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EFECTO DEL CO_2 ELEVADO SOBRE LA FOTOSÍNTESIS, LOS EXUDADOS RADICULARES Y EL RECLUTAMIENTO DE BACTERIAS DEL SUELO EN LA RIZOSFERA DEL TRIGO

EFFECT OF ELEVATED CO_2 ON PHOTOSYNTHESIS, ROOT EXUDATES, AND SOIL BACTERIA RECRUITMENT IN WHEAT RHIZOSPHERE

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Resumen

Las emanaciones de dióxido de carbono (CO_2) han incrementado en el último siglo, con proyecciones de hasta 900 ppm para finales del siglo XXI. Investigaciones estiman aumentos de hasta el 30% de biomasa vegetal debido a mayores tasas fotosintéticas en los cultivos agrícolas. En España, la biomasa de brotes y raíces de especies con metabolismo C3 aumentaron en un 53% y 14% respectivamente. A pesar de lo anterior estos resultados parecen contradictorios ya que algunos cultivos disminuyen la acumulación de carbono en hojas debido a una menor respiración celular. En cuanto a la actividad microbiana con elevado CO_2, se intensifica promoviendo la desnitrificación e inhibiendo la asimilación de nitrógeno en cultivos como el trigo, mientras que la diversidad bacteriana no varía significativamente a altas concentraciones de CO_2, pero se sostiene una mayor dispersión de bacterias en la rizosfera del cultivo. La hipótesis sostiene que el CO_2 atmosférico elevado incrementa la biomasa radicular del trigo y la exudación de ácidos orgánicos, elevando la biomasa microbiana, la respiración del suelo y la diversidad bacteriana. El objetivo general es evaluar los efectos del CO_2 elevado en la biomasa, actividad enzimática, exudados radiculares y microbiota de la rizosfera del trigo en distintas etapas de crecimiento, con objetivos específicos que comparan biomasa, producción de ácidos, actividad microbiana, actividad de enzimas y cambios en gases en diferentes niveles de CO_2.

Abstract

Carbon dioxide (CO_2) emissions have increased over the last century, with projections of up to 900 ppm by the end of the 21st century. Research estimates increases of up to 30% of plant biomass due to higher photosynthetic rates in agricultural crops. In Spain, the biomass of shoots and roots of species with C3 metabolism increased by 53% and 14%, respectively. Despite the above, these results seem contradictory since some crops decrease carbon accumulation in leaves due to lower cellular respiration. As for microbial activity at elevated CO_2, it intensifies promoting denitrification and inhibiting nitrogen assimilation in crops such as wheat, while bacterial diversity does not vary significantly at high CO_2 concentrations, but a greater dispersion of bacteria in the crop rhizosphere is sustained. The hypothesis holds that elevated atmospheric CO_2 increases wheat root biomass and organic acid exudation, raising microbial biomass, soil respiration and bacterial diversity. The overall objective is to evaluate the effects of elevated CO_2 on wheat rhizosphere biomass, enzyme activity, root exudates and microbiota at different growth stages, with specific objectives comparing biomass, acid production, microbial activity, enzyme activity and gas changes at different CO_2 levels.

CAPÍTULO I

Introducción general

El dióxido de carbono (CO_2) ha ido en aumento en el último siglo, presentando una concentración media a la fecha de 427 ppm de CO_2 atmosférico y se proyectan para finales del siglo XXI estará entre 700-900 ppm (Minervini et al., 2024). Investigaciones han previsto un aumento entre 20 % y 30 % en la biomasa vegetal, tanto aérea como radicular, esto producto de mayores tasas fotosintéticas y síntesis de compuestos carbonados (Van Der Kooi et al., 2016). Una investigación realizada en España, donde se analizaron especies invasoras de metabolismo C3, arrojó aumentos en la biomasa de brotes y raíces en un 53 % y 14 %, respectivamente (Caravaca et al., 2022).

Diferentes estudios concluyen resultados contradictorios sobre el efecto que posee el elevado CO_2 sobre los cultivos. En diferentes especies C3 se observó mayor acumulación de carbono en sus hojas, aumentando hasta en un 63 % producto de mayores tasas de asimilación de CO_2 (Markelz et al., 2014). En plantas de soja, bajo las mismas condiciones, ocurrió una disminución en la respiración celular, producto de la inhibición de proteínas involucradas en ese proceso (Cohen et al., 2018). Una investigación orientada a los sistemas radiculares concluyó que a altos niveles de CO_2 atmosférico se puede estimular la actividad microbiana en el sistema radicular, creando nichos anóxicos,

promoviendo la desnitrificación e inhibiendo la asimilación de nitrógeno (N) por parte de cultivos C3 como el trigo (Usyskin-Tonne, 2020).

La epidermis radicular, debido a sus propiedades exudativas, tiene la capacidad de crear zonas llamadas depósitos rizosféricos en los cuales ocurren procesos biogeoquímicos y se regula la asimilación de nutrientes, así como se controla el ciclo del carbono (C). Estos procesos son producidos por las exudaciones radiculares, las cuales se componen de enzimas y azúcares principalmente, pudiéndose excretar hasta un 30 % del C producido por la fotosíntesis, llegando a aumentar hasta en un 60 % la exudación de citrato en un ambiente de elevado CO_2 (O'Sullivan et al., 2021).

El análisis del trigo es esencial para evaluar su rendimiento en condiciones de CO_2 elevado, ya que las exudaciones radiculares son muy modificables por factores externos, lo que puede dar lugar a diferencias en sus resultados. Dado que estos efectos pueden diferir significativamente entre especies e incluso entre variedades, la investigación futura debería considerar el análisis de otros genotipos para obtener un conocimiento más completo y específico de la variedad de trigo. La influencia del CO_2 elevado en la diversidad bacteriana no varía a concentraciones más altas, según un estudio que analizó dos variedades de arroz; sin embargo, se informó de una mayor dispersión de bacterias asociadas con la rizosfera, que también tiene una mayor relación con el sistema radicular en las plantas de arroz (Xu et al., 2019).

Hipótesis

CO_2 atmosférico elevado aumenta la biomasa radicular de las plantas de trigo junto con una mayor exudación de ácidos orgánicos por la raíz, lo que conduce a un aumento de la biomasa microbiana, la respiración del suelo y la diversidad bacteriana.

Objetivo General

Evaluar los efectos del CO_2 elevado sobre la producción de biomasa aérea y radicular de trigo (*Triticum aestivum L.*), la actividad enzimática, los exudados radiculares y la microbiota de la rizosfera en dos etapas de crecimiento (Z2,2 y Z5,9).

