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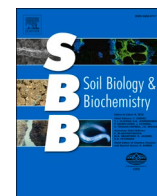
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Plant species specific effects of root exudates on the formation and destabilization of soil organic matter

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ABSTRACT

Root exudates are labile organic carbon (C) compounds released by plants into soil that can stimulate both the decomposition and formation of soil organic matter (SOM) through enhancing microbial activity. Drought alters root exudation, potentially shifting the balance between the loss and formation of SOM fractions of particulate organic matter (POM), which often turns over faster in soil, and mineral-associated organic matter (MAOM), with a comparatively slower turnover. Here, we tested how root exudates from three grassland species, *Lolium perenne*, *Ranunculus acris*, and *Trifolium pratense*, grown under drought and well-watered conditions, affect soil microbial activity, community composition, and SOM distribution over POM and MAOM. We collected and repeatedly applied exudates to unconditioned soils over five months. We found that root exudates from *R. acris* and *T. pratense* promoted the decomposition of POM and concomitantly increased MAOM formation. Root exudates from drought-stressed *R. acris* and *T. pratense* did not affect POM and MAOM pools but enhanced microbial respiration, potentially through increased microbial nitrogen (N) limitation, as evidenced by an increased C/N ratio in POM, or through reduced microbial C use efficiency. These findings advance our understanding of the dual role of root exudates in the loss and formation of SOM pools and the potential effect of drought on these processes.

1. Introduction

Soil is the largest terrestrial carbon (C) pool, storing C as soil organic matter (SOM) (Raich and Potter, 1995; Batjes, 1996; Schlesinger, 1997; Jobbágy and Jackson, 2000; Schmidt et al., 2011). The formation of SOM largely derives from plant C inputs, with the formation rate often attributed to plant litter quality (Monreal et al., 1997; Cotrufo et al., 2013; Roth et al., 2015). Plant litter undergoes a transformation from recalcitrant to labile forms, progressively accelerating the formation of SOM (Cotrufo et al., 2013; Kallenbach et al., 2016). Root exudates, composed of highly decomposable labile plant-derived OM compounds such as sugars, organic acids, and amino acids, are released into the soil and play a key role in mediating interactions with soil microbes (Kuzyakov, 2002a; Narula et al., 2012; Bradford et al., 2013; Huang et al., 2014; Pantigoso et al., 2025). By stimulating distinct microbial processes, root exudates influence both the liberation and formation of SOM (Kuzyakov, 2002a; Narula et al., 2012; Bradford et al., 2013; Huang et al., 2014; Na et al., 2025). Root exudates provide a readily bioavailable supply of energy, such as simple sugars, for the

decomposition of native soil C, and specific compounds, such as oxalic acids, promote C loss by liberating organic compounds from protective associations with minerals (term as “rhizosphere priming effect”) (Kuzyakov, 2002a, 2002b; Keiluweit et al., 2015). However, root exudates also favour the microbial biosynthesis of stable SOM (Bradford et al., 2013; Cotrufo et al., 2013) through sorption to soil mineral surfaces and binding with soil cations such as calcium, magnesium, and potassium (K) and metals such as iron and aluminium (Hättenschwiler and Vitousek, 2000; Hunt et al., 2007; Cheynier et al., 2013). Oppositely, a recent study reports that high-quality plant litter, causes less efficient formation of mineral-associated SOM when the soil microbial community has a low C use efficiency (Elias et al., 2024) or is limited by N (Na et al., 2025). Thus, root exudates can trigger opposing processes, but it remains unclear which mechanism dominates under which conditions, and what the consequences are for SOM dynamics.

