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DOI

[10.1016/j.soilbio.2025.109731](https://doi.org/10.1016/j.soilbio.2025.109731)

Publication date

2025

Document Version

Final published version

Published in

Soil Biology and Biochemistry

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Citation for published version (APA):

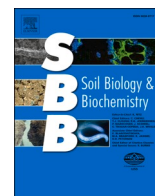
Hou, F., Hinojosa, L., Enderle, E., Jansen, B., Morriën, E., & de Vries, F. T. (2025). Root exudates from drought-affected plants increase soil respiration across a range of grassland species. *Soil Biology and Biochemistry*, 203, Article 109731. <https://doi.org/10.1016/j.soilbio.2025.109731>

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Root exudates from drought-affected plants increase soil respiration across a range of grassland species

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ARTICLE INFO

Keywords:

Drought
Root traits
Bacteria and fungi
Soil carbon cycling
Grasses
Forbs
Legumes

ABSTRACT

Root exudates play an important role in ecosystem carbon (C) cycling. Drought can alter the quality and quantity of root exudation, but it is not clear how root traits affect these changes, and what the implications are for soil C cycling. Seventeen common grassland species of three functional groups were subjected to a two-week drought followed by one week of recovery, after which root exudates were collected and analysed for their total C content, as well as for the respiration they triggered. Across all species but especially in legumes, drought increased specific root exudate-induced respiration rates. Both specific root exudation rate and specific respiration rate were positively correlated to root diameter and root nitrogen content, implying a link with “outsourcing” and “fast” strategies, and this correlation was strengthened after drought. These findings suggest that increased specific respiration rates as a result of drought-induced changes in root exudation is a plant strategy for coping with drought that may result in a loss of soil C after a drought has ended. These findings may help understand the impacts of drought on the capacity of soils to store C, and offer insight into the role of plants in this process.

1. Introduction

Root exudates are complex mixtures of molecules consisting of photosynthetically fixed high-quality carbon (C), such as sugars, amino acids, and organic acids (Whipps, 1990; Hamer and Marschner, 2005), which are released from roots into the soil. Altered root exudation is recognized as an important mechanism underlying the response of ecosystem C cycling to climate change – drought, elevated atmospheric CO₂ concentrations (Kuzuyakov, 2002a; Phillips et al., 2011), temperature increases (Yang et al., 2023) and nutrient imbalances (Kuzuyakov, 2002a). The C compounds in root exudates are key precursors of stable soil organic C (SOC) through efficient microbial processing, strong chemical bonding to the mineral soil matrix, and promoting aggregate formation (Bradford et al., 2013; Cotrufo et al., 2013; Sokol et al., 2019; Fossum et al., 2022). However, these labile dissolved C compounds can also stimulate microbial activity to increase the dissolution of minerals and accelerate soil organic matter (SOM) decomposition (“rhizosphere

priming effect”) (Kuzuyakov et al., 2000; Jones et al., 2004; Shahzad et al., 2015). Despite an increased understanding of the mechanisms through which root exudation affects SOC stabilisation and decomposition, we have limited understanding of which process – stabilisation or decomposition – dominates under which circumstances. Understanding this is key to predicting the consequences of changing environmental conditions for SOC content.

Recent experimental evidence shows that root exudation is linked to root traits and fits in the root economics space (Herz et al., 2018; Williams et al., 2022; Yin et al., 2023) both as an exploitative and an “outsourcing” trait (Iannucci et al., 2021; L. Sun et al., 2021; Williams et al., 2022; Rathore et al., 2023). The root economics space is defined by root strategies that help predict the responses of belowground plant economy to environmental ranges (Bergmann et al., 2020), and orthogonalizes a conservation axis (slow-fast plant resource acquisition strategies) and a collaboration axis (“do-it-yourself”-“outsourcing” plant resource uptake strategies) (Kong and Henry, 2019; Bergmann et al.,

Abbreviations: C, carbon; N, nitrogen; SOC, soil organic carbon; SOM, soil organic matter; SER, specific root exudation rate; SRR, specific (root exudate-induced) respiration rate; D, root diameter; SRL, specific root length; RNC, root nitrogen content; RTD, root tissue density; RCC, root carbon content; RDMC, root dry matter content.

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<https://doi.org/10.1016/j.soilbio.2025.109731>

Received 15 September 2024; Received in revised form 20 January 2025; Accepted 23 January 2025

Available online 25 January 2025

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2020). Along the slow-fast conservation axis, plants gradually reduce the cost of root construction by reducing root tissue density, and instead accelerate the metabolism of nitrogen (N) in root exudates and increase root nitrogen content (Comas et al., 2014; Erktan et al., 2018; McCormack and Iversen, 2019; Wen et al., 2022). Along the collaboration axis, root exudation reflects C allocation from phloem to roots (Canarini et al., 2016). “Do-it-yourself” plants invest more C in extending thin roots to explore soil resources themselves, while “outsourcing” plants exude C for attracting beneficial organisms or provide a larger cortex fraction and expand root diameter to facilitate colonisation by mycorrhizal fungi (Comas et al., 2014; B. Liu et al., 2015; H. Li et al., 2017; Guyonnet et al., 2018; McCormack and Iversen, 2019; Wen et al., 2022; Williams et al., 2022; Yin et al., 2023).

Drought is increasing in many regions as a result of global climate change and has widespread and pervasive impacts on ecosystems and their functioning (Pörtner et al., 2022). Grasslands cover ~40 percent of the Earth’s surface and contribute significantly to global SOC stocks (Scurlock and Hall, 1998; Song et al., 2018; Y. Sun et al., 2021). Under drought, grassland species reduced their growth but can invest more in root biomass and particularly in fine roots, which help them scavenge for water (Anjum et al., 2011; Guo et al., 2017; Liu et al., 2017). These impacts vary among plant functional groups, indicative for their position in the root economics space and their drought-coping strategy. Grass and forb species decrease specific root length due to reduced extension of fine roots under drought stress (Chapin et al., 2002; G. Zhou et al., 2018) but increase root tissue density, which helps store energy and maintain tissue integrity (De Vries et al., 2016; Reinelt et al., 2023). Drought differentially affects root diameter in legumes — *Medicago lupulina* has been shown to increase root diameter, while *Trifolium repens* exhibited a decrease (Lozano et al., 2020), but legumes generally retain thicker roots, allowing for mycorrhizal colonisation that supports efficient nutrient and water (Brundrett, 2002; Brundrett and Tedersoo, 2018). Consequently, drought shifts the root economics space across plant functional groups: grasses and forbs shift from “do-it-yourself” to “slow” strategies, and legumes show a tendency toward “outsourcing” strategy. Moreover, grasses, forbs, and legumes differ in their root exudate quantity and quality. Forbs and legumes generally release more root exudation than the grasses, and forbs and legumes also release more N-rich compounds, such as flavonoids and amino acids (Paynel and Cliquet, 2003; Fustec et al., 2010; Williams et al., 2022) than grasses with simple sugars (e.g., glucose and fructose) and low levels of organic acids (Williams et al., 2022). Thus, distinct growth and root investment responses in response to drought likely affect the quantity and quality of root exudation differently in grasses, forbs, and legumes.

