Short-term influences of C_4 versus C_3 plant growth on dissolved inorganic carbon in a carbonate-poor soil

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Abstract

Background and aims

Soil inorganic carbon (SIC) and its dissolved fraction are recognised as dynamic components of the terrestrial carbon (C) cycle. While they contribute to CO_2 sequestration and climate regulation, their short-term responses to plant-driven processes remain poorly understood. This study investigates how growth of a C_3 species (*Silphium perfoliatum* L.) and a C_4 species (*Zea mays* L.) affects dissolved inorganic and organic C (DIC/DOC) dynamics in a carbonate-poor soil under varying soil moisture conditions.

Methods

Leachates taken from a two-week lysimeter experiment were analyses for DIC and DOC concentrations, $\delta^{13}C$ isotope signatures, pH, and the data obtained were complemented with *in situ* CO_2 efflux measurements. A simplified two-endmember isotope mixing model was used to estimate the contribution of plant-derived C to the DIC pool.

Results

DIC concentrations exceeded DOC across all treatments. Increased CO_2 efflux and higher pH values indicated enhanced soil respiration. $\delta^{13}C_{DIC}$ values ranged from − 7‰ to − 20‰, while $\delta^{13}C_{DOC}$ remained nearly uniform at − 29 ± 0.7‰. Isotope modelling suggested that up to 62% of DIC could be derived from C_4 plant sources, despite the short timeframe.

Conclusion

Root and rhizomicrobial respiration may measurably influence short-term DIC dynamics in carbonate-poor soils. These findings suggest that DIC fluxes could play a more prominent role in soil C cycling than currently assumed, particularly over short temporal scales.

Introduction

Soil ecosystems represent the most significant carbon (C) reservoir in terrestrial ecosystems, harbouring more C than the atmosphere and global vegetation combined (European Commission 2011; Lal et al. 2021). While soil organic C (SOC) has been widely studied for its role in biogeochemical cycling and climate regulation (Billings et al. 2021), investigations of the soil inorganic C (SIC) fraction are less frequent (Dina Ebouel et al. 2024).

Within the upper two meters of the soil profile, approximately 700 to 1000 Pg C is stored as soil SIC, with an additional 2273 Pg C stored as SOC (Batjes 2016; Jackson et al. 2017; Sharififar et al. 2023). While SIC is generally regarded as stable over pedogenic timescales (Schlesinger 1985; Lal 2009), the biological origin of SOC introduces a high variability. Thus, SOC, originating from plant material, soil

microorganisms, and their metabolites, plays an essential role in regulating and stabilising ecosystem functions (Jackson et al. 2017; Billings et al. 2021). Moreover, this dynamic nature of SOC indicates that even minor alterations, such as turnover or increased C storage, can have significant repercussions on atmospheric CO_2 levels and, consequently, global warming (Billings et al. 2021). On the other hand, CO_2 emissions from the soil's dissolved inorganic C (DIC) pool can, depending on the soil type involved, substantially contribute to net C losses (Kindler et al. 2011; He et al. 2015; Sun et al. 2021; Ferdush and Paul 2021).

Besides soil properties, recent studies revealed that plant-soil interactions, especially through root respiration and microbial turnover, can affect DIC dynamics, even in poorly buffered soils and over short timeframes (Rovira and Vallejo 2008; An et al. 2019; Zhao et al. 2020). Hence, understanding the timescales over which C is transferred from plant to soil is crucial for interpreting soil C dynamics. Experimental evidence from ^{14}C-CO_2 pulse-labelling studies has shown that recently fixed C can be detected within hours to days in the plant's rhizosphere, appearing in root exudates, microbial biomass, as well as respired CO_2 (Biernath et al. 2008; Martens et al. 2009; Werth and Kuzyakov 2009; Pausch et al. 2013). These rapid fluxes imply that processes such as root respiration and microbial turnover could affect the inorganic C pool even over short durations. Considering that the specific processes governing the stability, distribution and depletion of both DOC and DIC remain elusive (Jackson et al. 2017; Billings et al. 2021; Krüger et al. 2024), short-term experiments might provide valuable insights to capture meaningful shifts in soil DIC.

Natural abundance measurements of DIC and DOC C-isotope ratios ($\delta^{13}C$) are widely applied to characterise soil systems, providing valuable insights into the predominant dynamics, which chaperone biotic, as well as abiotic, processes and thus shape ecosystem functioning (Balesdent and Mariotti 1996; Staddon 2004; Campeau et al. 2017). In established soil horizons, C concentrations tend to decline exponentially with depth, which corresponds to an increase in C age (Trumbore 2000). At the same time, there is a noticeable enrichment in ^{13}C (indicated by increases in $\delta^{13}C$) in SOC within the upper layer of the mineral soil (Diochon and Kellman 2008; Philben et al. 2022; Krüger et al. 2024). Various theories have been put forward to explain this phenomenon, mostly emphasising the impact governed by ongoing biological processes concerning C turnover (e.g., Diochon and Kellman 2008; Philben et al. 2022). While microbial decomposition is recognised as a central factor in C turnover (Freeman et al. 2024), the role of vertical transport of C by plant roots or fungi is also essential for understanding the observed $\delta^{13}C$ isotope profile (Basile-Doelsch et al. 2020; Philben et al. 2022).

Shifts in vegetation type, specifically from C_3 to C_4 plants, profoundly influence $\delta^{13}C$ isotope values, shedding light on SOC turnover patterns (Billings et al. 2002; Zang et al. 2018; Kirkels et al. 2022). Since the isotope signal remains unaffected by how C transitions from plant to soil or sediment, the $\delta^{13}C$ signature in the soil and its solution should mirror that of the overlying vegetation (Staddon 2004; Kirkels et al. 2022; Philben et al. 2022; Krüger et al. 2024). Consequently, plants that employ different photosynthetic pathways, such as C_4-plants utilizing the Hatch-Slack cycle, lead to distinct differences in

$\delta^{13}C$ isotope values, with C_3 plants ranging from ~-20 to -37‰, and C_4 plants from ~-10 to -16‰ for soil C from these sources (Balesdent and Mariotti 1996; Staddon 2004; Kirkels et al. 2022). In addition, numerous studies have demonstrated that environmental stressors (i.e., heat and drought) significantly affect plant performance. These changes are reflected in $\delta^{13}C$ isotope patterns observed in C_3 and C_4 plants (Dercon et al. 2006; Zang et al. 2018; Ouattara et al. 2021; Kirkels et al. 2022). The severity of drought conditions can influence C allocation patterns, as water stress leads to increased water-use efficiency and alters C inputs into the soil (Vadez et al. 2024).