Objetivos específicos

1. Comparar la biomasa radicular y aérea del trigo (variedad Pantera-INIA) en ambientes con diferentes concentraciones de CO_2 (550 ppm y 1000 ppm).
2. Evaluar la producción de ácidos orgánicos liberados por las raíces del trigo en ambientes contrastantes de CO_2 atmosférico (550 ppm y 1000 ppm).
3. Analizar la actividad y comunidad microbiana del suelo rizosférico (biomasa y respiración microbiana) en ambientes con diferentes concentraciones de CO_2 (550 ppm y 1000 ppm).

4. Evaluar la actividad de las enzimas nitrato reductasa y glutamato deshidrogenasa con diferentes niveles de concentración de CO_2 (550 ppm y 1000 ppm).
5. Examinar los intercambios de gases producidos por el trigo en ambientes con diferentes concentraciones de CO_2 (550 ppm y 1000 ppm).

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CAPÍTULO II (JOURNAL OF SOIL SCIENCE AND PLANT NUTRITION – 23/12/2024)

EFFECT OF ELEVATED CO2 ON PHOTOSYNTHESIS, ROOT EXUDATES, AND SOIL BACTERIA RECRUITMENT IN WHEAT RHIZOSPHERE

Abstract

Industrial growth has led to an increase in atmospheric CO_2, which is predicted to reach 900 ppm by the end of the 21st century compared with 427 ppm today. This mainly affects crops with C3 metabolism such as wheat. Wheat plants were evaluated in a closed system growth chamber under two CO2 conditions ($CO_{2\ 550}$ and $CO_{2\ 1000}$ ppm) at two growth stages (Z2.2 and Z5.9). Leaf enzymatic activity (GDH_a, GDH_d, and NR), gas exchange parameters, aerial and root biomass, organic acids exuded by roots, soil microbial activity, community composition and structure, and diversity (richness and Shannon index) were evaluated.

In the fourth week of growth, $CO_{2\ 1000}$ resulted in a decrease in net photosynthesis. However increased root biomass by 107 %, malic acid by 121% and enhanced soil bacterial activity and diversity compared to soil from plants grown under $CO_{2\ 550}$. In terms of richness and Shannon index, both treatments in the fourth week of growth were higher than those of $CO_{2\ 1000}$ in the eighth week. The three predominant genera of soil microbial community in the eighth week of growth under elevated CO_2 are Stenotrophomonas, Delftia, and Chryseobacterium, which are associated with soil denitrification processes. These

findings provide valuable insights into how the expected increase in CO_2 levels may influence wheat growth, photosynthesis, nitrogen acquisition, and carbon exudation, leading to alterations in the diversity and functioning of soil microorganisms. Such changes can significantly impact the functioning and sustainability of agroecosystems.

Keywords: organic acid; Andisol; Bacterial community; Root exudates

Introduction

Global industrial growth has led to sustained increases in atmospheric carbon dioxide (CO_2) levels. Currently 427 ppm of CO_2 is expected to increase between 700 and 900 ppm by the end of the 21st century (Minervini et al. 2024). This has been observed in wheat, by increasing the rate of CO_2 assimilation and water use efficiency, resulting in increased biomass production and yield under optimal growth conditions (Blandino et al. 2020; Broberg et al. 2019; Wang et al. 2022). Crops with C3 metabolism under elevated CO_2 have shown an increase in stomatal conductance of 26-38 %, and mesophyll conductance of 150 % (Xu et al., 2019), indicating that higher CO_2 assimilation is due to higher mesophyll conductance than stomatal conductance. However, under elevated CO_2, plant nitrogen (N) concentrations decrease, indicating lower assimilation by the crop (Feng et al. 2015; Wang et al. 2022). Therefore, improving N uptake capacity through larger or more efficient root systems could help mitigate the reduced plant access to nitrogen (Fan et al. 2023; Uddin et al. 2018a, b) under elevated CO_2

conditions. Increased root biomass potentially generates more exudates to attract microorganisms in the rhizosphere, influencing crop growth through nutrient solubilization, nutrient cycling, and decomposition of organic matter (Vives-Peris et al. 2020).

Root exudates contain a wide variety of compounds, including sugars, amino acids, organic acids, phenolic compounds, polysaccharides, and enzymes (Chai and Schachtman, 2022; Ma et al., 2022). Plant roots can exudate up to 30 % of the carbon produced by photosynthesis (O'Sullivan et al. 2021). Elevated CO_2 conditions increased the release of exudates into the rhizosphere. Increased citrate exudation up to 60 % (O'Sullivan et al. 2021) and malate exudation up to 87 % (Lal et al. 2019) have been reported. However, in wheat exposed to elevated CO_2, the exudation of organic acids, such as citric acid, may decrease, whereas in others, it could be doubled depending on the variety (Lal et al. 2019).

Nitrogen is essential for plant growth and development (Chamizo et al. 2017). In wheat, elevated CO_2 can stimulate microbial activity in the root system, create anoxic niches, promote denitrification, and inhibit N assimilation (Usyskin-Tonne et al. 2020). High CO_2 can inhibit nitrate assimilation due to nitric oxide-mediated uptake of nitrate reductase activity (Usyskin-Tonne et al. 2021), as well as decreased nitrifying activity of bacteria, and the suppression of the glutamine synthetase and glutamate synthase cycle in the plant (Adavi and Sathee 2021). This might have resulted in a decrease in this nutrient in the plant.

The analysis of wheat is essential to assess its performance under elevated CO_2 conditions because root exudations are highly modifiable by external factors, which can lead to differences in their results. As these effects can differ significantly between species and even between varieties, future research should consider the analysis of other genotypes to obtain a more complete and variety-specific understanding of wheat. The influence of elevated CO_2 on bacterial diversity does not vary at higher concentrations, based on a study that analyzed two rice varieties; however, a greater dispersion of bacteria associated with the rhizosphere was reported, which also has a greater relationship with the root system in rice plants (Xu et al., 2019).

The objective of this study was to evaluate the effects of elevated CO_2 on wheat (*Triticum aestivum* L.) above ground and root biomass production, enzymatic activity, root exudates, and rhizosphere microbiota at two growth stages (Z2.2 and Z5.9). It was hypothesized that elevated atmospheric CO_2 increases the root biomass of wheat plants along with increased exudation of organic acids by the root, leading to increased microbial biomass, soil respiration, and bacterial diversity.