Root exudation is responsive to changing environmental conditions, such as drought (Williams and De Vries, 2020; Chen et al., 2022), warming (Xiong et al., 2023), flooding (Meng et al., 2022), elevated CO₂ levels (Dong et al., 2021) and grazing (G. Sun et al., 2017; Wilson et al., 2018; Shen et al., 2020). Evidence is increasing that the amount and composition of root exudates are affected by drought (Canarini et al., 2016; Gargallo-Garriga et al., 2018; Preece et al., 2021; Staszal et al., 2022). Extreme drought stress generally decreases total root exudation C but increases root exudation per gram dry root (e.g. in the crop *Helianthus annuus* (Canarini et al., 2016), the grass *Holcus lanatus* and forb *Rumex acetosa* (De Vries et al., 2019) and the tree *Quercus petraea* Matt. (Sessile Oak) (Staszal et al., 2022) due to less photosynthetic C fixation and high photosynthetic C allocation to exudates rather than root biomass production under drought stress. Drought also affects the composition of root exudation through increasing the concentration of abscisic acid, organic acids (malic, lactic, aspartic, and fumaric acids) and amino acids (proline) (Song et al., 2012; Gargallo-Garriga et al., 2018; Chen et al., 2022; Jiang et al., 2023). These changes in root exudates in response to drought help adjust osmotic potential and maintain water retention (Song et al., 2012; Canarini et al., 2016; Z. Li et al., 2017; Chen et al., 2022), but also help solubilize minerals from soil for plant uptake (Hoffland et al., 1992; Wang et al., 2000; Henry et al.,

2007). Thus, root exudation is a plant strategy that helps plants cope with, and recover from, drought, but we do not know what the consequences of this altered root exudation are for soil C cycling.

Drought continues to disrupt ecosystem C cycling by reducing plant photosynthesis and C inputs, which diminishes plant productivity and triggers cascading effects throughout the ecosystem (Van der Molen et al., 2011; Lei et al., 2016; Sippel et al., 2018). As drought persists, microbial activity declines, limiting microbial physiological responses (including microbial death and cell lysis) to moisture stress and slowing SOC decomposition (Schimel et al., 2007; Cook and Orchard, 2008; Wang et al., 2014; Schimel, 2024). However, the role of root exudates in SOC cycling challenges this established view. Plants continuously release root exudates under drought (Karlowsky et al., 2018) that can maintain microbial activity and SOC decomposition (Chen et al., 2022). For example, malic acid increases soil microbial respiration and promotes the decomposition of SOC, thus causing a priming effect (Chowdhury et al., 2014). Moreover, altered root exudation can continue to affect processes of soil C cycling after a drought has ended. When soil is rewetted, microbial activity resumes and causes a flush in C and N mineralization (“the Birch effect”) (Birch, 1958). Root exudation contributes to this fast reinitiation of soil microbial activity and the pulse of CO₂ measured as specific respiration rates, which continues for weeks after rewetting (Karlowsky et al., 2018; Canarini et al., 2019; De Vries et al., 2019; Williams and De Vries, 2020). However, we do not know how drought affects root exudation rates across a range of plant species, what the implications are for specific respiration rates, and whether the strength of this response can be predicted based on the root economics space.

Here, we tested how drought affects root exudation rates across a range of plant species, and what the implications are for soil microbial respiration. We did this by growing 17 common grassland species under greenhouse conditions and subjecting them to drought vs. well-watered conditions before extracting their root exudates after a recovery phase and assessing specific root exudate-induced respiration rates. The 17 species were chosen to represent grasses, forbs, and legumes, covering the root economics space and covering a gradient from a “do-it-yourself” to an “outsourcing” strategy and from “slow” to “fast” (Williams et al., 2022). We expected the position of species in the root economics space to both explain the response of root exudation to drought, as well as the specific respiration rate that these root exudates trigger. We hypothesized that: (1) legumes have the highest root exudation rates because of their “outsourcing” strategy, (2) root exudates from droughted plants increase specific respiration rates across plant functional groups but especially in legumes, and (3) root exudation rates and specific root exudate-induced respiration rates link to the root economics space, and specifically with the “outsourcing” strategy.

2. Materials and methods

2.1. Experimental design

We purchased a clay loam soil (SOM = 5.5 ± 1.5%, pH = 7.75 ± 0.24, soil moisture = 16.7 ± 0.2%) (Den Ouden Group company, The Netherlands) on the December 18, 2019. Soil was sieved (4 mm mesh size), homogenized and stored at 4 °C until the start of the experiment. We selected 17 common grasslands species with a wide distribution across Europe, representing plant functional groups of grasses, forbs and legumes (Table S1). From the April 1, 2021, plants were germinated from seeds (ordered from Cruydt-Hoeck Seeds, The Netherlands) and planted out in the prepared soil in plug trays in the greenhouse of University of Amsterdam. After two weeks of growth, individual seedlings were randomly selected and transplanted into 640 ml trapezoidal pots (9 cm long and wide at the top, 7 cm long and wide at the bottom and 10 cm height) filled with 600 g fresh soil (soil moisture 16.7%). Each plant species was subjected to a drought and a control treatment. With five replicates per treatment, pots were arranged in a randomised block

design, with a staggered start with a ten-day gap for each block to allow enough time for harvest and lab analysis. Overall, 170 pots were set up (= seventeen species \times two treatments \times five replicates). Plants were watered every one or two days by maintaining weight to 60% of soil water-holding capacity (WHC) by calculating soil moisture contents at 100% WHC during the first 10 growing weeks. Then, soil WHC of drought treated plants was adjusted to 20% (equivalent to 7.3% gravimetric soil moisture) compared to 60% (equivalent to 21.9% gravimetric soil moisture) of control (Fig. S7). After two weeks of treatment, droughted plants were rewatered to the original moisture content (60% WHC), which was maintained for one week until plants and soil were destructively harvested. Previous work showed that the effect of drought on the soil respiration triggered by root exudates was most pronounced after plant recovery (De Vries et al., 2019). Three plants subjected to drought, *L. vulgare* in the first and fifth blocks and *T. repens* in the second block, did not survive during the rewater period after drought treatment and we removed these three plants in subsequent experimental steps and statistical analyses.