SOM can be separated into different physical fractions based on their origin (Smith et al., 2002; Angst et al., 2021) and the process of their formation and mineralization (Cambardella and Elliott, 1992; Christensen, 2001; Sollins et al., 2006; von Lützow et al., 2007; Lavalley et al.,

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2020; Cotrufo and Lavalley, 2022). Particulate organic matter (POM) is largely made up of partially decomposed plant residues (Six et al., 2001; Kögel-Knabner et al., 2008) with generally a relatively short residence time (less than ten years to decades) (Lavalley et al., 2020). POM enters the bulk mineral soil mainly through fragmentation and partial decomposition of plant litter into smaller sized particles and molecules (Wershaw, 1993; Piccolo, 2001; Sutton and Sposito, 2005), a process also driven by rapid microbial turnover (Leifeld and Kögel-Knabner, 2005) that can be inferred by measuring soil respiration rates (Alvarez and Alvarez, 2000). Mineral-associated organic matter (MAOM) is mainly formed by mineral adsorption of relatively low molecular weight compounds (Lehmann and Kleber, 2015) with generally a longer residence time in soil (decades to centuries) (Lavalley et al., 2020). Low molecular weight compounds are important components in root exudates, but can also leach from plant litter, or be produced by exoenzyme depolymerization of plant litter and associate directly with the mineral phase (the “ex vivo” modification pathway) (Sanderman et al., 2014; Liang et al., 2017). Low molecular weight compounds can also be produced by the “in vivo” microbial turnover pathway whereby microbiota decompose and transform organic material, resulting in necromass or exudates which are then incorporated into MAOM (Sanderman et al., 2014; Liang et al., 2017). Therefore, plant litter as well as root exudates can directly and indirectly affect POM and MAOM formation via microbial activity. However, the specific role of root exudates in regulating the dynamics of POM and MAOM remains unclear.

Grasslands cover ~40 percent of the Earth's surface and contribute significantly to global SOC stocks (Scurlock and Hall, 1998; Song et al., 2018; Sun et al., 2021). Grasses, forbs and legumes have been shown to differentially contribute to the formation of POM and MAOM, which may be explained by their root traits and exudates (Poirier et al., 2018; Rossi et al., 2020; Zhang et al., 2022). Thick roots generally undergo rapid transformation through microbial degradation (McCormack et al., 2015), thereby contributing to the formation of the MAOM pool (Rossi et al., 2020). Conversely, grasses generally have long, thin roots of low tissue quality and high carbon/nitrogen (C/N) ratio that reduce microbial C use efficiency and leave more C to be retained in POM form (Almeida et al., 2021; Bonanomi et al., 2021; Zhang et al., 2022). Moreover, forb and legume species with thick roots release more root exudates than grasses, and these exudates contain more N-rich compounds such as ureides and amino acids that are highly attractive to microbes and may promote the formation of stable SOM (Paynel and Cliquet, 2003; Fustec et al., 2010; Dietz et al., 2020; Williams et al., 2022). In contrast, grass root exudates are dominated by simple sugars (e.g., glucose and fructose) and low levels of organic acids (Dietz et al., 2020; Williams et al., 2022), resulting higher C/N ratios that generally limit microbial C use efficiency and potentially favouring POM pool over MAOM pool. In line with these differences, previous work showed that root exudates of forbs and legumes stimulated more microbial respiration than those of grasses (Hou et al., 2025). However, the consequences of these differences in root exudates for POM and MAOM pools remain unclear.

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). Drought reduces plant productivity and hence litter inputs to the soil, potentially causing subsequent decreases in POM (Sollins et al., 1996; Cotrufo et al., 2021). Simultaneously, drought stimulates microbial C allocation to the production of osmolyte or extracellular polymeric substances that accumulate as MAOM (Schimel et al., 2007; Sher et al., 2020; Sokol et al., 2022). Importantly, recent work shows that altered root exudation as a result of drought affects microbial activity, with potential consequences for the formation and loss of POM and MAOM. Drought reduced root exudation across a range of grassland species (De Vries et al., 2019; Hou et al., 2025), but these root exudates stimulated microbial respiration more than exudates from plants unexposed to drought, and this effect was strongest in forbs and legumes (Hou et al., 2025). This suggests that

drought might induce the priming of SOM through altered root exudation, but the implications for the formation and loss of POM and MAOM remain untested.

