such as *Pseudomonadota* suggest alterations in the biogeochemical cycling dynamics of macronutrients and micronutrients that likely contribute to the observed phenotypic responses of plants to eCO_2. Overall, this study underscores the complex interplay between atmospheric conditions, plant nutrition, and microbial ecology, providing a crucial understanding for future agricultural practices and food security in the context of climate change.

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Data availability Data will be made available on request.

Declarations

Competing interests The authors have no competing interests to declare that are relevant to the content of this article.

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