2.2. Root exudate collection

Each plant with intact roots and soil was gently removed from its pot. Rhizosphere soil was carefully shaken off, sieved to 2 mm mesh, and stored at 4 °C for later soil analysis. We collected root exudates following a previously developed hydroponics-hybrid method (De Vries et al., 2019; Williams and De Vries, 2020; Williams et al., 2021). Briefly, roots were carefully cleaned of adhering soil and plant residues using flowing water and tweezers and instantly transferred into a 100 ml SCHOTT glass bottle (DURAN®) containing 100 ml hydroponics solution. Hydroponics solution was made by weighing 100 g soil into 1000 ml MilliQ water, stirring for 2 h and filtering through 0.2 mm mesh. Bottles with plants were moved to a climate chamber (16 h of light at 22 °C and 8 h of dark at 20 °C, humidity 65%) for a week. All bottles were aerated to standardize growth conditions for each species, and hydroponics solution was replaced every two days (Fig. S8). This step can avoid pathogens to develop (Oburger and Jones, 2018) and offset chemical composition bias of root exudates caused by the stress and damage during root washing manipulation (De Vries et al., 2019; Williams and De Vries, 2020; Williams et al., 2021). Thus, combined with one week of recovery after drought in the greenhouse, root exudates were collected two weeks after ending the experimental drought. Then, roots were rinsed with MilliQ water several times and inserted into 100 ml SCHOTT

2.3. Root exudate concentration standardization and root exudate-induced respiration measurements

Root exudate samples were prepared for root exudate-induced respiration analysis as previously described in De Vries et al. (2019). Briefly, freeze-dried root exudates were resuspended and standardized to the targeted concentration (100 $\mu\text{g C ml}^{-1}$) through adding different volumes of sterile MilliQ water. These prepared root exudate solution samples and MilliQ water samples for standardization (2 ml) per block were stored at -80 °C until root exudate-induced respiration measurements. Next, we weighed 0.3 g unconditioned soil (in which no plants were grown) into each allocated position in a 96-well deepwell plate (12114172, Thermo Scientific™ Abgene™), and deepwell plates with soil were incubated at 20 °C for a week to homogenise soil moisture, avoiding potential local differences in soil moisture caused by water vapor circulation during storage, and restore soil microbial activities (see plate design per block in Fig. S1). We used unconditioned soil because in a previous experiment, soil conditioning history did not affect root exudate-induced respiration (De Vries et al., 2019) and because we wanted to isolate the effect of the root exudates. Then, indicator solution was prepared and fully integrated with agar in a microwave following the Microresp™ manual (Microresp™, UK) (Campbell et al., 2003) (<https://www.microresp.com/>), after which 250 μl agar solution was pipetted into clear detection plates (11349163, Thermo Scientific™ Sterilin™). After cooled and solidified, the light intensity of detection plates was absorbed at 570 nm on a spectrophotometer (601–0668, SPECTROstarNano, BMG LABTECH), recorded as Absorbance_{t1}. Then, 150 μl standardized root exudate containing 15 $\mu\text{g C}$, as well as sterile MilliQ water samples, were vortexed and pipetted into deepwell plates with six technical replicates (see plate design per block in Fig. S1). The detection plate was turned over to cover the deepwell plates connected by a Microresp™ seal. The whole Microresp™ equipment was then clamped to guarantee airtightness. After a 6-h incubation at 20 °C, detection plates were removed, and their light intensity was re-absorbed on the spectrophotometer and recorded as Absorbance_{t2}. Two absorbance readings (Absorbance_{t1} and Absorbance_{t2}) were matched to calculate root exudate solution-induced respiration and MilliQ water sample-induced respiration. Specific root exudate-induced respiration rate (SRR ($\mu\text{g CO}_2\text{-C} (\mu\text{g C added})^{-1} (\text{g dry soil})^{-1} \text{h}^{-1}$)) was defined as net soil microbial respiration rates induced per unit root exudation per gram dry weight soil per hour and calculated as formula 1:

$$\text{SRR} = \frac{\text{Root exudate solution-induced respiration } (\mu\text{g CO}_2\text{-C}) - \text{MilliQ water-induced respiration } (\mu\text{g CO}_2\text{-C})}{\text{Root exudate C } (\mu\text{g C}) \times \text{Dry soil weight } (\text{g}) \times \text{Incubation time } (\text{h})} \quad (1)$$

bottles containing 100 ml MilliQ water. Then, bottles with plants were shaken on ice to minimise turnover of collected exudates for 2 h at 60 rpm in light (Fig. S8). We removed plants from bottles and cut off the fresh roots, which were kept fresh in 20% ethanol solution for root traits analysis (Freschet et al., 2021). Collected root exudates were distributed through 0.2 μm filter paper (10401712, Whatman™, UK) into three 50 ml centrifuge tubes (227261, Greiner) evenly. The volume of each tube was recorded to calculate total root exudation. One of the three tubes of root exudate was used to analyze total organic carbon content (TOC) on a TOC analyzer (TOC-V CPH, SHIMADZU). The other two exudate samples were freeze-dried at -50 °C (CoolSafe™, Den Hartog SCIEN-TIFIC). All tubes with freeze-dried root exudate samples were stored at -80 °C before root exudate standardization.