Materials and methods

1.1. Plant material and experimental conditions

This study was conducted at the Soil Microbiology Laboratory of the Faculty of Agronomy, University of Concepción. The spring wheat variety Pantera-INIA was evaluated. The plants were pre-germinated in a germination room maintained at

22 °C with a humidity of approximately 60% and then grown in a controlled environment growth chamber (JSR, model JSPC-420C2, Korea) in two separate trials, with atmospheric CO_2 of 550 ppm ($CO_{2\ 550}$) and 1000 ppm ($CO_{2\ 1000}$). The temperature was maintained at 22 °C (day) and 17 °C (night), with a photoperiod of 16 h and relative humidity between 50 and 60 %. The average amount of light was 8680 LUX during the entire growth period. Seedlings (Z0.0.9) were kept in a growth chamber for eight weeks. Plants were established in 2 L plastic pots filled with 1.5 kg of soil at 60 % humidity. The soil belongs to the Diguillín series, with clayey silt texture, classified within the Andisol order as Typic Haploxerands, derived from modern volcanic ash (Soil Survey Staff 2014). The soil had a pH of 6.1, available N of 8.6 mg kg^{-1}, N-NO_3 of 5.1 mg kg^{-1}, N-NH_4 of 3.5 mg kg^{-1}, Olsen P of 14.4 mg $kg^{-1,}$ and available K of 132.8 mg kg^{-1}. Plants were supplemented with N when they were three weeks old by applying urea at 76.4 mg pot^{-1} (García 2008). Five seeds were sown in each pot, with a total of 10 pots for each treatment (20 pots in total), distributed in a completely randomized arrangement. Measurements were performed in the fourth (Z2.2) and eighth (Z5.9) weeks (Zadocks et al. 1974) (28 and 56 Days after sowing (DAS)), using five experimental units (pots), for all measurements. The germination was successful for the five seeds per pot in the 20 pots used.

1.2. Gas exchange measurements

It was measured using five Z2.2 and Z5.9 stage plants, with fully expanded and illuminated leaves. Measurements were performed using an infrared gas analyzer

(CIRAS – 2, PP Systems, MA, USA) with a light intensity of 400 PAR and airflow rate of 500 μmol s^{-1}. Records were obtained with a coefficient of variation of less than 0.1 % (Pearcy et al. 1991). Measurements were taken between 10:00 and 13:00 h.

1.3. Enzymatic activity in wheat leaf

The nitrate reductase (NR) activity was measured according to Eaglesham and Hewitt (1975), 100 mg of plant material (leaves) from Z2.2 and Z5.9 stage was extracted and incubated with 1 mL extraction buffer, after which a supernatant was extracted and incubated with 900 μL of reaction buffer. The reduced nicotinamide adenine dinucleotide (NADH) oxidation was measured at 340 nm at time 0 and 10 min. The glutamate dehydrogenase (GDH) enzyme activity was assessed in two processes: amination and deamination, as described by Kumar et al. (2000). For both processes, the absorbance was measured at 340 nm. Controls were measured by incubating the enzyme with the reaction buffer without NADH and NAD^+ and denatured enzyme with the reaction buffers.

1.4. Determination of plant biomass

To determine aerial and root biomass, plant material from Z2.2 and Z5.9 stage was dried at 60 °C for 72 h in a forced-air oven and dry weight was obtained with an analytical balance. Three plants per pot were used to measure the root biomass (15 samples per treatment) and one per pot for aerial biomass (five samples per treatment).

1.5. Determination of root exudates

Organic acid exudates were collected from the total root system of each plant following the methodology described by Delgado et al. (2014). Three root samples per pot were used to measure organic acids. Liquid samples containing exudates were frozen at -80 °C and then lyophilized using a Freeze Dryer (Operon, Korea). Identification and quantification were performed using high-performance liquid chromatography (HPLC) (Merck-HITACHI, Germany). Citrate, malate, oxalate, and succinate standards (Sigma Aldrich, USA) were used for quantification. Values were expressed as the rate of carboxylate exuded per gram of fresh weight (FW) per hour (μmol g^{-1} FW h^{-1}).

1.6. Soil biological parameters

The respiration rate was determined using the incubation method, which captures CO_2 produced by respiration from wet soil in a sodium hydroxide (NaOH) solution. The amount of NaOH remaining after a known incubation period (10 days) was determined by titration with a standardized acid (Rowell 1994).

The fluorescein diacetate hydrolysis (FDA) colorimetric method was used to quantify the total active microbiological activity in the soil at a general level (Alef and Nannipieri 1995). The absorbance of the samples and blanks was read at 490 nm; the blank consisted of 10 ml of acetone and 10 ml of distilled water.

1.7. Soil DNA Extraction

DNA from wheat soil was extracted according to Mandakovic et al. (2018) using 5 g of soil for wheat soil DNA extractions, these samples were re-suspended in 5 mL extraction bufer [100 mM Tris–HCl; pH 8, 100 mM Na EDTA; pH 8, 100 mM

Na2HPO4, 1.5 M NaCl, 1% (w/v) CTAB], and then, 10 mg/mL of lysozyme (final concentration) was added and mixed by vortexing, followed by incubation at 37 °C for 1 h with shaking. After centrifugated at 2000 × *g* for 5 min at room temperature, the supernatant was transferred to a new tube. Next, 3 μL of proteinase (100 mg/mL) was added, followed by incubation at 37 °C for 1 h with gentle shaking. This mixture was transferred in triplicate using MOBIO PowerSoil (Mobio Laboratories Inc., Carlsbad, CA, USA). The quality of extracted DNA was checked on a 1% agarose gel stained with red gel, and its concentration was measured using a Qubit fluorometer® dsDNA Assay kit (ThermoFisher) according to the manufacturer's instructions.

1.8. Bacterial community analyses

The V4 region of the 16S rRNA gene was amplified using the primers 515F and 806R. The reads were processed using Qiime 2 v2022.2 plugins (Bolyen et al. 2019).The barcodes and primers were removed from the demultiplexed reads using the Cutadapt *trim-paired* plugin. Amplicon Sequence Variants (ASVs) were generated using the DADA2 (Callahan et al. 2016) *denoise-paired* plugin and assigned a taxonomy using the *classify-sklearn* plugin with a naive Bayes classifier trained on the SILVA 138 database (Quast et al. 2013) sequences of the V4 region. Only ASVs detected in at least two samples, which were classified as belonging to the phylum Archaea or Bacteria, excluding mitochondria and chloroplasts, were retained.

For diversity analysis, the ASVs samples were rarefied to 55,000 counts. The R package vegan v2.6-4 (Oksanen et al. 2022) was used to calculate the alpha diversity indices for each sample and the Bray-Curtis dissimilarity between samples.