While not all assimilated C is retained in plant biomass, significant fractions are released during growth as e.g., root exudates or respiratory CO_2 (Sokol et al. 2019; Wen et al. 2022). Hence, these compounds may contribute to the dissolved C pool both inorganic and organic, yet the magnitude and timing of these contributions remain elusive. Particularly in short-term studies and in soils with low carbonate content, plant-derived CO_2 might dominate DIC formation pathways. Thus, to assess whether short-term (i.e., hours, days) plant activity measurably affects the DIC pools in a carbonate-poor soil system, a lysimeter-based field experiment was established. Rather than testing a priori hypotheses, this study aimed to characterise the relative contributions of C_3 and C_4 plants to soil DOC and DIC using natural abundance $\delta^{13}C$ isotope patterns. Given the short duration of the experiment, it was unclear whether isotopic ratios from recent plant-derived C would be traceable in the dissolved C pools. However, plant-specific differences in root activity and soil moisture conditions could leave detectable signatures in DIC or DOC composition. To evaluate these dynamics, leachates from lysimeters planted with either *Silphium perfoliatum* L. (C_3) or *Zea mays* L. (C_4), grown under moderate drought and optimal irrigation conditions, were compared. Stable isotope analyses of DIC and DOC, combined with a simplified isotope mass balance model, were used to estimate plant contributions to the DIC pool.

Materials and Methods

Study area

The experiment was part of a four-year investigation (see Abdalla et al. 2024), conducted at the Ecological Botanical Garden (49˚5519N, 11˚3455E, 365 m a.s.l) at the University of Bayreuth, Germany. The climate of this area lies within a transition zone where oceanic and continental climates meet with 741 mm and 8.2°C long-term (1980–2019) mean annual precipitation and temperature, respectively (Zsolnay et al. 2023). The soil used for the experiment represents carbonate-poor soils typical of Central European floodplain landscapes, allowing focused investigation of biologically driven DIC formation.

A total of 28 built-in lysimeters with cement-smoothened concrete walls and inner side sealing, (coating material (Inertol 49W)) to prevent horizontal water movements, were deployed (Fig. 1A). The lysimeters were partially equipped with a mobile greenhouse, that was able to automatically position itself over ten lysimeters to exclude rain and to allow for simulations of droughts.

Each lysimeter consisted of two parts: A funnel-shaped bottom filled with gravel and an upper, rectangular prism part (width: 1.3 m, length: 1.3 m, depth: 1 m) filled with soil (Fig. 1B). The bottom was connected to a tank via a polyethylene pipe that led to a leachate collector, which was accessible through a cellar for regular sampling (see Fig. 1B). A drainage fleece sheet was deployed between gravel and soil, acting as a filter for soil particles. At the top, lysimeters were connected to an automatic drip irrigation system that controlled the amount of water applied to the treatment. Soil water contents were monitored via soil moisture sensors (TERSO10, Meter Group AG, Munich, Germany) at soil depths of 25, 50 and 75 cm below ground.

Soil characterisation and experimental design

Soil characterization – The soil of this study was collected from the floodplain of the Red Main River near Bayreuth, Germany. The soil developed within a fluvial deposit, has no horizon structure and is classified as Fluvisol (IUSS Working Group WRB 2024). It has a neutral to slightly acidic soil reaction with moderate SOC and sandy loam texture (71.7% sand, 21.3% silt and 7% clay content, see Table 1).

Table 1
Baseline soil properties of the Fluvisol (Auenboden), obtained from the floodplain of the Red Main River (Bayreuth, DE). Values are averaged and given with standard error (n = 5).

Soil properties	Mean ± SE
pH (1:5, water)	6.5 ± 0.03
Total nitrogen (g kg^{-1} soil)	1.24 ± 0.01
Soil organic carbon (g C kg^{-1} soil)	14.88 ± 0.05
$\delta^{13}C_{bulk}$ [‰]	26.58 ± 0.12
$\delta^{15}N_{bulk}$ [‰]	5.33 ± 0.06
Clay content (%)	7.0 ± 0.16
Silt content (%)	21.3 ± 0.34
Sand content (%)	71.7 ± 0.03
Soil texture class	Sandy loam

Before launching the experiment, four composite soil samples were collected for baseline soil property analyses (Table 1). The soil samples were air-dried, sieved (2 mm mesh) and finely ground for chemical analysis. Soil pH was measured in soil-water mixtures (1:5) using a pH/mV meter (FiveEsy F 20, Mettler-Toldo GmbH, Switzerland). Total soil carbon (C), nitrogen (N), as well as their natural abundance isotope values ($\delta^{13}C$ and $\delta^{15}N$) were determined by coupled Element Analysis-Isotope Ratio Mass Spectrometry

(EA-IRMS, NA 1108, CE Instruments, Milano, Italy; Interface: ConFlo III, Finnigan MAT, Bremen, Germany to IRMS delta S, Finnigan MAT, Bremen, Germany). Soil particle size distribution was determined via an integrated suspension method using the PARIO device (UMS AG, Munich) as described by Durner et al. (2017).

Experimental design – Four treatments (i.e., optimum watered maize, moderate drought-stressed maize, optimum watered cup plant, moderate drought-stressed cup plant) were implemented in the presence of an unplanted control. Control lysimeters were kept under optimum moisture conditions, and no fertiliser was applied. As previously described by Abdalla et al. (2024), the soil moisture content was adjusted to a volumetric water content (VWC) of 25% and 12.5% to represent optimum moisture and moderate drought conditions, respectively. These values are equal to 100% (i.e., 25% VWC) and 50% (i.e., 12.5% VWC) of the soil water content at the field capacity, as determined from the initial soil water retention curve. The treatments were established in a randomised design, and replicated five times (25 lysimeters), with the moderate drought treatments kept together. The complete experiment lasted from 2019 to 2022. Note, however, that for this study the focus was set exclusively on the last two weeks of the growing season of 2020.

In 2019, ten lysimeters were planted with maize (*Zea mays* L.) within the first week of May, with 14 plants per lysimeter and a spacing of 32.5 cm between plants, and 28 cm between rows (i.e., plant density of 8 plants m^{-2}, approx. 80000 plants ha^{-1}). For the cup plant, ten lysimeters were planted with six cup plants (*Silphium perfoliatum* L.), with 55 and 40 cm spacing between plants and rows (plant density of 4 plants m^{-2}, approx. 40000 plants ha^{-1}). As a perennial plant, the cup plant grows as leaf rosettes in its first growing season. To ensure shoot formation, pre-planting was required in May 2019.

NPK(S) fertilizer (Complex 15 EG-fertilizer containing 15% N (6% NO_3^-, 9% NH_4^+), 15% P_2O_5, 15% K_2O, 7,5% SO_3, 0.01% Zn) was added (146 Kg N ha^{-1} total) in three dosages (i, same day of planting; ii, four weeks after maize sowing and iii, 3 to 4 weeks after dose ii) to maize and cup plants. After the plant's emergence in 2020, weeds inside or between the lysimeters were removed manually. Fertilisation, plant densities and spacing were, according to the Bavarian State Research Centre for Agriculture (LfL), Bavaria, Germany.

Seepage water samples – The seepage water drained in the leachate collector tank located underneath the lysimeters was recorded per lysimeter as leachate volume per litre (see also Fig. 1B). Homogeneous subsamples of about 250 mL were collected in polyethylene plastic bottles and transferred to the laboratory for chemical analyses. Subsamples were used to measure water pH with the above-mentioned pH/mV meter. Additionally, subsamples were filtered (0.45 µm PES filter syringes) to determine dissolved organic C (DOC) and dissolved inorganic C (DIC), as well as stable isotopes. Concentrations were analysed with a multi N/C 2100S instrument (Analytik Jena, Jena, Germany).