Here, root exudate solution-induced respiration represents the total amount of C respired by each root exudate sample dissolved in MilliQ water sample per well of the deepwell plates; MilliQ water-induced respiration represents the total amount of C respired by each MilliQ water sample per well of the deepwell plates with a volume equivalent to that of the root exudate solution sample; Root exudate C represents the amount of root exudate C contained in each root exudate solution sample added per well of the deepwell plates. For this study, the root exudate C was uniformly standardized to 15 μg per well; Dry soil weight represents dry weight of soil per well of the deepwell plates; Incubation time represents the duration for which the root exudate solution samples and MilliQ water samples were incubated in the sealed Microresp™ system. In this study, the incubation time was uniformly set to 6 h, which was established as a standard duration.

$$\text{Total root exudate-induced respiration (TR } (\mu\text{g CO}_2\text{-C (g dry soil)}^{-1}$$

h^{-1}) was defined as the total amount of C respired by the total collected root exudate C by a single plant and was calculated by multiplying specific root exudate-induced respiration rate by total root exudation (formula 2).

$$\text{TR} = \text{SRR} \left(\mu\text{g CO}_2\text{-C} \left(\mu\text{g C added} \right)^{-1} \left(\text{g dry soil} \right)^{-1} \text{h}^{-1} \right) \times \text{Total root exudation (mg C)} \times 1000 \quad (2)$$

Here, SRR represents the result derived from formula 1; Total root exudation represents the total collected root exudate C by a single plant; 1000 is used to convert the unit mg to μg .

2.4. Root trait analyses

After root exudate collection, shoot dry biomass for each plant was weighed after drying at 70 °C for 48 h. Fresh roots were carefully spread out in demineralized water on a transparent tray (25 cm × 30 cm) and detected based on grey level using an Epson flatbed scanner (STD4800, REGENT). WINRHIZO® ROOT ANALYSIS software was used to analyze root other traits, including root diameter (D mm), total root length (cm) and root volume (cm^3). After this, we blotted fresh roots dry using paper towel and weighed dry biomass (g) after drying at 70 °C for 48h. Dry roots were ground using a ball mill (ZM 200 Retsch®), and root carbon content (RCC %) and root nitrogen content (RNC %) were determined using an Elemental analyzer (vario EL CUBE). Specific root length (SRL mm g^{-1}) was calculated as total root length divided by root dry biomass. Root tissue density (RTD g cm^{-3}) was calculated as root dry biomass divided by root volume. Root dry matter content (RDMC) was calculated as root dry biomass divided by root fresh biomass. Total root exudation was calculated as the total amount of C exuded per single plant during a 2-h root exudation collection, and specific root exudation rate (SER mg C g^{-1}) was defined as the amount of C exuded per unit root dry biomass and was calculated by dividing the total root exudation by root dry biomass. Specific root exudate-induced respiration rate (SRR ($\mu\text{g CO}_2\text{-C} \left(\mu\text{g C added} \right)^{-1} \left(\text{g dry soil} \right)^{-1} \text{h}^{-1}$)) was also defined as a root trait in this study.

2.5. Soil analyses

Water dissolved C and nutrients were extracted by shaking 15 g fresh soil sample with 40 ml MilliQ water in a 50 ml centrifuge tube at 150 rpm for 2 h. Soil samples were filtered through 0.45 μm filter papers (0424A00023, Fisher Scientific) using filter boxes. Part of filtered samples measured dissolved organic C in the TOC analyzer (TOC-V CPH, SHIMADZU) and the remaining was determined for water-dissolved nutrients (NO_3^- , NH_4^+ and PO_4^{3-}) in the Auto Analyzer (SAN⁺⁺ SYSTEM, SKZLAR). Plant available nutrients (NO_3^- , NH_4^+ and PO_4^{3-}) were extracted by shaking 4 g fresh soil in 20 ml 1 M KCl at 150 rpm for 1 h. KCl extracted samples were filtered through 0.45 μm filter papers and diluted 10 times before measurement using the Auto Analyzer (SAN⁺⁺ SYSTEM, SKZLAR).

2.6. Statistic analyses

First, we tested whether our data were normally distributed by inspection of density plots, QQ plots and Shapiro-Wilk test ($p > 0.05$) using the package gridExtra (Auguie, 2017), and log-transformed variables that did not meet these criteria. To assess the impact of drought and plant functional group and their interaction on root traits, total root exudation, specific root exudation rates, raw root exudate solution-induced respiration and soil properties, we fitted lmer linear mixed effects models with fixed effects of drought treatment (Treatment) and plant functional groups (FunctionalGroup) and random effects for block and plant species in the packages Matrix (Bates and Maechler, 2009), lme4 (Bates et al., 2015) and lmerTest (Kuznetsova et al., 2017) in R, and checked the residuals. We also analyzed the

interactive effects of treatment and plant functional groups on total root exudate-induced respiration and specific respiration rates using lmer linear mixed effects models, this time with three independent random factors, namely pot nested in each block where root exudates originated, the deepwell plate nested in each block and plant species.

Root traits were normalized by \log_{10} -transformation and auto-scaled for data standardization to perform principal component analysis (PCA) using the packages ggplot2 (Wickham, 2016), factoextra (Kassambara and Mundt, 2020), dplyr (Wickham et al., 2023) and ggpubr (Kassambara, 2023). Permutational multivariate analysis of variance (PERMANOVA) was used to detect differences in root traits in response to treatment and plant functional groups using the package vegan (Oksanen et al., 2022). Linear regression models were fitted in the packages ggplot2 (Wickham, 2016), Matrix (Bates and Maechler, 2009), lme4 (Bates et al., 2015), lmerTest (Kuznetsova et al., 2017), tidyverse (Wickham et al., 2019) and mlmtools (Jamison et al., 2022) for testing how individual root traits predict specific root exudation rates and specific respiration rates. All analyses and figures were done in with R v.4.3.1 (2023-06-16 ucr) (R Core Team, 2023) and RStudio v. 2023.03.0–386.

3. Results

3.1. Effects of drought and plant functional group on plant biomass and soil C and nutrients

Drought strongly reduced shoot and root dry biomass across plant functional groups, and this reduction was strongest in legumes (Treatment: Functional group interaction, $F_{2,143.1} = 13.1$, $p < 0.0001$ for shoot dry biomass; Treatment: Functional group interaction, $F_{2,143.1} = 5.2$, $p = 0.0064$ for root dry biomass; Fig. 1).

Plant growth did not affect the concentration of soil dissolved organic carbon, but drought increased the concentration of plant available nitrate (Treatment: Functional group interaction, $F_{2, 143.3} = 2.4$, $p = 0.0098$) in legumes and phosphate in grasses (Treatment: Functional group interaction, $F_{2, 143.2} = 3.6$, $p = 0.0306$; Table S3).