1.9. Statistical analysis

The normality and homoscedasticity of the data were checked before statistical analyses using the Shapiro-Wilk and Levene tests. Two-way analysis of variance (ANOVA) and post-hoc Tukey's HSD tests ($p < 0.05$) were performed. Analyses were conducted with the Infostat software (Di Rienzo et al., 2020). The statistical significance of comparing alpha diversity indices between microbials groups was evaluated using the Kruskal-Wallis test, followed by Dunn's test

Results

1.10. Biomass determination

The aboveground biomass was not significant at 4 weeks; however, at eight weeks, the biomass of plants under the $CO_{2\ 1000}$ treatment was 43 % lower than that under the $CO_{2\ 550}$ treatment (Figure 1 A). A different pattern was observed in root biomass (Figure 1 B), which was significantly increased under $CO_{2\ 1000}$ treatment (107 %) compared to $CO_{2\ 550}$ by the fourth week of growth, whereas in the eighth week, no significant differences were observed.

1.11. Gas-exchange parameters of wheat plants

The plant transpiration rate (E) in the fourth week was significantly higher in the $CO_{2\ 550}$ treatment (25 %); however, in the eighth week, this treatment was

significantly lower than the ambient with the highest CO_2 and both CO_2 treatments in the fourth week. There were no differences in the $CO_{2\ 1000}$ treatment between the measurement time points (Figure 2). The photosynthetic capacity of the wheat leaves showed a pattern similar to that of leaf transpiration. In the fourth week, the Pn of the $CO_{2\ 550}$ treatment was 41 % higher, and by the eighth week, the $CO_{2\ 1000}$ treatment was significantly higher with 17.2 µmol m^{-2} s^{-1}, while the $CO_{2\ 550}$ treatment was only 2 µmol m^{-2} s^{-1}. Stomatal conductance (Gs) did not show significant differences in the fourth week of growth, whereas, in the eighth week, Gs was significantly higher in the $CO_{2\ 1000}$ treatment. The leaf respiration did not show significant differences in the fourth week, but a significantly higher Rd was observed in the eighth week in the $CO_{2\ 1000}$ treatment.

1.12. Enzymatic activity in wheat leaf

The foliar enzymatic activity patterns varied for glutamate dehydrogenase amination (GDHa), glutamate dehydrogenase deamination (HDHd), and nitrate reductase (NR) (Figure 3). At four weeks of growth, GDHa activity was significantly higher (148 %) under the $CO_{2\ 1000}$ treatment, while no significant differences were observed between CO_2 conditions in GDH deamination capacity. By the eighth week, GDH activity was significantly lower in the $CO_{2\ 1000}$ treatment for both amination and deamination, with reductions of 55% and 60%, respectively; however, there were no significant differences in GDH activity between measurement periods in the $CO_{2\ 1000}$ treatment. The nitrate reductase

activity exhibited significant differences only in the eighth week, being 75 % lower in the $CO_{2\ 1000}$ treatment.

1.13. Chemical and biological soil properties

Significant differences were observed at four weeks of plant growth in basal soil respiration, with an increase of 48 % in $CO_{2\ 1000}$, whereas in the eighth week, no significant differences were observed (Figure. 4 A). The biomass of the soil microorganisms showed a similar trend to basal soil respiration. In the fourth week, significantly higher microbial biomass was observed in $CO_{2\ 1000}$ (109 %), whereas in the eighth week, there were no significant differences (Figure. 4 B).

The availability of nitrogen and its different forms in the soil were also affected by the CO_2 environment (Figure 5). Available N did not show significant differences in the fourth week of growth, whereas in the eighth week, it was significantly lower (49 %) in the $CO_{2\ 1000}$ treatment. Soil nitrate was significantly higher in $CO_{2\ 1000}$ in the fourth and eighth weeks (109 % and 143 %, respectively), whereas ammonium soil content showed the opposite effect, being significantly higher in $CO_{2\ 550}$ at both measurement times by 49 % and 87 %, respectively.

1.14. Roots exudation

The levels of oxalic, malic, and citric acids in the wheat root exudates were affected by the concentration of CO_2 in the growth chamber (Figure 6). Oxalic acid and citric acid contents showed a similar pattern; they were significantly higher in the fourth week under $CO_{2\ 550}$, with increases of 55 and 79 % for oxalic acid and citric acid, respectively, whereas no differences were found for both acids in the

subsequent measurement. In contrast, malic acid content was significantly higher (121 %) in $CO_{2\ 1000\ in}$ the fourth week, and no significant differences were found in the eighth week.

1.15. Bacterial community analyses

The data obtained did not indicate significant differences in the analysis of Pielou's evenness but showed significant differences in the analysis of richness and Shannon index (Figure 7). In terms of richness, $CO_{2\ 550}$ and $CO_{2\ 1000}$ treatments in the fourth week of growth revealed higher values, which were significantly different compared to $CO_{2\ 1000}$ in the eighth week, whereas $CO_{2\ 550}$ in the eighth week remained without significant differences. The same pattern was observed in Shannon index analysis. To assess whether bacterial community composition differed between the CO_2 treatments, we performed a Non-metric MDS analysis (Figure 8). Replicates between the fourth and eighth weeks had distinct areas between them, showing little variability between them, while samples of $CO_{2\ 550}$ and $CO_{2\ 1000}$ conditions showed overlaps between their replicates. For the microbial order, family, and genus analyses (Figure 9), higher dominance and, therefore, lower diversity was observed in the eighth week for $CO_{2\ 1000}$, whereas the other treatments showed higher microbial equity, which is similar to what was observed in the richness and Shannon index analyses.

Discussion

Biomass analysis indicated increased root development in the fourth week under $CO_{2\ 1000}$, suggesting greater allocation of carbon compounds to the roots during

the initial stages of growth under these conditions. Under CO_2 (700) conditions, crop transpiration is reduced by up to 50 % during the first 3 min; however, this reaction does not limit root uptake, which continues to take up water for 5-6 h with the same intensity, thus maintaining an adequate hydration status in the upper and lower roots (Suslov 2020).

