



Effects of short-term exposure to elevated atmospheric CO₂ on yield, nutritional profile, genetic regulatory pathways, and rhizosphere microbial community of common bean (*Phaseolus vulgaris*)

Rafael D. C. Duarte · Marta Nunes da Silva · Gianuario Fortunato · Juan Quirós-Vargas · Onno Muller · Célia M. Manaia · Marta W. Vasconcelos

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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 CO₂ (eCO₂) 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 CO₂ (control, aCO₂, 400 ppm) or eCO₂ (600 pm) from the start of pod filling until plant maturity and analyzed for several morphophysiological and nutritional parameters.

Results Compared with aCO₂, eCO₂ 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 eCO₂-induced alterations in microbial community structure.

Conclusions This research demonstrates how eCO₂ impacts the nutritional quality of common beans regarding micronutrients and phenolic content, while also affecting soil microbiome composition. Highlighting the value of shorter term eCO₂ 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 eCO₂ conditions.

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R. D. C. Duarte · M. Nunes da Silva · G. Fortunato · C. M. Manaia · M. W. Vasconcelos (✉)
CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Rua Diogo Botelho 1327, Porto 4169-005, Portugal
e-mail: mvasconcelos@ucp.pt

J. Quirós-Vargas · O. Muller
Institute of Biogeosciences, IBG2: Plant Sciences, Forschungszentrum Jülich GmbH, 52425 Jülich, Germany

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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 (CO₂) levels, temperature fluctuations, and erratic rainfall, threatens this goal (Solomon et al. 2009; Mirzabaev et al. 2023). By 2050, CO₂ 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 CO₂ 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 eCO₂ 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 CO₂ 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 eCO₂ 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 eCO₂ 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 CO₂ 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 eCO₂ 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 eCO₂ 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 eCO₂. *P. vulgaris* cv. Logan, in particular, has previously shown an ability to cope with long-term eCO₂ without significant nutritional losses in field conditions (in a free air CO₂ enriched – FACE – platform) under aCO₂ (400 ppm) or eCO₂ (600 pm) (Soares et al. 2019a). Nonetheless, we hypothesize that a shorter-term exposure of *P. vulgaris* to eCO₂, 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 eCO₂ 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 CO₂ levels, providing insights into the immediate impacts of eCO₂ 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 Alten-dorf 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 eCO₂ environment (Supplementary Material—Fig. 1). The CO₂ 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 m²) with small openings each 20–30 cm for the ejection of CO₂ in the opposite direction of the wind. Two small cabinets are placed close to the ring, one to distribute the CO₂ 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 CO₂ concentration were installed. Intended to detect which pipes have to be activated, and to monitor the amount of CO₂ that was reaching the furthest point from the pipes. Additionally, four CO₂ 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 CO₂ levels (aCO₂, 400 ppm) and one fumigated with eCO₂ (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/m². 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, Ca²⁺ 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 CO₂ conditions (aCO₂ at 400 ppm and eCO₂ at 600 ppm)

for one month, from pod filling (R8, Supplementary Material—Fig. 2) until harvest (R). The fixed infrastructure required for CO₂ 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 CO₂ 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 aCO₂ or eCO₂, 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 °C 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 (Ca²⁺), magnesium (Mg²⁺), 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) ($\text{ABTS}^{\bullet+}$), adapted from (Gonçalves et al. 2009). The $\text{ABTS}^{\bullet+}$ 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 °C for 3 min, followed by 38 cycles of 95 °C for 15 s, primer specific annealing temperature for 30 s and 72 °C for 30 s. Melt curve profiles were analysed for each gene tested. The comparative CT method ($\Delta\Delta CT$) (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 aCO₂ or eCO₂. Total DNA was extracted from 250 mg of rhizosphere soil, following the previously described sampling method, and we used a DNEasy Power-Soil Kit (Qiagen Laboratories, the Netherlands). DNA concentration was measured with the Qubit™ 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 Illumina 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 aCO₂ and eCO₂ 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). Heat-map 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 aCO₂ and eCO₂, but plant exposure to eCO₂ led to a significant increase of pod biomass (250%) as compared with aCO₂ (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 eCO₂ (by 30% and 20%, respectively) (Fig. 1).

The CO₂ 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 CO₂ 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 eCO₂ there is a significant increase of Fe (200%), as compared with aCO₂ (Fig. 2B). Contrastingly, eCO₂ did not significantly decreased or increased any of the other minerals accumulation. Grain Zn and Fe concentrations significantly decreased in plants grown under eCO₂ (up to 51% and 41%, respectively).