3.2. Effects of drought and plant functional group on root exudation and root exudate-induced respiration

Total root exudation was reduced by drought (Main effect of Treatment $F_{1, 143.3} = 1.5$, $p < 0.0001$; Fig. 2a) (Main effect of Treatment $F_{1, 143.1} = 19.2$, $p < 0.0001$; Fig. 2a) but did not differ between plant functional groups. However, drought increased specific root exudation rates in legumes (Treatment: Functional group interaction, $F_{2, 143.1} = 3.7$, $p = 0.0283$; Fig. 2b).

Root exudates from droughted plants induced higher specific respiration rates from soil compared to those from control plants across all 17 species, and more so in forbs and legumes than in grasses (Treatment: Functional group interaction, $F_{2, 71.6} = 9.8$, $p = 0.0002$; Fig. 3). Total root exudate-induced respiration was higher in legumes than forbs followed by grasses (Main effect of Functional group, $F_{2, 15.0} = 4.0$, $p = 0.0396$; Fig. S3).

3.3. Links between root traits and specific root exudation rates and specific respiration rates

Plant functional groups clearly differed in their root traits (Main effect of Functional group, $F_1 = 190.5$, $R^2 = 0.5368$, $p = 0.001$; Fig. 4). Grasses had the highest specific root length in both control and drought treatments (Treatment: Functional group interaction, $F_{2, 143.1} = 6.0$, $p = 0.0032$; Table S2; Fig. S2c). Forbs displayed high specific root exudation rates (Fig. 4), and legumes were related to root diameter (Treatment: Functional group interaction, $F_{2, 143.1} = 13.8$, $p < 0.0001$; Fig. 4; Table S2; Fig. S2a) and root nitrogen content (Treatment: Functional group interaction, $F_{2, 143.1} = 7.2$, $p = 0.0011$; Table S2; Fig. S2d).

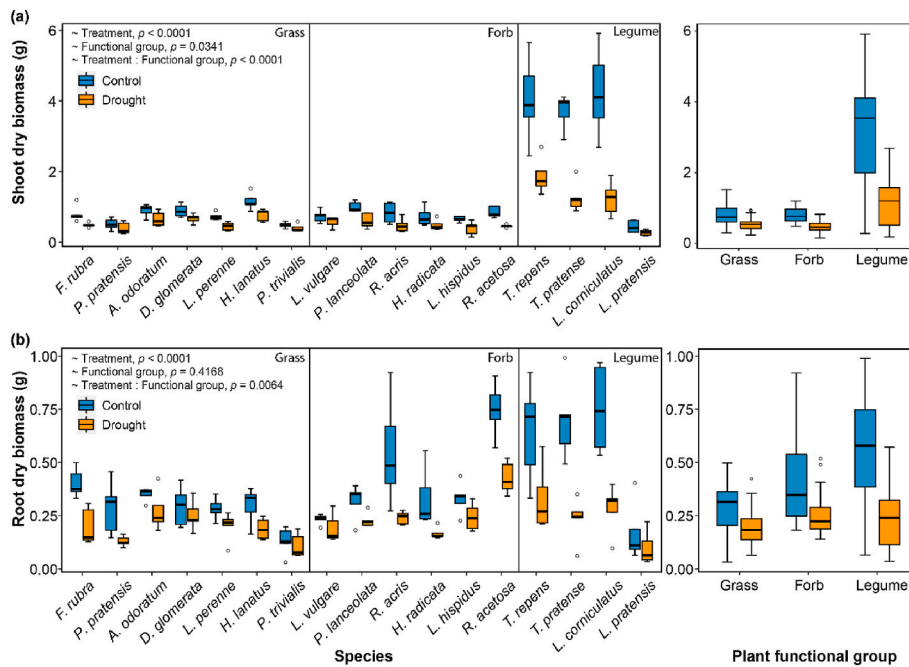


Fig. 1. Shoot dry biomass (a) and root dry biomass (b) for each species as affected by treatment and plant functional groups (grasses, forbs and legumes). Blue and orange boxes represent control and drought treatments, respectively. The central line in boxes marks the median value of the data, and dots placed past the line edges are outliers. See [Supplementary Table S1](#) for Latin names of 17 plant species. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

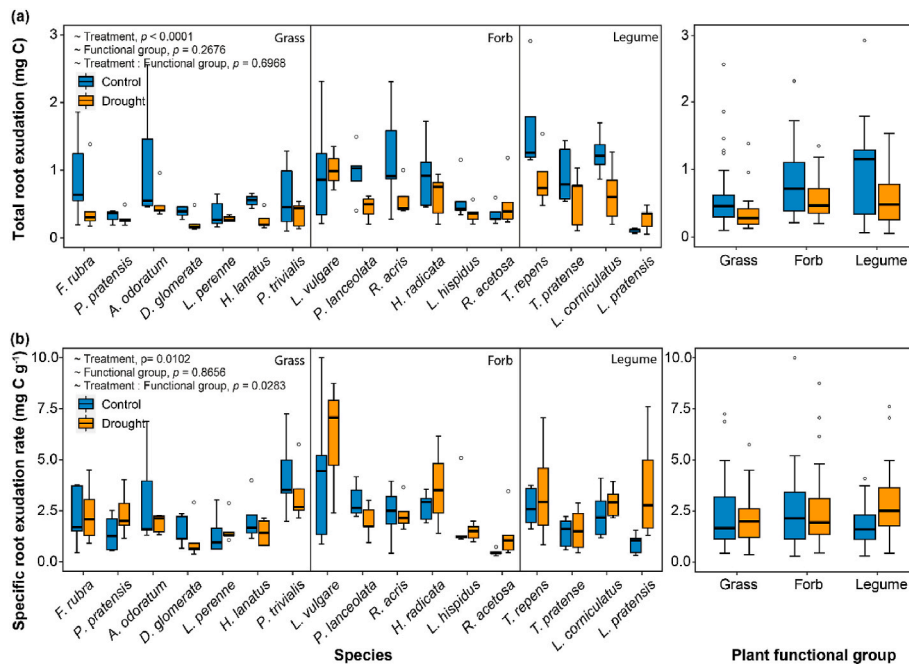


Fig. 2. Total root exudation (a) and specific root exudation rate (b) for each species as affected by treatment and plant functional groups (grasses, forbs and legumes). Blue and orange boxes represent control and drought treatments, respectively. The central line in boxes marks the median value of the data, and dots placed past the line edges are outliers. See [Supplementary Table S1](#) for Latin names of 17 plant species. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Drought affected the relationship between individual root traits and specific root exudation rate. Drought strengthened the positive relationship between root diameter and specific root exudation rate (root diameter: Treatment interaction: $R^2 = 0.0409$ explained by fixed effect, $R^2 = 0.5273$ explained by fixed and random effect, $F_{1, 144.3} = 10.0$, $p = 0.0019$; [Fig. 5a](#)) and between root nitrogen content and specific root

exudation rate (root nitrogen content: Treatment: $R^2 = 0.1513$ explained by fixed effect, $R^2 = 0.6243$ explained by fixed and random effect, $F_{1, 147.2} = 9.9$, $p = 0.0020$; [Fig. 5b](#)). Drought also changed the relationship between specific root length and specific root exudation rate from positive in control plants to negative in droughted plants (specific root length: Treatment interaction: $R^2 = 0.0788$ explained by