The aboveground biomass during the eighth week was significantly higher under $CO_{2\ 550}$, indicating higher mobilization towards the stems in this treatment. Higher biomass production is usually associated with higher photosynthesis rates (Simkin et al. 2022). However, plants exposed to $CO_{2\ 500}$ showed higher aboveground biomass and significantly lower values of P_n, G_s, and E in the eighth week, possibly due to the onset of physiological senescence. The results suggest that the increase in biomass observed in this study at $CO_{2\ 500}$ could be due to a higher efficiency in the mobilization of carbon compounds as it has been reported that under high CO_2 conditions (550 ppm), higher mobilizations of up to 24 % can occur (Fang et al. 2022), in addition, higher CO_2 assimilation is mainly due to higher mesophyll conductance than stomatal conductance (Xu et al., 2019). However, the higher aerial biomass in the eighth week under $CO_{2\ 500}$ could be explained, in part, by the higher activity of GDH_a, GDH_d, and NR, and consequently, higher N assimilation. This is suggested by the fact that the $CO_{2\ 1000}$ treatment, despite maintaining a higher P_n, obtained lower values of aerial biomass in the eighth week, together with lower expression of its GDH_a, GDH_d, and NR activity. This may indicate that an elevated $CO_{2\ 700}$ condition is able to

inhibit nitrate assimilation in wheat proteins (Adavi and Sathee 2021) due to nitric oxide-mediated uptake of nitrate reductase activity, decreased nitrite reductase activity and suppression of the glutamine synthase and glutamate synthase cycle, causing a decrease of this nutrient in the plant and a decrease in growth rate. Therefore, the lower biomass accumulation in $CO_{2\ 1000}$ in the eighth week could be associated with lower N assimilation by the crop due to lower GDH_a, GDH_d and NR activity.

The highest root biomass in the $CO_{2\ 1000}$ treatment in the fourth week occurred along with the highest root exudation of malic acid. However, the higher levels of oxalic acid and citric acid in $CO_{2\ 550}$ in the same week were not associated with an increase in aerial or root biomass. The increase in malic acid in $CO_{2\ 1000}$ in the fourth week was associated with higher soil respiration and microbial biomass. In the eighth week, the exudation of organic acids by roots, soil respiration, and microbial biomass were not affected by the CO_2 environment. However, the levels of organic acids are highly variable even for the same wheat varieties. Research (Lal et al. 2019) evaluated two wheat varieties with different ploidy under elevated CO_2 (700 ppm) and phosphorus deficit (5 μM) and obtained 55 % more malate exudation in the hexaploid variety than in the tetraploid. Therefore, the hexaploid variety showed higher malate production, similar to the effect observed in Pantera-INIA under $CO_{2\ 1000}$ in the fourth week of growth. We cannot rule out the possibility that other exudation compounds not measured here were secreted by $CO_{2\ 1000}$ plants.

Root exudation influenced soil bacterial diversity, which was higher in the fourth week of growth under both CO_2 conditions, possibly increasing the root growth rate at this stage, specifically under $CO_{2\ 1000}$ and high levels of exuded organic acids. The use of elevated CO_2 (600 ppm) under water deficit conditions led to an increase in bacterial abundance of up to 51 % in wheat (Krause et al. 2023). In this study, the soil bacterial structure under both CO_2 conditions was different between measuring moments, with lower bacterial diversity in the fourth week. This effect can be attributed to direct competition between soil microorganisms because they can promote the creation of anoxic niches, which vary depending on the form of available N, either as ammonium or nitrate, thus decreasing the diversity of other microorganisms (Usyskin-Tonne et al. 2021).

The three predominant orders of soil microorganisms in the eighth week belonged to Xanthomonadales, Burkholderiales, and Flavobacteriales, which belong to the three predominant families Xanthomonadaceae, Comamonadaceae, and Weeksellaceae, respectively. The Giessen free-air CO_2 enrichment (Gi-FACE) experiment also found increased levels of Xanthomonadaceae and Comamonadaceae in the rhizosphere of the plant community. Soil analyses showed less available N in the eighth week of the $CO_{2\ 1000}$ treatment, despite the use of a buffer fertilizer at the beginning of tillering in the third week, and less assimilation of nitrogen compounds by the crop. Soil N was found to be more nitrate than ammonium available because of the inhibition of wheat enzymes related to soil N assimilation, due to a decrease in nitrite

reductase activity and suppression of the glutamine synthetase and glutamate synthase cycle (Adavi and Sathee 2021). This is supported by observations in the $CO_{2\ 550}$ treatment, which, because of maintaining the activity of NR and GD_H enzymes in the eighth week, managed to assimilate more nitrate and therefore more ammonium was found in the soil. The decay in soil N availability that occurred in the eighth week in the $CO_{2\ 1000}$ environment is indicative of an alternative loss process such as denitrification, possibly by bacteria of the order Burkholderiales, specifically the genera Stenotrophomonas, Delftia, and Chryseobacterium, which have been associated with soil denitrifying processes under elevated CO_2 (850 ppm) (Usyskin-Tonne et al. 2020).

Conclusions

This study showed that wheat grown under $CO_{2\ 1000}$ conditions experienced an increase in root biomass, mainly due to an enhanced allocation of carbon compounds to the root zone during early growth stages. However, inhibition of N assimilation due to the reduced activity of GDH_a, GDH_d, and NR enzymes appears to counteract biomass assimilation. Concentrations of $CO_{2\ 550}$ were able to maintain enzyme activity and obtain higher biomass accumulation.

The increased allocation of carbon compounds to the root zone leads to the increased exudation of malic acid, which can increase microbial activity and diversity. This indicates that variations in organic acids over time can have a significant impact on soil microorganisms. However, $CO_{2\ 1000}$ did not directly

influence bacterial diversity but rather intensified the activity of microorganisms, possibly increasing their dispersal through the root zone.

Elevated CO_2 levels do not directly affect microbial communities, but were altered through crop phenology and exudate composition. In addition, lower enzyme activity and thus higher availability of N compounds in the soil may reduce bacterial diversity and is indicative of an alternative loss process such as denitrification, possibly by bacteria of the order Burkholderiales, specifically the genera Stenotrophomonas, Delftia, and Chryseobacterium, which have been associated with soil denitrifying processes.

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Figures

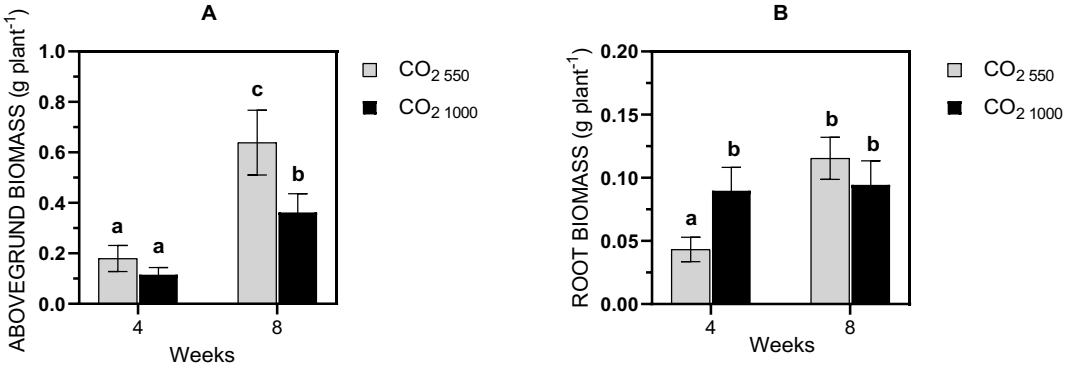

Fig. 1. Biomass production in wheat grown under two CO_2 environments (550 and 1000 ppm) was measured four and eight weeks after sowing. A) Above-ground biomass and B) root biomass. Each bar represents the mean ± standard error (n=5). Means with the same letter are not significantly different (Tukey HSD $p < 0.05$).