Soil respiration

Rhizomicrobial soil respiration measurements were taken every two weeks during the growing seasons. The measurement was conducted between 09.00 and 13.00 (CET) using a LI-COR 6400XT portable photosynthesis gas exchange system (LI-COR, Lincoln, NE, USA), equipped with a LI-COR 6400-09 soil respiration chamber (Norman et al. 1992, 1997). The closed chamber had an internal volume of 991 cm^3 and a surface area of 71.6 cm^2 (Healy et al. 1996). For measurement, the closed chamber was positioned on top of PVC collars (11.7 x 4.5 cm) that were inserted 2.5 cm into the soil and left another 2 cm above the soil surface. The PVC collars were inserted in the soil two weeks before the first measurements to eliminate the effect of soil disturbance. The measurements were performed by inserting two collars between three plants in each lysimeter, yielding a total of ten measurement points per treatment.

Carbon stable isotopes ($\delta^{13}C_{DIC}$ and $\delta^{13}C_{DOC}$) in water – Gasbench-CF-IRMS

To prevent biotic processes, samples were filtered (0.45 µm, Nylon) directly into 40 mL amber vials that were prepared with 20 µL of saturated $HgCl_2$ solution and were kept at 4°C in the dark until analysis. Analyses for DIC and DOC concentrations and their isotope values ($\delta^{13}C_{DIC}$ and $\delta^{13}C_{DOC}$) were carried out with an OI Analytical Aurora 1030W TIC/TOC analyser (OI Analytical, College Station, Texas), which was coupled in continuous flow mode to a Thermo Fisher Scientific Delta V Plus isotope ratio mass spectrometer (IRMS). The sample was reacted with 1 mL of 5% H_3PO_4 at 70°C for 2 minutes to turn DIC into CO_2. In a second step, 2 mL of 10% $Na_2S_2O_8$ were reacted with a subsample for 5 minutes at 98°C to oxidise DOC to CO_2. In both cases, the resulting CO_2 was purged from the solution by helium, cleaned, quantified by non-dispersive infrared (NDIR) and finally brought to the IRMS for isotope analyses. Details regarding the coupling between TIC/TOC analyser and IRMS are described in St-Jean (2003).

All values are reported in the standard permille-notation (‰) versus the Vienna Pee Dee Belemnite (VPDB) according to

$$\delta^{13}C = \left(\frac{R_{Sample}}{R_{Reference}} - 1 \right) * 1000$$

1

where R is the ratio of the numbers (n) of the heavy and light isotope of an element (e.g., $n(^{13}C)/n(^{12}C)$) in the sample and the reference (Coplen 2011). Data were corrected for instrumental drift during the run and for linearity. Repeat analyses of control standards yielded precisions better than ± 0.3‰ (1-σ).

Mass balance calculations

A mass balance was applied to assess the contribution of plant (C_4)-derived C to the DIC pool (Clark and Fritz 2013; Barth et al. 2017). The conceptual model and assumptions underlying this calculation are illustrated in Fig. 2 and are further described below.

Calculations were based on $\delta^{13}C$ endmember values of -28.5‰ for C_3 plants and − 12.5‰ for C_4 plants (Cerling et al. 1997; Marshall et al. 2008; Kirkels et al. 2022). During turnover, CO_2 from these endmember materials retains the same isotope value, however, during soil mitigation, it experiences diffusion enrichment in ^{13}C by 4‰ (Cerling et al. 1991; Clark and Fritz 2013). Consequently, adjusted $\delta^{13}C_{CO2}$ end member values become − 24.5‰ for C_3 and − 8.5‰ for C_4 plants (e.g., Marshall et al. 2008). According to Drever et al. (1998), relative proportions of temperature- and pH-dependent DIC species (i.e., CO_{2aq}*, HCO_3^-, and CO_3^{2-}) were determined. Subsequently, temperature-dependent equilibrium isotope values between endmember CO_2 and each species served to calculate individual $\delta^{13}C$ values (see Clark and Fritz 2013). With known relative proportions of each species, a theoretical $\delta^{13}C_{DIC}$ value for equilibration with CO_2 from either C_3 or C_4 plants was established. Our measured $\delta^{13}C_{DIC}$ values fell between these end members, allowing for the following mass balance to be formulated:

$$\delta^{13}C_{measured} = \frac{\left(\left(PC_{C4} * \delta^{13}C_{DICC4}\right) + \left(PC_{C3} * \delta^{13}C_{DICC3}\right)\right)}{(PC_{C4} + PC_{C3})}$$

2

Here, $\delta^{13}C_{DICmeasured}$ is the measured isotope composition of DIC, PC_{C4} is the proportion of C_4 contributions to the DIC pool, while $\delta^{13}C_{DICC4}$ refers to the assumed $\delta^{13}C_{DIC}$ isotope endmember for equilibration with CO_2 from C_4 material. Similarly, PC_{C3} is the relative proportion of C_3 contribution to the DIC pool, and $\delta^{13}C_{DICC3}$ is the assumed $\delta^{13}C$ isotope end member for equilibration with CO_2 by C_3 material. This simplification is justified by the short experiment duration, the low carbonate content of the soil, and minimal isotopic contrast between SOM and C_3 respiration, which would implicate their separation. Therefore, under the assumption that all DIC originates from either a C_3 or a C_4 source, we can postulate that PC_{C4} + PC_{C3} equals 1 (i.e., 100%). This results in: $PC_{C3} = 1 - PC_{C4}$(3).

When substituting Eq. (3) into (2), it can be rearranged for the per cent contribution of C_4 plants to the DIC pool with:

$$PC_{C4}\,[\%] = \frac{(\delta^{13}C_{DICmeasured} - \delta^{13}C_{DICC3})}{\left(\delta^{13}C_{DICC4} - \delta^{13}C_{DICC3}\right)} * 100$$

4

Statistical analysis

Normality testing of the experimental data was carried out using a Shapiro-Wilk normality test, confirming that the data is normally distributed ($p > 0.05$), except for the data of leachate amounts. Leachate amount data transformation did not improve data normality; therefore, the non-parametric Mann-Whitney U test was used for statistical analyses. A significant threshold of $p \leq 0.05$ was used for treatment mean comparisons and documented as mean ± standard error. For the statistical evaluation of the DIC and DOC isotope ratio and concentration data, Leven's test was employed to assess equality of variances, followed by the Shapiro-Wilk normality test, with a two-way ANOVA and Tukey test applied for significance ($p < 0.05$), using RStudio (RStudio 2023.06.0) for analyses and figures generated in Sigma Plot (Version 14.5, Systat Software Inc, Richmond, California, USA, 2021).