Grain phenolic content and antioxidant capacity

Comparing with aCO₂, plant growth under eCO₂ 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 CO₂ 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 CO₂ 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 eCO₂ for 15 days (T1) was

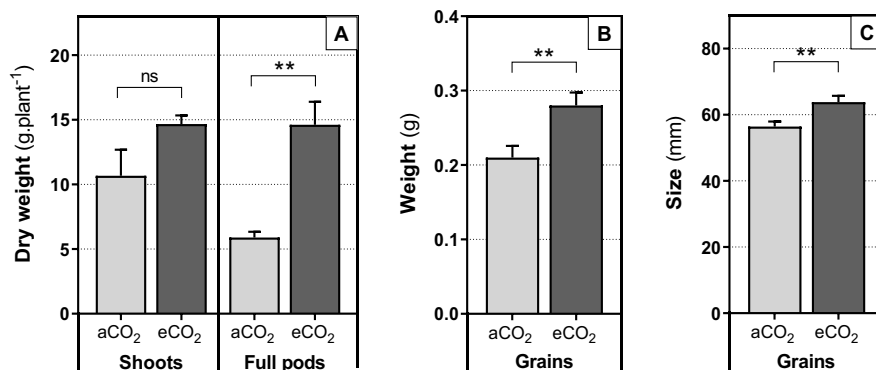


Fig. 1 Shoot and full pods (A) biomass, seed weight (B) and size (C) of *Phaseolus vulgaris* cv. Logan, grown under ambient (400 ppm, aCO₂) or elevated atmospheric CO₂ concentration (600 ppm, eCO₂) from pod filing to harvest (1 month).

Each value represents the average of 3 sub-plots composed of ten plants each ($N=3$) $\pm$ SEM and asterisks indicate significant differences between aCO₂ and eCO₂. **= $p < 0.01$; 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 eCO₂. *Euryarchaeota* was only recovered at T1 (regardless of the CO₂ conditions) and FCPU426 and WS4 bacteria were only detected under aCO₂ at T1, while *Halanaerobiaeota* was only recovered at T2 (under both aCO₂ and eCO₂) (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 eCO₂ (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 eCO₂.

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 eCO₂ was associated with significant variations in the relative abundance of 26 orders, most of them belonging to the phyla *Acidobacteriota* and *Pseudomonadota* (Table 1). At T1, eCO₂ 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 *Gammatimonadales* decreased (from 29 to 87%). In addition, GWA2-38-13b was only detected in plants grown under aCO₂, whereas *Acidimicrobiales* was only observed in plants exposed to eCO₂. At T2, most classes were observed to have reduced relative

abundance under eCO₂ conditions, including *Armatimonadales*, *Gaiellales*, *Gammaproteobacteria*, *Glycomycetales*, *Isosphaerales*, *Rhodospirillales* among other, which decreased from 27 to 81%. Also, the class *Erysipelotrichales* was not detected at eCO₂. In contrast, the relative abundance of *Enterobacterales* was significantly increased under eCO₂ (150%).

Discussion

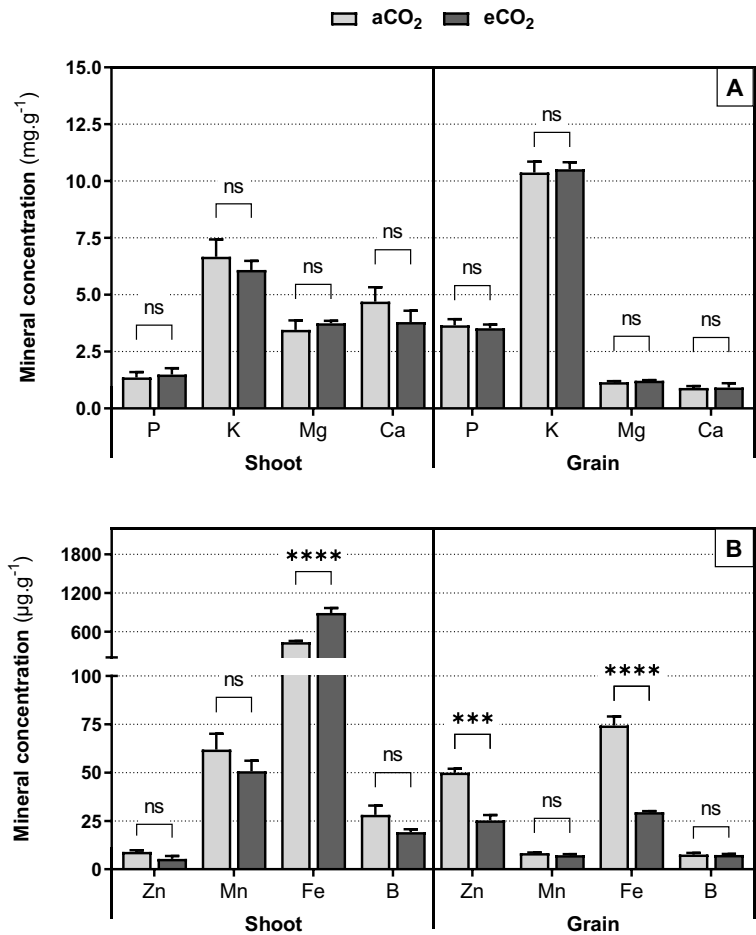
Implications of eCO₂ in *P. vulgaris* yield and quality