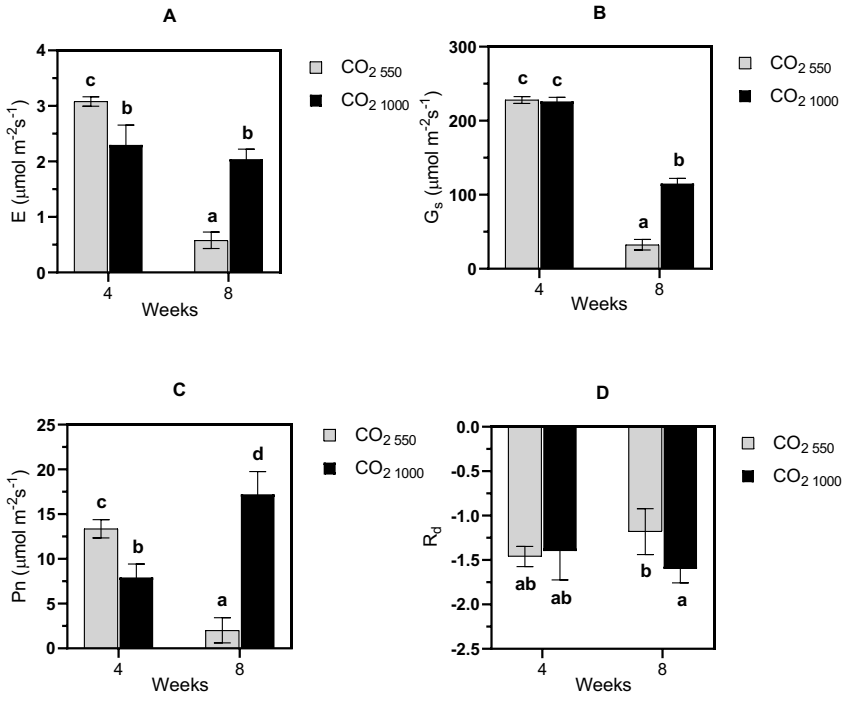

Fig. 2. Gas exchange parameters in wheat grown under two CO_2 environments (550 and 1000 ppm) measured at four and eight weeks after sowing A) Transpiration rate (E), B) stomatal conductance (G_s), C) net photosynthesis (P_n), and D) respiration (R_d). Each bar represents the mean ± standard error (n=5). Means with the same letter are not significantly different (Tukey HSD $p < 0.05$).

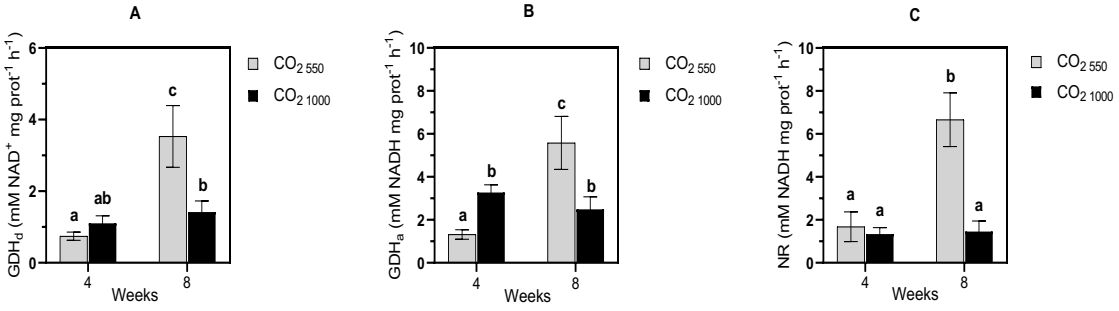

Fig 3. Enzymatic activity in wheat leaves grown under two CO_2 environments (550 and 1000 ppm) measured at four and eight weeks after sowing. A) glutamate dehydrogenase deamination (GDH_a), B) glutamate dehydrogenase amination (GDH_d), and C) nitrate reductase (NR). NADH (Nicotinamide Adenine Dinucleotide reduced). Each bar represents the mean ± standard error (n=5). Means with the same letter are not significantly different (Tukey HSD $p < 0.05$).

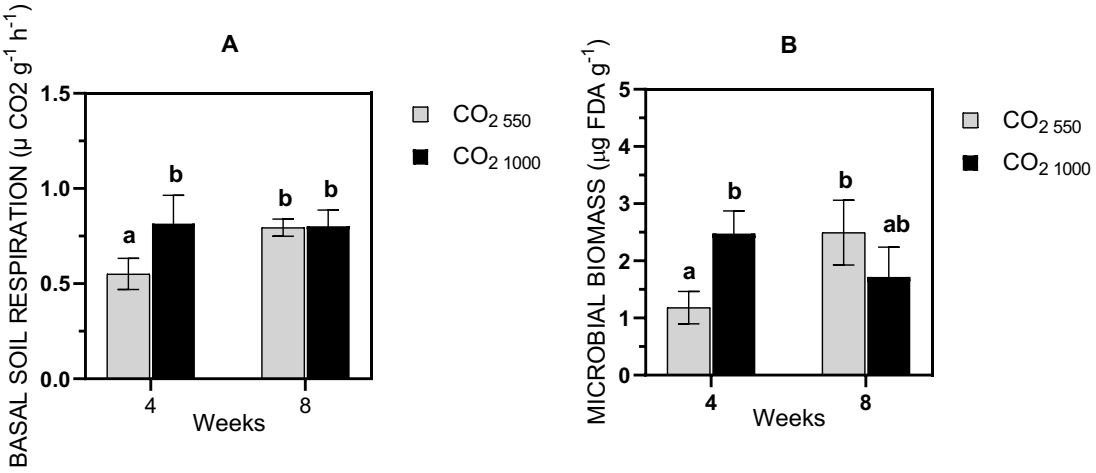

Fig. 4. Soil analysis of wheat grown under two CO_2 environments (550 and 1000 ppm) measured at four and eight weeks after sowing. A) Basal soil respiration and B) Microbial biomass. Means with the same letter are not significantly different (Tukey HSD $p < 0.05$). Each bar represents the mean ± standard error (n=5).