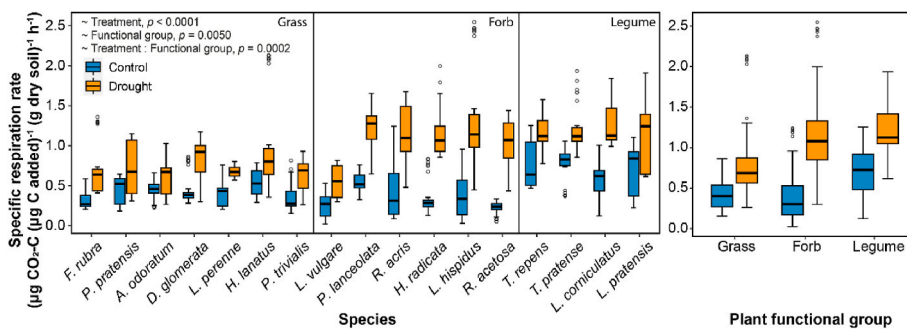


Fig. 3. Specific root exudate-induced respiration rate for each species as affected by treatment and plant functional groups (grasses, forbs and legumes). Blue and orange boxes represent control and drought treatments, respectively. The central line in boxes marks the median value of the data, and dots placed past the line edges are outliers. See [Supplementary Table S1](#) for Latin names of 17 plant species. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

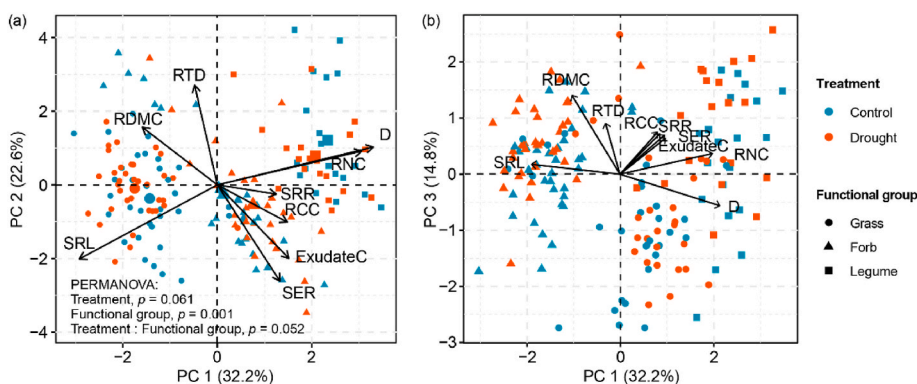


Fig. 4. PCA plots with PC1 and PC2 (a) and with PC1 and PC3 (b) of root traits as affected by treatment and plant functional groups (grasses, forbs and legumes). Symbols represent plant functional groups. Blue and red dots represent individual observations of control and drought treatments. Arrows indicate projections for each individual root trait. Abbreviations: D, root diameter (mm); SRL, specific root length (cm g^{-1}); RTD, root tissue density (g cm^{-3}); RNC, root nitrogen content (%); RCC, root carbon content (%); RDMC, root dry matter content; SER, specific root exudation rate (mg C g^{-1}); Exudate C, total root exudation (mg C); SRR, specific respiration rate ($\mu\text{g CO}_2\text{-C } (\mu\text{g C added})^{-1} (\text{g dry soil})^{-1} \text{h}^{-1}$). See [Supplementary Fig. S1](#) for root traits analysis for each species. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

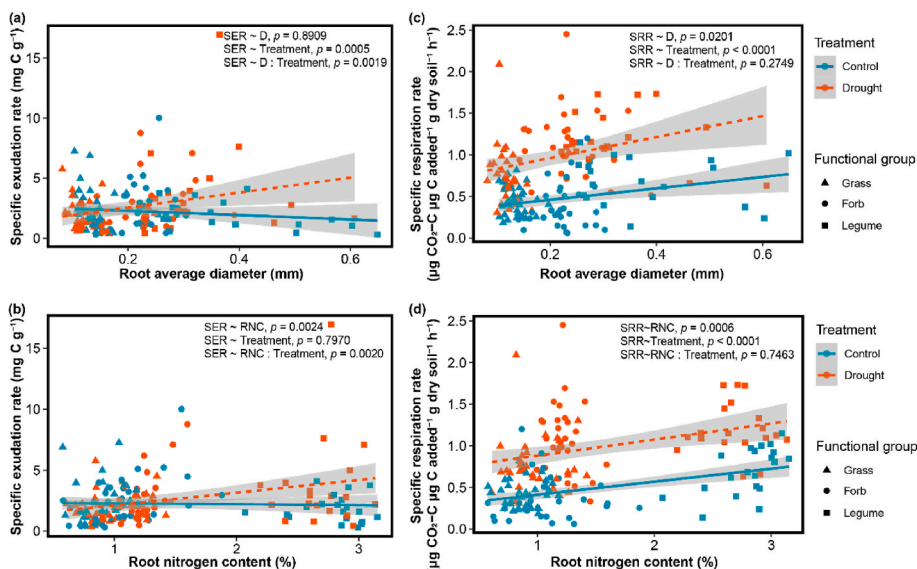


Fig. 5. Relationships between root traits and specific root exudation rate (a, b) and specific respiration rate (c, d) as affected by treatment and plant functional groups (grasses, forbs and legumes). Blue and red dots represent individual observations of control and drought treatments, respectively. Lines and shading represent linear regression and 95% confidence interval. See the linear table in [Table S4](#) for the full model output. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

fixed effect out of $R^2 = 0.5641$ explained by fixed and random effect, $F_{1, 144.7} = 16.5, p = 0.0001$; Fig. S6a) (See the linear table in Table S4 for the full model output).