Here, we aimed to test how root exudates of different plant species affect soil microbial respiration and pools of POM and MAOM, and how drought alters this effect. We chose three common temperate grassland plant species that represent three functional groups, the grass *Lolium perenne* (*L. perenne*), the forb *Ranunculus acris* (*R. acris*) and the legume *Trifolium pratense* (*T. pratense*). We grew these plants under well-watered (Control) and drought conditions (Drought), after which we collected their exudates, and repeatedly added these to an unconditioned soil to assess their impact on soil respiration, SOM pools, and soil microbial communities. We hypothesized that: (1) *L. perenne* root exudates increase the POM pool, while *R. acris* and *T. pratense* root exudates result in an increase of the MAOM pool, (2) Droughted root exudates increase microbial respiration in particular for *R. acris* and *T. pratense* exudates, and (3) Drought-affected root exudates result in a decrease in POM through the priming of its decomposition and an increase in MAOM through direct sorption and microbial turnover, especially in *R. acris* and *T. pratense*.

2. Materials and methods

2.1. Experimental design

We selected three common grassland species representing grasses, forbs, and legumes: *L. perenne*, *R. acris*, and *T. pratense*, which are widely distributed across Europe. Individuals of these species were grown in a sandy loam soil (SOM = $19.28 \pm 0.73 \text{ mg g}^{-1}$, pH = 8.35 ± 0.18 , soil moisture = $16.04 \pm 0.05\%$; Den Ouden Group, The Netherlands) for the purpose of collecting root exudates. These exudates were then used in the subsequent experiment, in which we assessed the effect of repeatedly adding root exudates from these species, grown under droughted (Drought) and well-watered (Control) conditions, on soil microbial respiration, bacterial and fungal communities, and POM and MAOM pools (Supplementary Fig. 6). To achieve repeated additions of root exudates, we calculated how many individual plants we needed to yield sufficient exudates based on previous studies (De Vries et al., 2019; Hou et al., 2025). De Vries et al. (2019) added 0.17–1.59 μg root exudate C collected from two grassland species subjected to control vs. drought treatments to around 0.3 g of each individual soil sample in deepwell plates (De Vries et al., 2019). Hou et al. (2025) increased the amount of root exudates and standardized C content of each root exudate sample collected from 17 grassland species, namely adding 15 μg root exudate C in 0.3 g soil sample (Hou et al., 2025). In this study, we scaled up the amounts of exudates based on this root exudate to soil weight ratio ($15 \div 0.3 = 50$) (Table 1). Specifically, we weighted around 20 g of soil in a two-digit balance to ensure enough soil materials for subsequent observation of POM and MAOM dynamics, soil microbial community composition and soil nutrient analyses, and calculated that the total root exudate C required was 1000 μg . To achieve the experimental aim of repeated additions of root exudates, we added root exudates five times and established five replicates. This setup resulted in a total of 25 blocks (five additions $\times$ five replicates = 25 blocks).

2.2. Experimental execution

From March 1, 2022, plant species were grown from seeds ordered from Cruydt-Hoeck Seeds (The Netherlands). These seeds were germinated in the prepared soil in plug trays in the greenhouse of the University of Amsterdam. After two weeks, the specified number of seedlings of each species were randomly selected (Table 1) and assigned to Control and Drought treatments. Each individual seedling was transplanted into a 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 of fresh soil. Pots were arranged in a randomized block design with a

Table 1Background information about the average total root exudation C of *L. perenne*, *R. acris* and *T. pratense* under drought and control treatments, along with the number of plant species used for this experiment.

Plant species	Treatment	The amount of root exudate C added in (Hou et al., 2025) (μg)	Soil weight incubated (g)	Root exudate C/Soil weight ratio	Soil weight in the Respicond system in this manuscript (g)	Estimated root exudate C needed (μg)	Average amount of total root exudate C per plant species in Hou et al. (2025) (μg)	The number of plants to yield the estimated total root exudate C	The number of plants to allow for five times addition into the Respicond system	The number of plants needed with five replicates
<i>L. perenne</i>	Control	15	0.3	50	20	1000	360.05	e.g. 1000 ÷ 360.05 ≈ 3	15	75
	Drought	15	0.3	50	20	1000	281.88	4	20	100
<i>R. acris</i>	Control	15	0.3	50	20	1000	1191.40	1	5	25
	Drought	15	0.3	50	20	1000	574.26	2	10	50
<i>T. pratense</i>	Control	15	0.3	50	20	1000	930.56	2	10	50
	Drought	15	0.3	50	20	1000	574.41	2	10	50
Total (five additions × five replicates = 25 blocks)								14	70	350