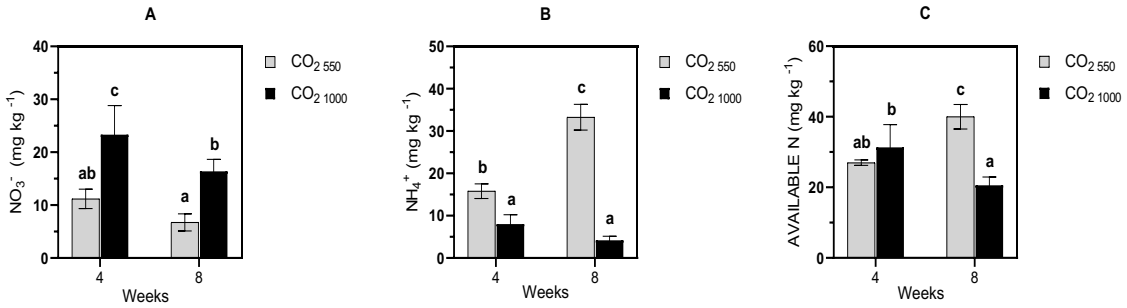

Fig. 5. Soil analysis of wheat grown under two CO_2 environments (550 and 1000 ppm) measured at four and eight weeks after sowing. A) Nitrate (NO_3^-), B) ammonium (NH_4^+), and C) available N in soil. Means with the same letter are not significantly different (Tukey HSD $p < 0.05$). Each bar represents the mean ± standard error (n=5).

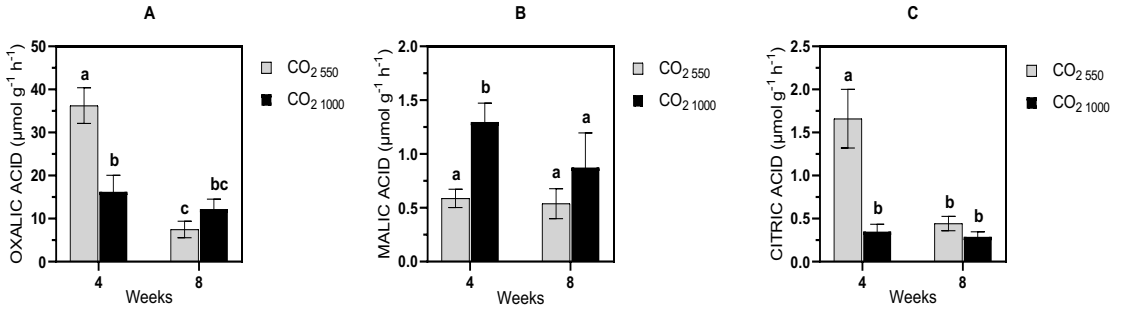

Fig. 6. Organic acids in root exudates of wheat grown under two CO_2 environments (550 and 1000 ppm) measured at four and eight weeks after sowing. A) Oxalic acid. B) Malic acid. C) Citric acid. Each bar represents the mean

± standard error (n=5). Means with the same letter are not significantly different (Tukey HSD $p < 0.05$).

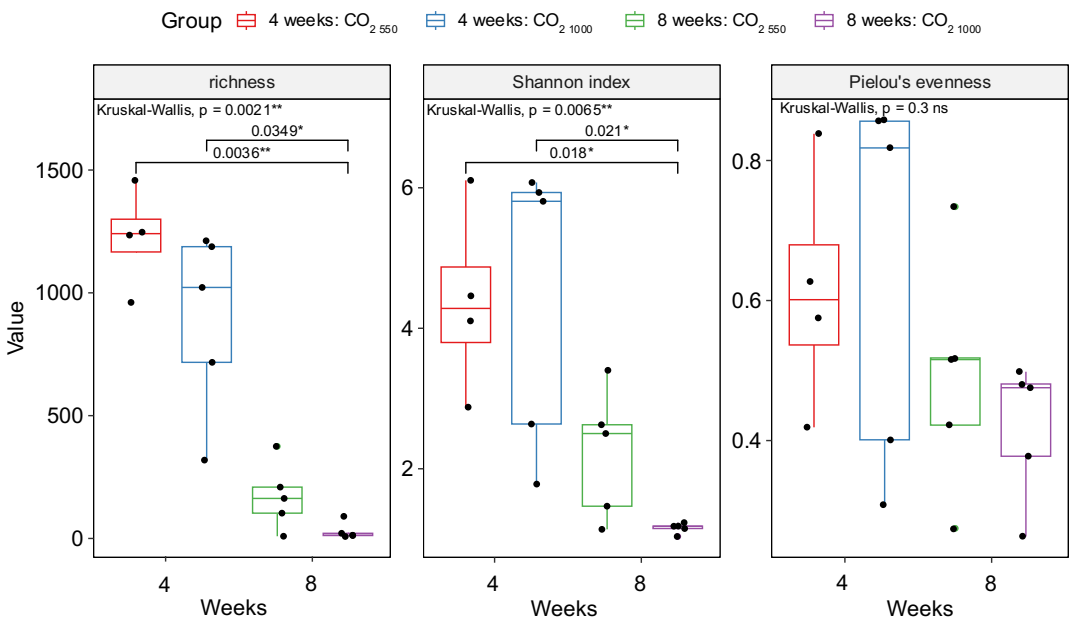

Fig. 7. Soil bacterial community analyses of wheat grown under two CO_2 environments (550 and 1000 ppm) measured at four and eight weeks after sowing. The data were subjected to richness, Shannon index, and Pielou's evenness tests. The Kruskal-Wallis test ($p < 0.05$).