Results

DIC and DOC characteristics

The concentrations of dissolved inorganic C (DIC) and dissolved organic C (DOC) were measured, showing consistently higher levels of DIC (Fig. 3A) than DOC (Fig. 3B). While this pattern was consistent across all treatments and the unplanted control, it reflects a short-term (i.e., two-week) snapshot under controlled conditions in a carbonate-poor soil. Nevertheless, DIC levels exhibited slight variations, yet were significantly (p-value < 0.05) influenced by the type of plant (C_3 or C_4). Soil moisture did not impact DIC content (Fig. 3A). Conversely, DOC concentrations ranged between three to four times less than those of DIC. For DOC, higher soil moisture content is associated with reduced concentrations in both the C_3 and C_4 planted lysimeters (Fig. 3B). Despite this observation, the system response to DOC levels was notably consistent in both moderate-drought-stressed C_3 and C_4 lysimeters (Fig. 3B).

CO_2 efflux was generally higher at pH levels exceeding 7.5 (Fig. 4). When compared to the unplanted control, the soil pH measured in leachates from the lysimeters showed an increase greater than 6.5 ± 0.03 (Table 1) across all lysimeters (Figure S1). The presence of C_3 plants resulted in elevated pH values (> 7.5), whereas the controls and C_4 planted lysimeters exhibited slightly lower pH levels, ranging from 7.4 to 7.5. Additionally, increased soil moisture contents contributed to marginally higher pH values (Fig. 4). However, due to the high standard error associated with the pH measurements, a definite effect of soil moisture content on pH could not be discerned (Fig. S1).

To evaluate C consumption and turnover, DOC and DIC increments were assessed by subtracting the values of the controls from the final value of each treatment (Fig. 5). Optimum moisture levels facilitated microbiological activity, resulting in a net decrease in DOC. In contrast, lysimeters experiencing moderate drought stress showed an increase in DOC (Fig. 5A). The presence of C_3 plants appeared to enhance DOC consumption (p-value < 0.05), whereas DOC accumulation was more apparent in C_4-planted lysimeters (Fig. 5A). Additionally, at optimal moisture conditions, higher CO_2 efflux was associated with lower DOC concentrations (Fig. S2 B). Conversely, the accumulation of DIC was observed in all

lysimeters, with a more pronounced increase in the C_4-planted lysimeters. Notably, C_4 lysimeters displayed clear DIC accumulation regardless of the soil moisture content (Fig. 5A).

$\delta^{13}C$-based contribution of plant-derived C to the DIC pool

The build-up of DIC governed by C_4 plants could be further substantiated by isotope analyses of $\delta^{13}C_{DIC}$ (Fig. 6). The $\delta^{13}C_{DIC}$ values derived from C_4 sources are generally more enriched in ^{13}C with values around − 8.3 ± 0.3‰. This contrasts $\delta^{13}C_{DIC}$ values from C_3 plant-treated lysimeters with values around − 16.7 ± 0.3‰. In all treatments, only minor variations in $\delta^{13}C_{DOC}$ values were observed, on the order of ± 0.3‰ (Fig. 6A).

Maize plant-derived contributions to the overall DIC pool were determined with the above-described two endmember mixing model (Fig. 6B, see Methods for model assumptions). Here, lower $\delta^{13}C_{DIC}$ values are associated with a lower contribution to the DIC pool. In contrast, lysimeters with C_4 plants display more enriched $\delta^{13}C_{DIC}$ values, thus indicating a higher ^{13}C content. This C_4 signal contributed between 40% and 62% of C to the DIC pool (Fig. 6B).

Discussion

Leachate DIC concentrations consistently exceeded those of DOC across both C_3- (*Silphium perfoliatum* L.) and C_4- (*Zea mays* L.) planted lysimeters (Fig. 3). This contrasts with expectations based on SOC turnover, where plant litter and root exudates generally contribute, and thus increase, DOC concentrations (Pollierer et al. 2007; Gosling et al. 2013; Sokol et al. 2019). However, similar patterns of DIC > DOC have been reported in carbonate-rich soils (Kindler et al. 2011; Yamanaka 2012; Sun et al. 2021), suggesting that in certain systems, plant-derived CO_2 and buffering conditions can favour inorganic C formation even under low-carbonate conditions.

Plant-derived C inputs have been shown to occur rapidly within the rhizosphere through root respiration and microbial activity (Biernath et al. 2008; Pausch et al. 2013). Organic C is released as root exudates (Sokol et al. 2019; Wen et al. 2022; Lei et al. 2023) and can subsequently fuel microbial turnover, thus contributing to the overall soil CO_2 pool. Recently published studies (e.g., Kuzyakov et al. 2019; Sun et al. 2021) have shown that rhizosphere processes, including root and microbial respiration and acidification, promote DIC formation via increased CO_2 flux and soil solution alkalinity. These processes are particularly relevant in acidic soils, which commonly exhibit lower DIC concentrations (Kindler et al. 2011; Meesenburg et al. 2019), since their low buffer capacity would most likely amplify the effects of localized CO_2 production. Here, CO_2 originating from root and rhizomicrobial respiration, dissolves in soil water, leading to the formation of aqueous species such as carbonic acid and dissolved CO_2 (collectively referred to as CO_2*), as well as bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) (Clark and Fritz 2013). These

species reach equilibrium with the gaseous CO_2 present in the soil, thereby contributing to an overall $\delta^{13}C_{DIC}$ signal that is primarily governed by the gas phase.

Low DIC backgrounds of the carbonate-poor Fluvisol tested here, which exhibited an initial pH of 6.5 ± 0.03 (see Table 1), enabled insights into biologically induced inorganic C formation. Hence, pH values measured in leachates, ranging from 7.2 to 7.6 for C_3 and 7.5 to 7.9 for C_4 plants (Fig. 4), are most likely attributed to respiration-driven HCO_3^- formation, which, in turn, caused the observed pH increases (Poschenrieder et al. 2018). While higher pH was also associated with elevated CO_2 efflux, the precise contributions of plant, microbial, and faunal respiration to this pattern remain uncertain. Still, the combination of CO_2 outgassing and a limited buffering system likely explains the pH dynamics.

DOC concentrations were generally low, particularly in C_3-planted lysimeters, and may reflect plant adaptation to the prevailing moderate drought conditions (Ishida et al. 2015; Wang et al. 2020). Plants adjust root exudate composition and quantity under stress (e.g., Gargallo-Garriga et al. 2018), which may explain the elevated DOC concentrations detected in drought-treated lysimeters (Fig. 4B). Additionally, microbial consumption of DOC usually has a huge impact on soil DOC concentrations (Campbell et al. 2022); thus, lower soil microbial activity could result in the observed build-up of DOC. While soil microbial communities are known for their rapid response towards environmental fluctuations, alterations in temperature, soil moisture content and availability of C could, however, induce shifts in the microbial community with regards to their composition or function, and thus impact their overall performance (Fierer et al. 2007; Bian et al. 2022; Díaz-González et al. 2025). Hence, the more pronounced response in C_4-planted lysimeters may indeed reflect a physiological strategy to cope with water limitation. Although drought typically increases DOC losses (Ritson et al. 2017; Basile-Doelsch et al. 2020), the moderate drought conditions applied here may not have been severe enough to elicit a distinct stress response. Additionally, elevated temperatures during the main growing season accelerate DOC turnover (Werth and Kuzyakov 2009). In contrast, under optimum moisture conditions, C_3 plants showed low DOC but high CO_2 efflux and pH, suggesting higher C turnover under increased moisture conditions.