two-to three-day gap between blocks to allow enough time for processing samples during harvest (14 single plants × 25 blocks = 350 pots) (see [Supplementary Fig. 1](#) for the whole experimental setup and [Table 1](#) for information about the number of individuals). Plants were watered by weight to maintain 60% water-holding capacity (WHC) during the next 10 weeks. After 10 weeks, control and droughted plants were monitored daily by weight and kept at 60% and 20% WHC for two weeks, respectively. After that, the moisture content of all pots was re-adjusted to 60% WHC and maintained for a week in a climate chamber (16 h of light at 22 °C and 8 h of dark at 20 °C, humidity 65%) to standardize growth conditions until plant harvesting. Each plant with intact roots and rhizosphere soil was gently removed from pots and roots were soaked in water. Then, adhering soil and plant residues were carefully cleaned using flowing water and tweezers, and each plant was instantly transferred into a 100-ml plastic SCHOTT bottle containing 100 ml hydroponics solution made by stirring 100 g soil in 1000 ml MilliQ water for 2 h and filtering through 0.2 mm mesh ([De Vries et al., 2019](#); [Williams et al., 2021](#)). All bottles with plants were brought back to the climate chamber (16 h of light at 22 °C and 8 h of dark at 20 °C, humidity 65%) and aerated for a final week of recovery to avoid pathogen illness ([Oburger and Jones, 2018](#)) and to 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](#)). Plants were then ready for root exudate collection and standardization.

2.3. Root exudate collection

We collected root exudates according to a hydroponics-hybrid method described by ([De Vries et al., 2019](#); [Williams and De Vries, 2020](#)). Each individual root system was rinsed with MilliQ water several times, and we inserted clean roots into 100 ml glass SCHOTT bottles that contained 100 ml MilliQ water. Bottles with intact plants were gently shaken on ice for 2 h at 60 rpm under natural light before removing the plants from the bottles. Root exudate solution from each bottle was filtered through 0.22 μm filter paper (10401712, WhatmanTM, UK) into three 50 ml falcon tubes (227261, Greiner). Then, all root exudate sample tubes were frozen at −80 °C before being freeze-dried at −50 °C (CoolSafeTM, Den Hartog SCIENTIFIC). Finally, freeze-dried root exudate samples were stored in −80 °C.

2.4. Root exudate concentration standardization and root exudate-induced respiration measured in the respicond system

We merged freeze-dried root exudate samples per treated plant species from every five blocks by resuspending them in sterile MilliQ water. Based on the feasibility of merging root exudates, we standardized the exudate sample concentration to 333.33 μg ml^{−1} with a sample volume of 3 ml, ensuring that 1000 μg (=1 mg) of root exudate C could be applied in each addition for each treatment (representing the three species grown under Control and Drought treatments). These standardized root exudate samples and MilliQ water samples (Water) were stored at −80 °C ready for root exudate-induced respiration measurements, and soil samples without addition (Soil) was also considered until addition step and respiration measurement in the Respicond VIII System (Zürich, <https://www.respicond.com/>) ([Nordgren, 1988](#)) (three Species exudate addition applications × two Treatments + 1 Water + 1 Soil) × five replicates = 40 addition samples). Then, we weighed 20 g fresh unconditioned soil (soil moisture 16.04%, the same soil that was used for plant growth) using a 2-digit balance and transferred them into 40 individual soil sample cups. All 40 soil sample cups were carefully and randomly placed in 40 experimental jars in 20 °C water bath for temperature consistency. Each experimental jar was connected to a conductometer via a multiplexer containing 30 ml KOH solution (39.60 g L^{−1}), where accumulated CO₂ production (mg CO₂) was calculated by transmitting the KOH conductivity every 30 min and automatically