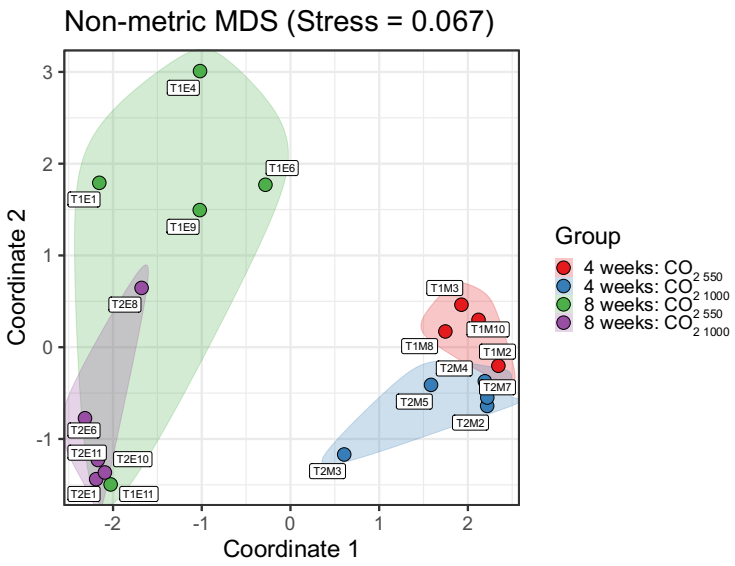

Fig. 8. Non-metric multidimensional scaling (NMDS) ordination of the surface soil and leaf litter bacterial community composition of wheat grown under two CO_2 environments (550 and 1000 ppm) measured at four and eight weeks after sowing. Circles represent red (fourth week, $CO_{2\ 550}$), blue (fourth week, $CO_{2\ 1000}$), green (eighth week, $CO_{2\ 550}$), and purple (eighth week, $CO_{2\ 1000}$).

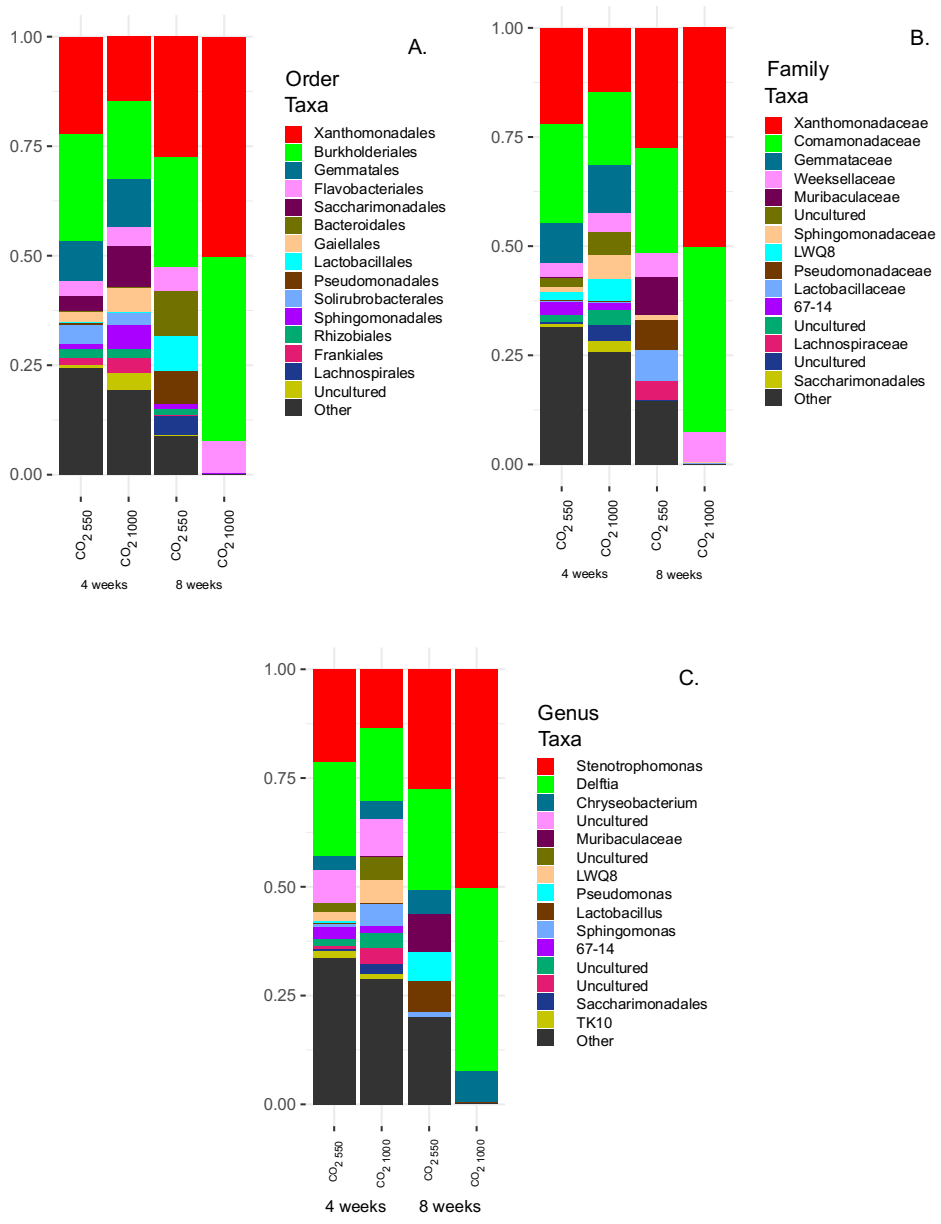

Fig. 9. Soil bacterial community analyses of wheat grown under two CO_2 environments (550 and 1000 ppm) measured at four and eight weeks after sowing. Bacterial order (A), family (B), and genus (C) were identified and organized according to the relative abundance of bacteria.

Conclusión

Este estudio demostró que el trigo cultivado en condiciones de $CO_{2\ 1000}$ experimentó un aumento de la biomasa radicular, debido principalmente a una mayor asignación de compuestos de carbono a la zona radicular durante las primeras etapas de crecimiento. Sin embargo, la inhibición de la asimilación de N debida a la actividad reducida de las enzimas GDHa, GDHd y NR parece contrarrestar la asimilación de biomasa. Las concentraciones de $CO_{2\ 550}$ fueron capaces de mantener la actividad enzimática y obtener una mayor acumulación de biomasa.

La mayor asignación de compuestos de carbono a la zona radicular conduce a una mayor exudación de ácido málico, que puede aumentar la actividad y diversidad microbianas. Esto indica que las variaciones de los ácidos orgánicos a lo largo del tiempo pueden tener un impacto significativo en los microorganismos del suelo. Sin embargo, el $CO_{2\ 1000}$ no influyó directamente en la diversidad bacteriana, sino que intensificó la actividad de los microorganismos, aumentando posiblemente su dispersión por la zona radicular.

Los niveles elevados de CO_2 no afectan directamente a las comunidades microbianas, pero se vieron alterados por la fenología de los cultivos y la composición del exudado. Además, una menor actividad enzimática y, por tanto, una mayor disponibilidad de compuestos de N en el suelo puede reducir la diversidad bacteriana y es indicativa de un proceso de pérdida alternativo como la desnitrificación, posiblemente por bacterias del orden Burkholderiales,

concretamente los géneros Stenotrophomonas, Delftia y Chryseobacterium, que se han asociado a procesos de desnitrificación del suelo.