Specific respiration rate was positively related to root diameter (Main effect of root diameter: $R^2 = 0.3680$ explained by fixed effect, $R^2 = 0.5872$ explained by fixed and random effect, $F_{1, 19.9} = 6.4, p = 0.0201$; Fig. 5c) and root nitrogen content (Main effect of root nitrogen content: $R^2 = 0.4186$ explained by a fixed effect, $R^2 = 0.5950$ explained by a fixed and a random effect, $F_{1, 20.0} = 16.5, p = 0.0006$; Fig. 5d) in both control and drought treatments. Specific respiration rate was not related to specific root length (Fig. S6e; Table S4) (See the linear table in Table S4 for the full model output).

4. Discussion

We set out to test how specific root exudation rates and the specific respiration rates triggered by root exudates are altered by drought, and whether this response is consistent across plant functional groups. We hypothesized that specific root exudation rates would be highest in legumes and would be reduced by drought, and that exudates from drought-recovered plants trigger higher specific respiration rates, especially in legumes. We also expected that specific respiration rates would be linked to the root economics space; specifically, we hypothesized that root exudates from roots with high root diameter (i.e. linked to the “outsourcing” strategy) would trigger the highest specific respiration rates. We found that specific root exudation rates were increased by drought across all species, and more so in legumes. In line with our hypothesis, drought increased specific respiration rates across all 17 species, and again especially in legumes. Specific respiration rates were positively correlated to root diameter and negatively correlated to specific root length, thus confirming our hypothesis that specific respiration rate is not only part of the “outsourcing” strategy but also of the “fast” strategy. These findings suggest that alterations in root exudation in response to drought are a plant strategy with implications for soil C cycling after drought.

4.1. Drought increases specific root exudation rates particularly in legumes

Total root exudation was reduced by drought across all plant functional groups, but specific root exudation rates were increased, especially in legumes. Legumes were also the plant functional group that was reduced in root dry biomass most under drought (Carlsson et al., 2017; Lozano et al., 2019). Our findings suggest that the increased specific root exudation rate is compensating for the loss of total root exudation as a result of root biomass reduction. This increase in specific root exudation rates might be a result of the accumulation of metabolic compounds in roots due to a slower phloem transport during drought, which are then flushed out of the plant after rewetting (Canarini et al., 2016; Hikino et al., 2022). The quick flush of root exudates can help moisten the soil and help plants recover from drought. For example, legumes tend to allocate more C in their roots and alter hormones that increase root cell wall permeability to intercept N and reduce N losses from the system (Brophy et al., 1987), which can help legume to take up soil N under drought (Qiao et al., 2022). The limited response of grasses to drought, both in terms of root dry biomass and specific root exudation rates, may be because grasses maintain tissue water content through the loss of root dry biomass and total root length (Fig. S2b), and these two processes may balance out to maintain fine root investment (Brunner et al., 2015; Da Silveira Pontes et al., 2015; Liu et al., 2020; Keep et al., 2021; Chandregowda et al., 2022). Similarly, drought likely did not affect total root exudation and specific root exudation rates of forbs because forbs are less sensitive to environmental changes due to their ability to store nutrients in their roots (Herz et al., 2017; Dietz et al., 2020). Combined, these findings suggest that legumes in particular alter their root exudation to overcome drought stress, potentially for stimulating

microbial activity or attracting beneficial microbes.

4.2. Increased specific root exudate-induced respiration rates after drought may have implications for SOM

We found that across all 17 species, drought did not affect total root exudate-induced respiration but increased specific respiration rates, and more so in forbs and legumes than in grasses (Fig. S3 and Fig. 3). These findings are consistent with the study of De Vries et al. (2019), with a similar set up but only including two species, and our finding indicates that increased specific respiration rates triggered by droughted root exudates occurs across a range of species. Moreover, total root exudation decreased under drought, but specific respiration rates increased, suggesting that the increased respiration per unit C compensated for the loss of total C exuded. We suggest that drought-altered root exudation is an active plant strategy to overcome adverse environmental conditions through stimulating microbial activities. While we did not measure the root exudate profiles of different plant species after drought, our findings clearly show that drought alters specific root exudate-induced respiration, i.e. the amount of respiration triggered by the same amount of root exudate, which can only stem from differences in root exudate composition. We suggest further research to identify the changes in root exudate composition in response to drought across plant species or functional groups, as well as to explore the broader implications of these alterations.

Drought was previously found to decrease plant N uptake and microbial N immobilization and nitrification, leading to an accumulation of available N in the soil (Austin et al., 2004; Fisk et al., 2015), which was supported here by an increase in soil nitrate concentrations under drought (Table S3). During recovery after drought, a quick flush of root exudates may reinitiate rhizosphere microorganisms (i.e. saprotrophic fungi and bacteria) (Karlowsky et al., 2018), leading to increased N mineralization rates (Kuzyakov, 2002a; Karlowsky et al., 2018) and a respiration pulse (Kuzyakov, 2002b; Ingrisch et al., 2020). Moreover, legumes have a stronger positive effect on rhizosphere microbial respiration than forbs and grasses, induced by their N-rich root exudates (such as ureides and amino acids in white clover) (Paynel and Cliquet, 2003; Fustec et al., 2010; Williams et al., 2022). Therefore, legume exudates may limit microbial C but increase microbial N availability. This imbalance in stoichiometry induced by legume exudates can thus stimulate microbial activity in the rhizosphere and are compensated by microbial respiration and nutrients mineralization (Cheng and Coleman, 1990; Paungfoo-Lonhienne et al., 2017; Wobeng et al., 2020; Pausch et al., 2024). This mechanism explains our finding that increased specific respiration rates in response to drought, especially in legumes, compensate for the loss in total root exudation (Fig. 2a; Fig. 3) (Canarini et al., 2016; De Vries et al., 2019). Moreover, in conjunction with our finding, droughted root exudates resulted in more C release than was added (i.e. a priming effect at specific respiration rates $>1 \mu\text{g C added}$). While without the use of ^{13}C -labelled root exudates we cannot reliably assess the priming of SOM, we unequivocally find that across a range of plant species setup in a short-term pot experiment, root exudates from plants that have experienced drought trigger more specific respiration rates and thus stimulate microbial activity, with potential consequences for soil C content formation and loss. Our finding that this increased respiration triggered by root exudates persisted for at least two weeks after drought, raises the question for how long this increased respiration persists, and what the implications are for SOM dynamics after drought.