Natural abundance $\delta^{13}C_{DOC}$ ranged from − 28 to -30‰, with no significant differences between plant types or treatments (Fig. 6). The expected C_4 isotope signal (approximately − 12.5‰, (Kirkels et al. 2022)) was not evident in the DOC pool. This may be attributed to two factors: First, the larger mass of SOC is predominantly composed of C_3 plants, even after multiple growing seasons of C_4 plants; and second, the freshly produced DOC from C_4 plants may be rapidly converted into CO_2, which subsequently equilibrates with the existing DIC. In contrast, $\delta^{13}C_{DIC}$ values exhibited clear separation between C_3 and C_4-planted lysimeters, clustering around − 15 to − 20‰ and − 5 to − 10‰, respectively (Fig. 6). This was further supported by a simplified two-endmember model, which determined that up to ~ 62% of DIC was derived from C_4-plant sources. This estimate assumes negligible input from carbonate dissolution and minor SOM contribution—reasonable considering the carbonate-poor conditions and short experiment duration. While this simplified two-endmember model provides a useful first-order estimate of C_4-derived contributions, it cannot resolve potential overlaps with respired CO_2 from pre-existing SOM. Future work

incorporating radiocarbon or labelled ^{13}C tracers could help disentangle recent root inputs from background SOM mineralisation. Still, these results indicate that $\delta^{13}CDIC$ has the potential to reflect plant-derived C inputs over short timescales. However, the underlying processes may involve both root respiration and microbial priming of SOM (Werth and Kuzyakov 2009), although distinguishing between them was not possible here.

The observed DIC patterns point to a dynamic role of plant-derived C in soil mineralisation, even in shallow horizons and over short periods (weeks). Although long-term changes in total soil C were not measured here, the three-year study on the very same system conducted by Abdalla et al. (2024) reported only minor shifts in SOC despite high root inputs. Our findings suggest that part of the plant-derived C input may transiently contribute to DIC, thus influencing CO_2 emissions and leaching losses. Consequently, integrating DOC and DIC dynamics is essential for capturing the full C budget in soils, particularly under changing climate conditions. The exclusion of DIC from most C models could lead to underestimation of C losses and misinterpretation of soil C sink/source dynamics. Albeit not being designed to generalise beyond this system, our short-term experiment highlights that DIC fluxes can be substantial and isotopically traceable even within weeks, particularly in soils with low carbonate content and high biological activity. Thus, future work combining natural abundance and labelled isotope approaches could further disentangle root- versus microbial-derived DIC and clarify the stability and fate of this pool. Such knowledge is crucial for accurate C cycle modelling, greenhouse gas forecasting, and assessing soil contributions to climate regulation.

Conclusions & Outlook

Leachates obtained from a short-term lysimeter experiment conducted on a carbonate-poor soil revealed substantially higher concentrations of DIC compared to DOC across both C_3- and C_4-planted soils. These findings suggest that, under certain conditions, inorganic C formation via plant-driven respiration may be a more significant contributor to short-term soil C fluxes than commonly acknowledged, particularly in systems with low carbonate buffering capacity. Particularly in the C_4 treatment, enriched $\delta^{13}C_{DIC}$ signatures and elevated DIC concentrations underscore the responsiveness of the inorganic C pool to short-term plant-derived C inputs.

The implications of such findings are specific to the conditions examined here but have broader relevance for understanding C cycling in shallow soil systems. Hence, accurate assessments of soil C budgets must encompass the processes influencing the inorganic C pool, an undertaking of particular relevance in soils exhibiting fluctuating pH and limiting carbonate buffering. Our study emphasises the necessity of integrating short-term (seasonal) and long-term (multi-year) datasets to assess how transient DIC formation contributes to CO_2 fluxes and C sequestration potential. Future research should incorporate labelled isotope and radiotracers, centimetre-scale depth profiling, and diel sampling to better resolve the temporal and vertical dynamics of DIC. Coupling such methods with process-based modelling could help to clarify the fate and mobility of plant-derived inorganic C in soil systems, thus providing the representation of DIC in C cycle projections under changing climate conditions.

Declarations

Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Author contributions

JP and KA initiated the project. Field work was supervised by KA and JP. Laboratory analysis was conducted by KA and supported by JACB. Manuscript preparation was performed by ANV and KA. All experiments were designed, coordinated, conducted and executed by KA and JP. ANV interpreted the data provided by JACB, JP and KA, and prepared the manuscript with inputs from all co-authors.

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Data availability

We are currently working on archiving our data at the publicly accessible Zenodo data repository. A temporary file containing the data used for this manuscript is included as supplementary information file.

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Figures

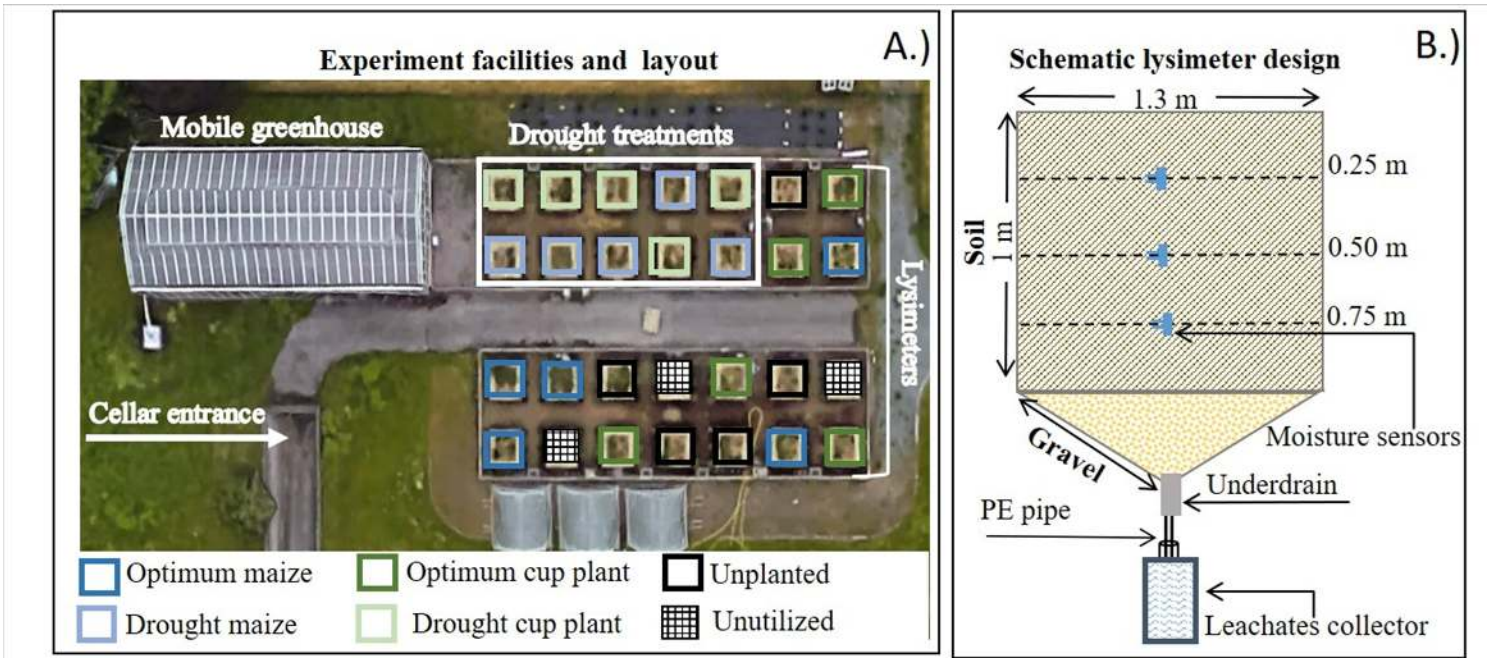

Figure 1

Field site overview and experimental setting. Arial view (Google Earth Pro) of the experimental facilities and treatments layout (A) and schematic lysimeter design in longitudinal section (B).