In this study, eCO₂ 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 eCO₂ 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 eCO₂ (Fig. 2), except for a pattern of decreased shoot K accumulation after eCO₂ 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 eCO₂ (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, eCO₂ 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, aCO₂) or elevated atmospheric CO₂ concentration (600 ppm, eCO₂) from pod filling to harvest (1 month). Each value represents the average of 3 sub-plots composed of ten plants each ($N=3$) $\pm$ SEM and asterisks indicate significant differences between aCO₂ and eCO₂. ****= $p < 0.0001$; ***= $p < 0.001$; 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 eCO₂ 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 eCO₂ (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 eCO₂, in contrast with the non-significant differences observed in the ABTS assay results regarding the antioxidant activity. These results

contrast with findings that eCO₂ 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 CO₂ 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 eCO₂ 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 CO₂ 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, aCO₂) or elevated atmospheric CO₂ concentration (600 ppm, eCO₂) for 15 (T1) or 30 days (T2)

Order	aCO ₂	eCO ₂	<i>p</i> -value
15 days (T1)			
Acetobacterales	0.13 ± 0.03 ^b	0.22 ± 0.03 ^a	0.014
Acidimicrobiales	0 ± 0 ^b	0.013 ± 7 × 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 × 10 ⁻³ ± 4 × 10 ^{-3b}	0.024
Candidatus Yanofskybacteria	0.020 ± 6 × 10 ^{-3a}	3.0 × 10 ⁻³ ± 5 × 10 ^{-3b}	0.017
Chlamydiales	0.065 ± 0.01 ^a	0.024 ± 9 × 10 ^{-3b}	0.007
Corynebacteriales	0.51 ± 0.1 ^b	0.89 ± 0.1 ^a	0.017
Fimbriimonadales	0.27 ± 8 × 10 ^{-3a}	0.17 ± 0.04 ^b	0.016
GWA2-38-13b	6.0 × 10 ⁻³ ± 3 × 10 ^{-3a}	0 ± 0 ^b	0.027
Latescibacteriales	0.073 ± 6 × 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 × 10 ⁻³ ± 4 × 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 ± 3 × 10 ^{-3a}	4.0 × 10 ⁻³ ± 5 × 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 ± 7 × 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 ± 7 × 10 ^{-3b}	0.005

Each value represents the average of three plants ± SEM and different letters indicate significant differences between aCO₂ and eCO₂ within each class

Contribution of plant molecular mechanisms to *P. vulgaris* resilience to eCO₂

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.

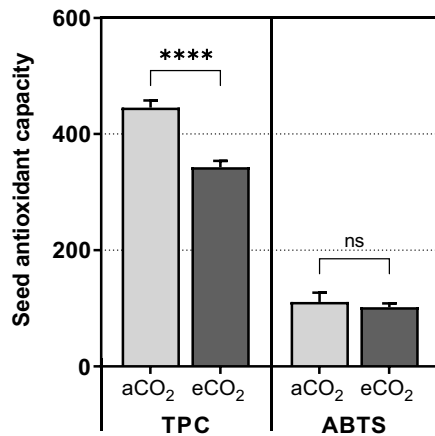


Fig. 3 Total phenolic content (TPC) and antioxidant capacity (ABTS equivalent) in grains of *Phaseolus vulgaris* cv. Logan, grown under ambient (400 ppm, aCO₂) or elevated atmospheric CO₂ concentration (600 ppm, eCO₂) from pod filling to harvest (1 month). Each value represents the average of 3 sub-plots composed of ten plants each ($N=3$) $\pm$ SEM and asterisks indicate significant differences between aCO₂ and eCO₂. ****= $p < 0.0001$; 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 eCO₂

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 CO₂ is known to impact microbial properties,