4.3. Specific root exudation rates as well as specific root exudate-induced respiration rates are part of the “outsourcing” and “fast” root strategies

We found that specific root exudation rates increased with root diameter and root nitrogen content. A high mean root diameter implies a larger proportion of cortex and is recognized to be part of the “outsourcing” strategy that relies on mycorrhizal fungi colonisation for

foraging nutrients (Kong et al., 2014; Paterson et al., 2016; Ma et al., 2018; Bergmann et al., 2020; Sweeney et al., 2021). Drought reduced root diameter, potentially weakening the “outsourcing” strategy and reducing the colonisation by arbuscular mycorrhiza. However, specific root exudation rates and specific respiration rates were increased, which points to a likely strategy of drought-altered root exudates re-activating root-associated microbiome activities and compensating for the reduction of root diameter (Santana et al., 2020; Chen et al., 2022). For example, drought has been shown to increase the concentration of strigolactones and flavonoids in root exudates, which stimulate fungal growth (Besserer et al., 2006; Nakabayashi et al., 2014; Gargallo-Garriga et al., 2018; Li et al., 2019). Drought also increased the concentration of flavonoids in *Quercus ilex* root exudates to tolerate drought (Nakabayashi et al., 2014; Gargallo-Garriga et al., 2018), and biochanin A and formononetin (flavonoids) were reported to stimulate hyphal growth in *Trifolium repens* (Nair et al., 1991). Similarly, root nitrogen content is linked to the “fast” strategy in the root economics space (Kong et al., 2019; Bergmann et al., 2020; Ding et al., 2020) and indicates fast plant root metabolic activities (Reich, 2014; Tang et al., 2019). Recent studies have reported that root nitrogen content is closely related with high root exudation rates (L. Sun et al., 2017, 2021; Jiang et al., 2023). Moreover, root nitrogen content has been found to be correlated to specific root exudate compounds, such as certain sugars, amino acids and organic acids (malic acid, benzoic acid and possibly succinic acid) (Y. Liu et al., 2015; Guyonnet et al., 2018; Canarini et al., 2019; Williams et al., 2022). Drought was also found to increase root exudation rates of sugars and amino acids to maintain cellular osmotic pressure (Javot and Maurel, 2002; Martínez-Vilalta and García-Fórner, 2017; Gargallo-Garriga et al., 2018), and increased exudation rates of organic acids (such as malic acid and oxalic acids), which can interact with drought-resistant plant-growth-promoting bacteria to enhance drought tolerance and recovery (Allard-Massicotte et al., 2016; Santana et al., 2020; Jiang et al., 2023). Thus, our results suggest that the specific root exudation rate is part of the “outsourcing” and “fast” root strategy, which can potentially aid plant recovery after drought.

Specific respiration rates increased with root diameter and root nitrogen content, which is in line with previous findings (M. Zhou et al., 2018; Han et al., 2020; Henneron et al., 2020; Han and Zhu, 2021; Chao et al., 2023). M. Zhou et al. (2018) found that across 15 temperate grassland species root respiration rates were positively correlated with average root diameter and negatively with specific root length. Chao et al. (2023) found that mean diameter of first-order roots was the main driver of the rhizosphere priming effect through C allocation to mycorrhizal fungal symbionts and through high root exudation and derived respiration in thick roots. While these studies highlight the importance of mycorrhizal fungi for the priming effect, we excluded this mechanism by directly adding root exudates to the soils, and still observed a positive relationship with root diameter, most likely through altered root exudate composition. The positive relationship between root nitrogen content and specific respiration rate is in line with results reported by Han et al. (2020), M. Zhou et al. (2018), Tang et al. (2019) and Han and Zhu (2021), who found that the rhizosphere effect on SOM decomposition was positively correlated with the root nitrogen concentration, reflecting the intensity of the plant root metabolism. Moreover, specific root exudate compounds associated with root nitrogen content can contribute to the rhizosphere priming effect: drought increases the amounts of organic acids (malic acids, oxalic acids, fumaric acids) and amino acids (ABA, aspartic acid and leucine) released into soil (Allard-Massicotte et al., 2016; Gagné-Bourque et al., 2016; Gargallo-Garriga et al., 2018; Sasse et al., 2018). These compounds provide energy and stimulate microbial activity and exoenzyme production; and lead to high metabolic rates and a high rhizosphere priming effect (Kuzayakov, 2002a; Cheng, 2005). Overall, these findings suggest that the rhizosphere priming effect may be related to the root economics space and linked to both the “outsourcing” strategy as well as to the “fast” strategy.

5. Conclusion

Taken together, our results show that drought decreases total root exudation but increases specific root exudation rates in all 17 species of three plant functional groups, suggesting that this is a general pattern in grassland species and implying that altered root exudation may be the embodiment of a plant strategy to cope with drought (De Vries et al., 2019; Williams and De Vries, 2020). Root exudates from plants that experienced drought triggered higher specific respiration rates, showing that these root exudates stimulate microbial activity and may have consequences for soil C formation and loss. Importantly, this increased respiration induced by root exudates was linked to “outsourcing” as well as to “fast” strategies in the root economics space, and drought strengthens this relationship. These findings underline that root exudation is part of the root economics space, and that we may be able to predict the consequences for soil C cycling based on functional groups or root traits.

CRedit authorship contribution statement

Fangbin Hou: Writing – review & editing, Writing – original draft, Visualization, Methodology, Funding acquisition, Conceptualization. **Leonardo Hinojosa:** Writing – review & editing, Resources. **Eileen Enderle:** Writing – review & editing, Resources. **Boris Jansen:** Writing – review & editing. **Elly Morriën:** Writing – review & editing. **Franciska T. de Vries:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Data availability

The raw data and code used in this study are provided in figshare (<https://doi.org/10.21942/uva.26083612>).

Declaration of competing interest

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

Acknowledgements

We would like to acknowledge the support provided by Faculty of Science in University of Amsterdam throughout this project. We thank Eva de Rijke, Rutger van Hall and Michel van Son for assistance with all lab analysis and Ludek Tikovsky for greenhouse management. This research was funded by the scholarship under the State Scholarship Fund of China (File No. 202006300028). Franciska T. de Vries was supported by ERC-StG SHIFTFEEDBACK (851678).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.soilbio.2025.109731>.

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