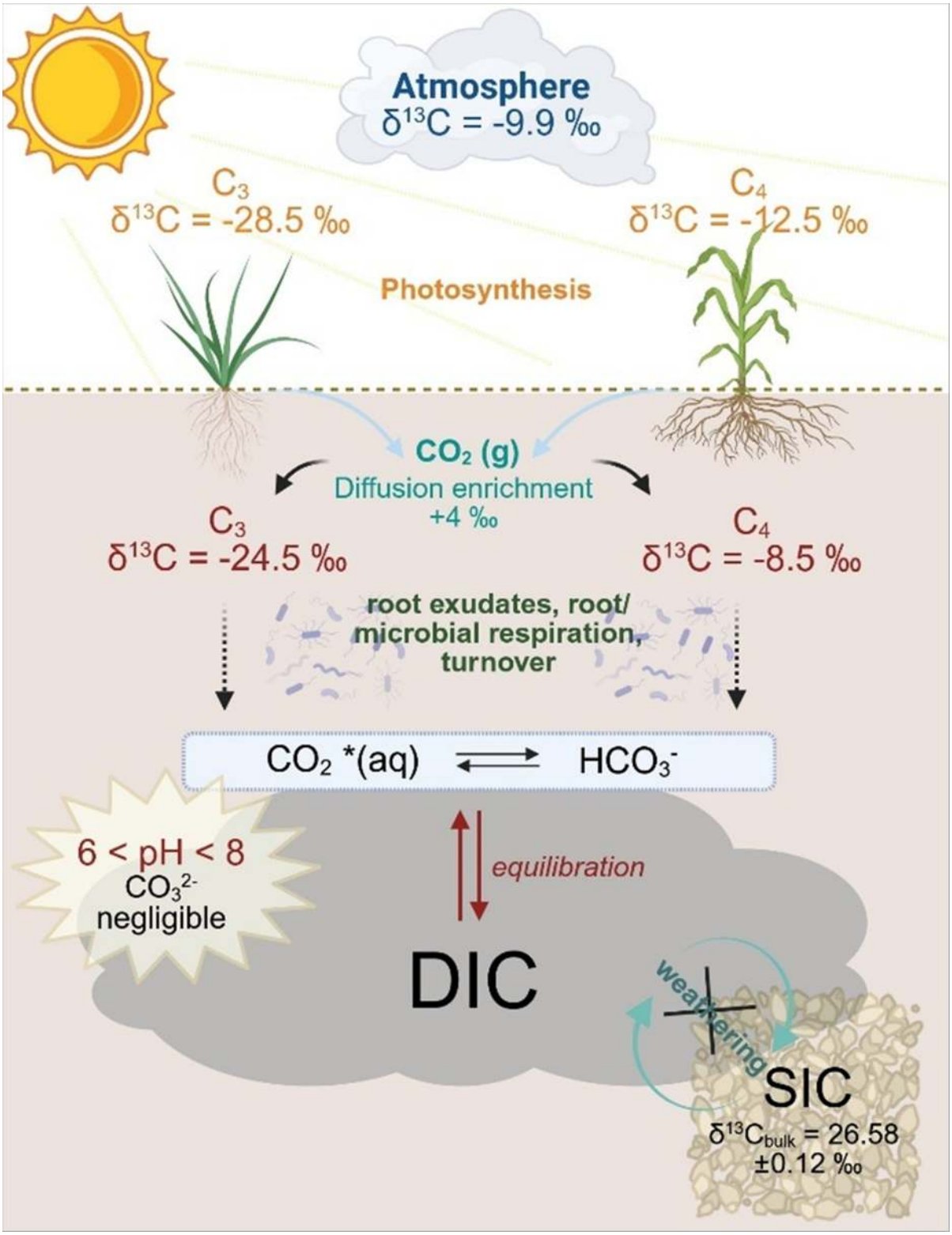

Figure 2

Conceptual model of the mass balance approach used to estimate plant-derived C contributions to soil dissolved inorganic C (DIC). The schematic outlines root respiration, microbial turnover, and CO_2 diffusion as contributors to the DIC pool, with $\delta^{13}C$ endmembers applied in a two-source mixing model. The approach assumes minimal input from carbonate weathering, justified by the carbonate-poor soil and short experimental duration.