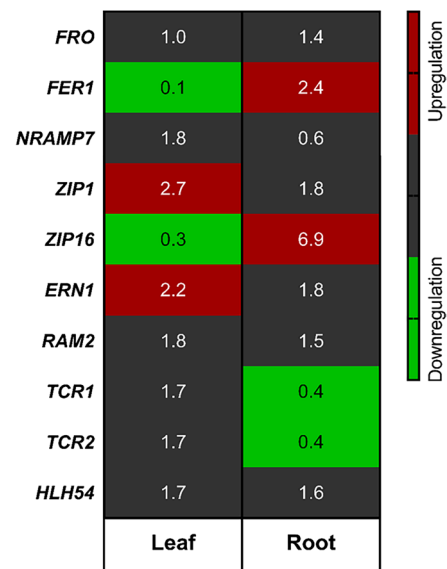


Fig. 4 Heatmap of relative fold of expression of genes *FRO2*, *FER1*, *NRAMP7*, *ZIP1*, *ZIP16*, *ERN1*, *RAM2*, *TCR1*, *TCR2*, and *HLH54* in shoots and roots of *Phaseolus vulgaris* cv. Logan, grown under ambient (400 ppm, aCO₂) or elevated atmospheric CO₂ concentration (600 ppm, eCO₂) from pod filling to harvest (1 month). Values represent the fold of expression relative to the housekeeping genes *TUB9* and *ACT11*

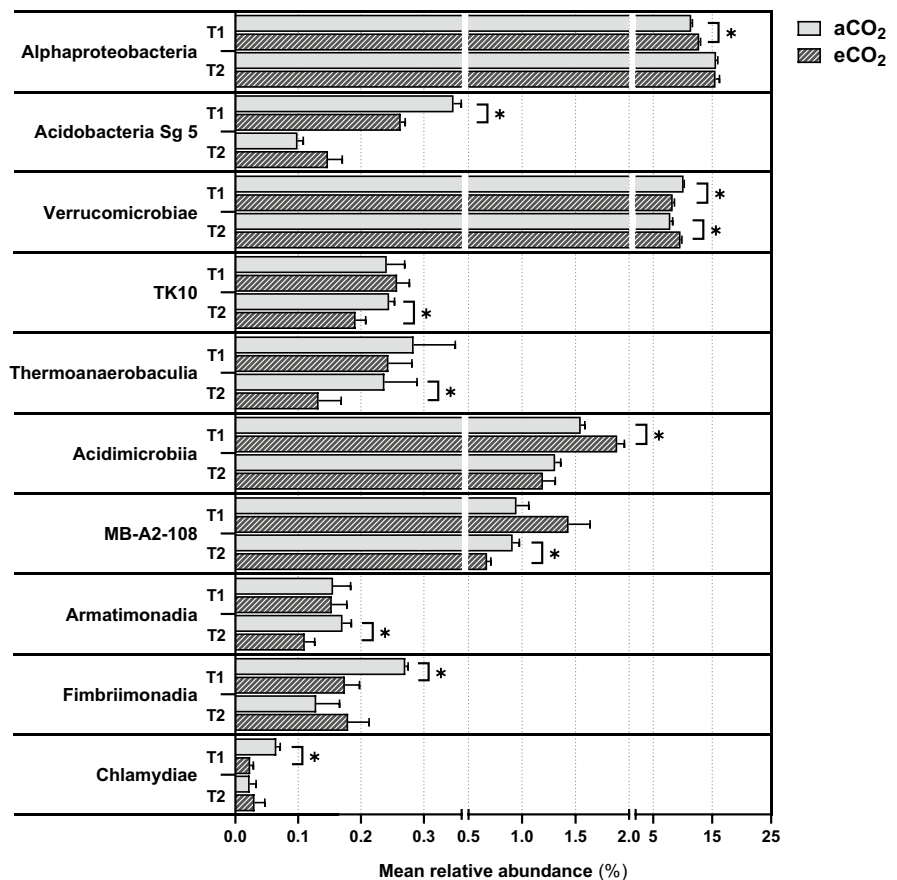
rhizosphere behaviour and overall soil health (Williams et al. 2018). In line with this, our findings revealed that eCO₂ exposure significantly increased the relative abundance of *Pseudomonadota*, which is involved in carbon, nitrogen, and sulfur cycling (Supplementary Material – Fig. 4). This microbial shift correlates with the observed increase in plant productivity under eCO₂, 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 eCO₂ 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*, *Betaproteobacterales*, 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, aCO₂) or elevated atmospheric CO₂ concentration (600 ppm, eCO₂), 15 days after pod filling (T1, 15 days of exposure to CO₂) and at harvest (T2, 30 days of exposure to CO₂). Each value the average of three plants ± SEM and asterisks indicate significant differences between aCO₂ and eCO₂ 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 eCO₂. 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 eCO₂ 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 eCO₂ 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 eCO₂ 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 eCO₂. 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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