saved. We also set two extra jars with empty soil sample cups to reference the temperature consistency of the water bath. After observation of stable temperatures and no CO₂ accumulation in the empty jars, we pipetted raw root exudate solution samples and MilliQ water samples into their corresponding sample cups from September 3, 2022 (see [Supplementary Table 1](#) for the order of samples addition). Root exudate solution and water samples were added monthly (five times in total), with pH measured for both at each addition ([Supplementary Fig. 5](#)). After each addition, we took out soil sample cups and placed them at 20 °C for about 48 h to allow the soil moisture return to its original level before the next addition. Captured root exudate solution-induced respiration (CO₂ exudate; mg CO₂-C) and MilliQ water-induced respiration (CO₂ water; mg CO₂-C) were acquired from the Respicond system ([Supplementary Figs. 3 and 4](#)). Root exudate-induced respiration (mg CO₂-C (mg C added)⁻¹ (g dry soil)⁻¹) was defined as net soil microbial respiration induced per unit root exudate C per gram dry weight soil and calculated as [formula 1](#) below:

$$\text{Root exudate-induced respiration} = \frac{\text{CO}_2 \text{ exudate (mg CO}_2\text{-C)} - \text{CO}_2 \text{ water (mg CO}_2\text{-C)}}{\text{Root exudate C (mg)} \times \text{Dry soil weight (g)}} \quad (1)$$

Raw root exudate solution-induced respiration (mg CO₂-C (mg C added)⁻¹ (g dry soil)⁻¹) was defined as the raw soil microbial respiration respired per unit root exudate solution C per gram dry weight soil and was calculated as [formula 2](#) below:

$$\text{Raw root exudate solution-induced respiration} = \frac{\text{CO}_2 \text{ exudate (mg CO}_2\text{-C)}}{\text{Root exudate C (mg)} \times \text{Dry soil weight (g)}} \quad (2)$$

Some CO₂ production data during the period between December 25, 2022 and January 2, 2023 were not successfully recorded, but the Respicond system operated normally.

2.5. Soil DNA extraction, amplification and amplicon sequencing

Around 0.25 g fresh soil from each sample was frozen at -20 °C until DNA extraction. We extracted soil DNA using DNeasy® PowerSoil® Pro Kit (QIAGEN N.V., Germany) following the Quick-Start protocol. The DNA quantification was done using the Implen NanoPhotometer® N60/N50; then the DNA concentration for each sample was normalized to 5 ng µl⁻¹. We amplified the bacterial 16S ribosomal DNA region with set of primers CS1_515FB (ACACTGACGACATGGTTCTACAGTGYCAGCMGC CGCGGTAA) and CS2_806RB (TACGGTAGCAGAGACTTGGTCTGG ACTACNVGGGTWTCTAAT) ([Apprill et al., 2015](#); [Parada et al., 2016](#)) and the fungal internal transcribed spacer 1 region (ITS1) with the primer pair CS1_ITS1F (ACACTGACGACATGGTTCTACACTTGGTCATTT AGAGGAAGTAA) and CS2_ITS2R (TACGGTAGCAGAGACTTGGTC TGCTGCGTTCTTCATCGATGC).

Our 20 µL PCR mixture consisted of 13.6 µL of autoclave MQ water, 2 µL of DreamTaq™ Buffer (10 ×), 2 µL of 1 mM dNTPs, 0.2 µL of DreamTaq DNA Polymerase (5 U µL⁻¹) from Thermo Fisher Scientific, 0.6 µL of each primer (10 µM for both forward and reverse), and 1 µL of normalized extracted DNA. The PCR protocol for 16S involved an initial denaturation at 95 °C for 5 min, followed by 28 cycles of 95 °C for 30 s,

55 °C for 45 s, 72 °C for 30 s, and a final extension at 72 °C for 7 min. For ITS amplification, the protocol included an initial denaturation at 95 °C for 5 min, followed by 28 cycles of 95 °C for 30 s, 52 °C for 30 s, 72 °C for 30 s, and a final extension at 72 °C for 7 min. The DNA amplification fragments were visualized through electrophoresis on a 1.5% agarose gel. The PCR products were sent to the Core Research Resource Center at the University of Illinois (Chicago, USA) for a second round of PCR and the preparation of Illumina libraries. The libraries were then pooled into two groups: one for 16S amplicons, which were sequenced using an Illumina MiniSeq MO 2 × 150, and another for ITS amplicons, which were sequenced using an Illumina MiSeq with a V3, 600-cycle kit (2 × 300).