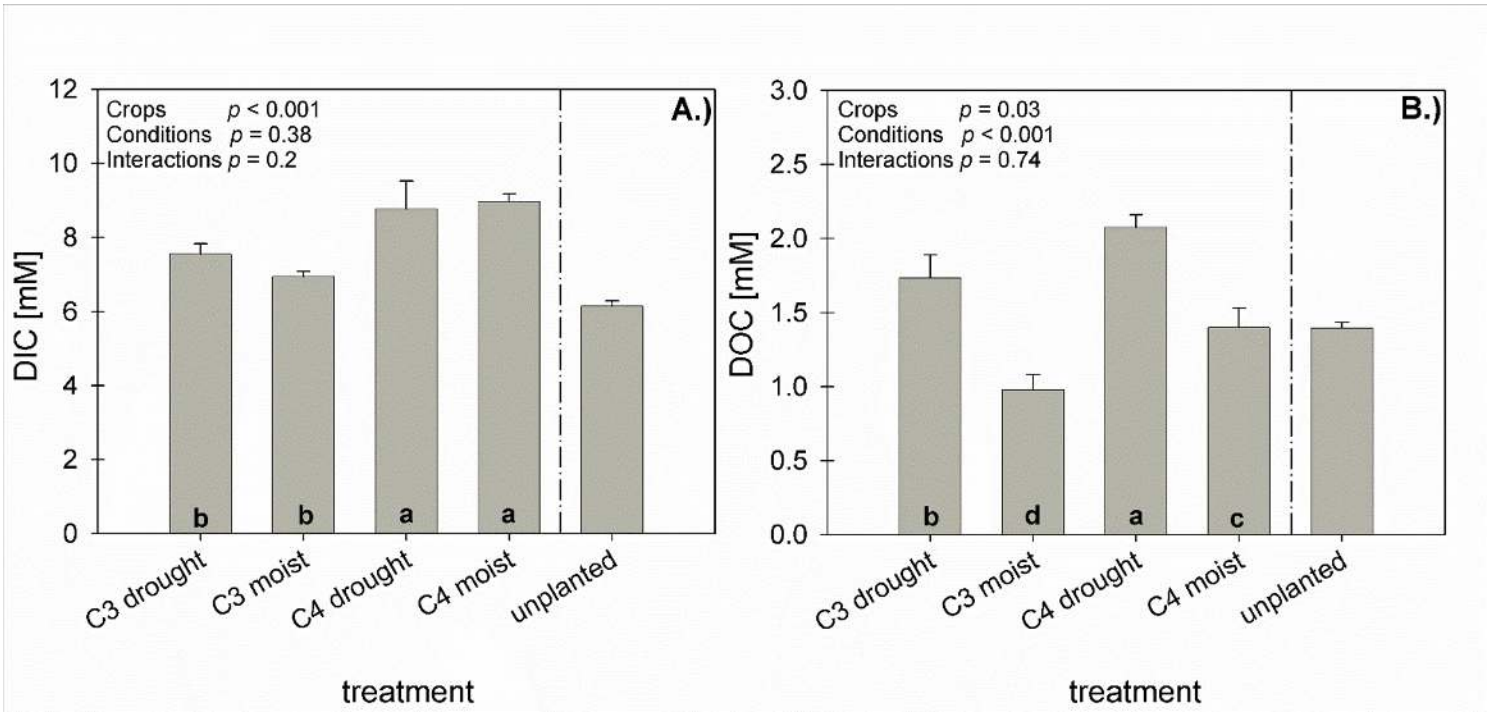

Figure 3

Seepage water DOC (A) and DIC (B) concentrations measured for all treatments and an unplanted control. Standard errors are depicted as error bars, and different letters indicate significant differences ($p < 0.05$, $n = 5$) between groups. p values result from two-way ANOVAs, excluding the control (dashed line). Note the different scales of the y-axes. Note that drought conditions mentioned in the graphs refer to a moderate drought treatment as described in the method section.

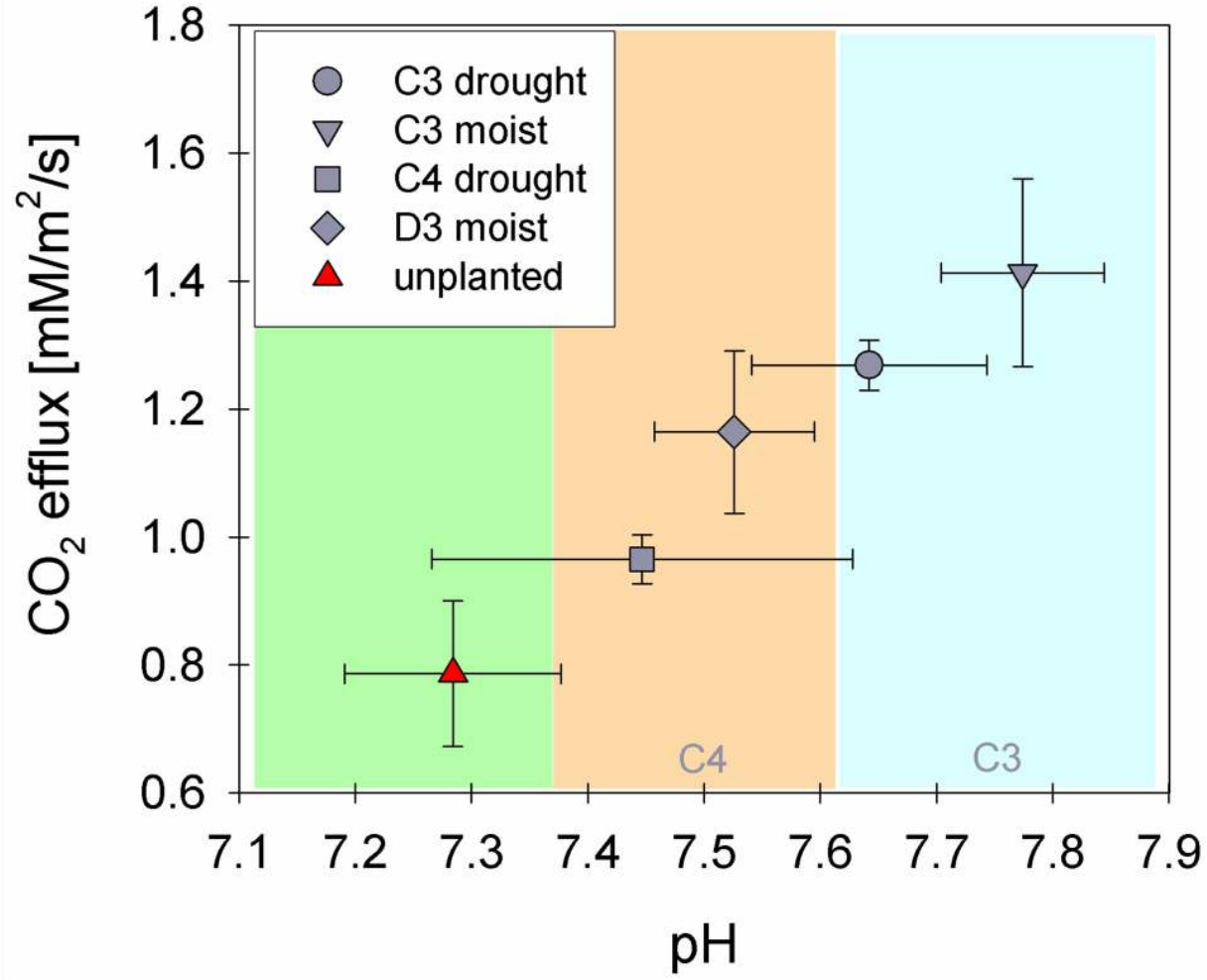

Figure 4

Soil CO_2 efflux in correlation to the seepage water pH. C_3-planted lysimeters (blue area) exhibited higher CO_2 flux and pH, while C_4-planted lysimeters were characterised by medium pH and CO_2 efflux (orange area). Lowest pH and medium to lowest CO_2 efflux were detected for the unplanted control (green area). Standard deviations for both CO_2 efflux and pH measured are depicted as error bars. Note that drought conditions mentioned in the graphs refer to a moderate drought treatment.

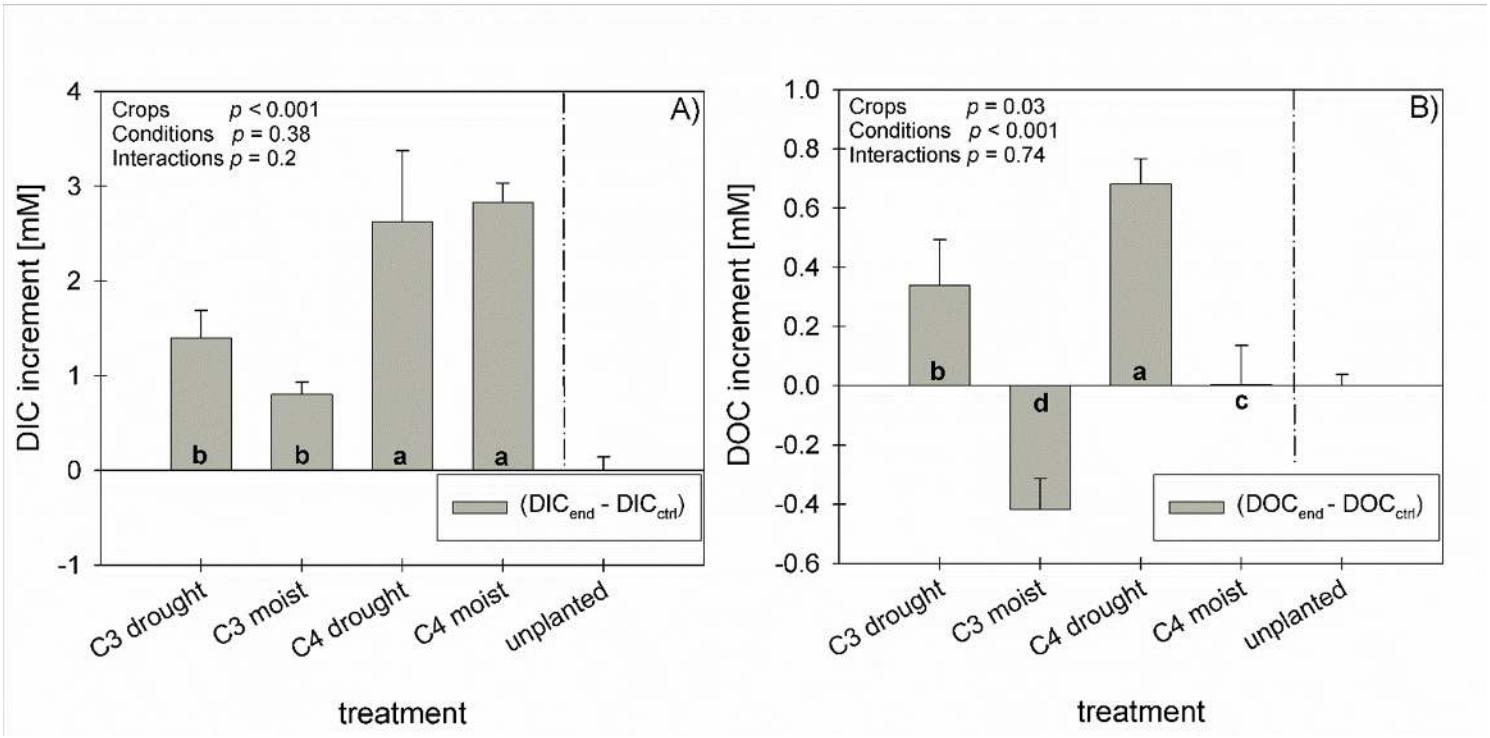

Figure 5

Carbon consumption evaluation by calculating DIC (A) and DOC (B) increments for each treatment. Standard errors are provided as error bars, and different letters indicate significant differences ($p < 0.05$, $n = 5$) between groups. p values result from two-way ANOVAs, excluding the control (dashed line). Note the different y-axis. Note that drought conditions mentioned in the graphs refer to a moderate drought treatment.

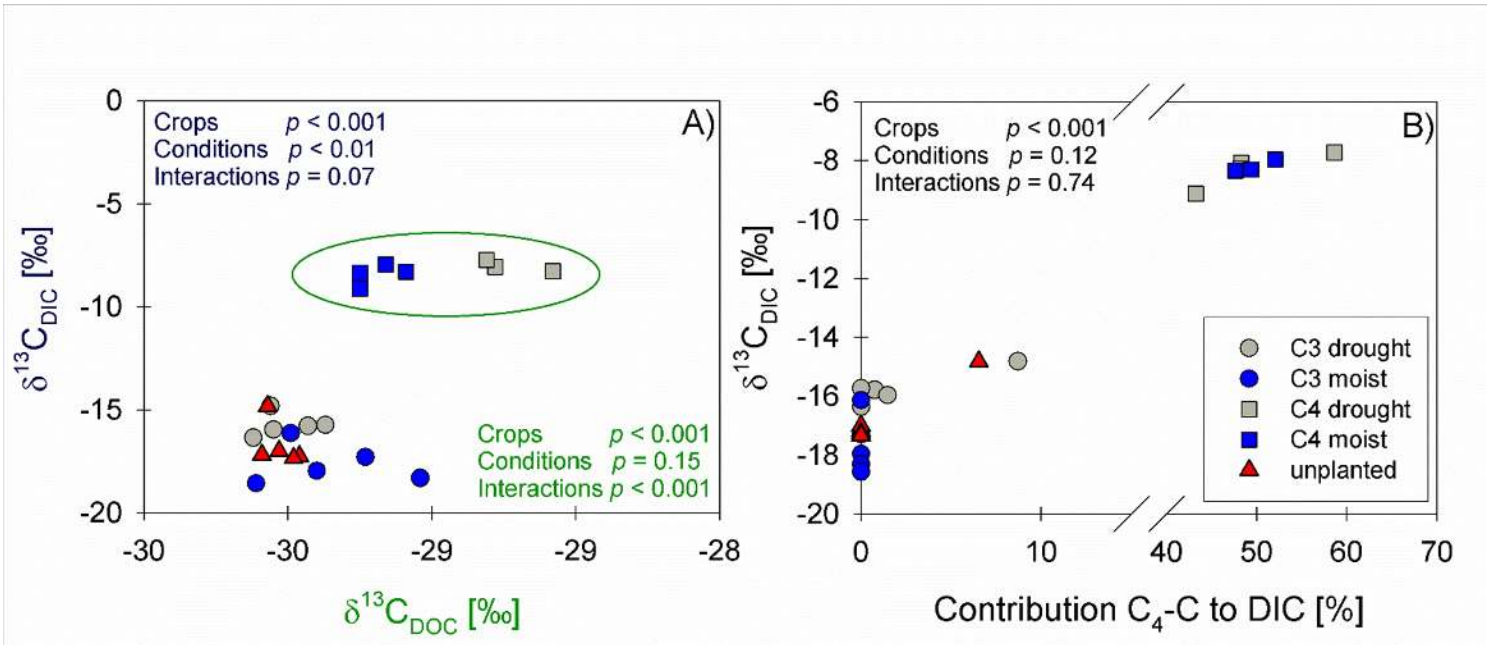

Figure 6

Evaluating the C_4 plant-governed built-up of DIC by plotting $\delta^{13}C_{DIC}$ versus $\delta^{13}C_{DOC}$ isotope ratios (A) and the percentage contribution (mass balance calculation-based approach) of C_4 plant-derived C to the $\delta^{13}C_{DIC}$ pool (B). Two-way ANOVAs were performed, and p-values were provided to evaluate the influence of plant type (crop), moisture conditions and the interaction between them. Note that drought conditions mentioned in the graphs refer to a moderate drought treatment.

Supplementary Files

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- DataAll202412new.xlsx
- SPSSuppInfoVisser.docx