2.6. Soil nutrient analyses

Water-soluble nutrients and C were extracted by shaking 8 g of fresh soil with 20 ml MilliQ water in a 50 ml centrifuge tube at 150 rpm for 2 h

([Supplementary Fig. 2](#)). Extracts were filtered through 0.45 µm filter papers (0424A00023, Fisher Scientific) using filter boxes. The filtered samples were analysed for water-dissolved nutrients, including dissolved organic carbon (DOC), nitrate (NO₃⁻) and ammonium (NH₄⁺) in the Auto Analyzer (SAN++ SYSTEM, SKZLAR). Plant available nutrients (NO₃⁻, NH₄⁺ and PO₄³⁻) were extracted by shaking 1 g of fresh soil in 4 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) ([Supplementary Fig. 2](#)).

2.7. Density fractionation

After microbial DNA extraction and soil nutrient analyses, the remaining soil samples were air-dried, and we separated POM and MAOM by density fractionation ([Golchin et al., 1994a, 1994b](#)). We weighed around 12 g dry soil of each sample into one centrifugation tube and slowly added 60 ml of sodium polytungstate (NaPT, TC-Tungsten Compounds GmbH) solution (1.8 g cm⁻³) to moisten the soil. All the material stood for 1 h. During this period, we prepared a filtration Erlenmeyer-shaped flask for each individual sample and pre-wetted a glass microfibers filter (ø 55 mm; VWRI516-0346, VWR) in its plastic filter holder with NaPT solution to allow the adhesion of the filter to the holder, avoiding losing soil materials with the solution. One hour later, we placed each centrifugation tube containing soil-NaPT solution on the ice-bath and inserted a cylindrical sonotrode (ø 13 mm; SH 213 G) of an ultrasound generator (SONOPULS, BANDELIN) 1 cm below the liquid level to disperse the energy at 100 J ml⁻¹ for 4 min and 20 s. Then, centrifugation tubes were centrifuged at 6800 g for 20 min (HiGen 21G, Herolab). We steadily removed tubes from the centrifuge and translocated the floating material using a rubber spatula to the filter. The obtained fraction was rinsed with MilliQ water several times until the electrical conductivity dropped below 50 µS cm⁻¹ (LF 90, WTW). The supernatant was slowly aspirated with a suction device, and

the soil fraction left at the bottom of centrifugation tubes was rinsed with MilliQ water and centrifuged at 7500 g for 20 min. This process was repeated around five to seven times until the electronic conductivity of the supernatant below $50 \mu\text{S cm}^{-1}$. All obtained fractions were completely transferred using a wash bottle containing MilliQ water into weighed dry brown bottles using a 4-digital balance (ABT 220-50 M, KERN). Brown bottles containing soil fractions were frozen-dried at -50°C (CoolSafeTM, Den Hartog SCIENTIFIC) and weighed again. The weight of POM and MAOM was calculated by the difference between the two weights. All fractions were fully homogenized ground manually. 5 mg POM fraction and 40 mg MAOM fraction were weighed (MX5, METTLER TOLEDO) and folded with two technical duplicates to determine C contents in POM (POC), N contents in POM (PON), C contents in MAOM (MAOC) and N contents in MAOM (MAON) using an Elemental analyzer (vario EL CUBE).

2.8. Statistical analyses

All statistical analyses and plots were done in R v.4.4.1 (2024-06-14 ucrt) (R Core Team, 2024) and RStudio v.2024.04.2 + 764. We tested whether our data were normally distributed by Shapiro-Wilk test ($p > 0.05$) using the package gridExtra (Auguie, 2017) and log-transformed variables that did not fit normalization ($p < 0.05$) (including MAOC, root exudate-induced respiration, dissolved organic carbon and plant available ammonium). To assess the impact of drought treatment (Treatment) and plant species (Species) on soil C and N pools (POC, PON, C/N of POM, MAOC, MAON and C/N of MAOM), water dissolved organic C (DOC) and nutrients (nitrate (NO_3^-) and ammonium (NH_4^+)), plant available nutrients (nitrate (NO_3^-), ammonium (NH_4^+) and phosphate (PO_4^{3-})) and root exudate-induced respiration (cumulative amount of C respired), we fitted linear mixed-effect models with two fixed effects of Treatment and Species and two independent random effects, namely the row and the column of soil sample cups assigned to

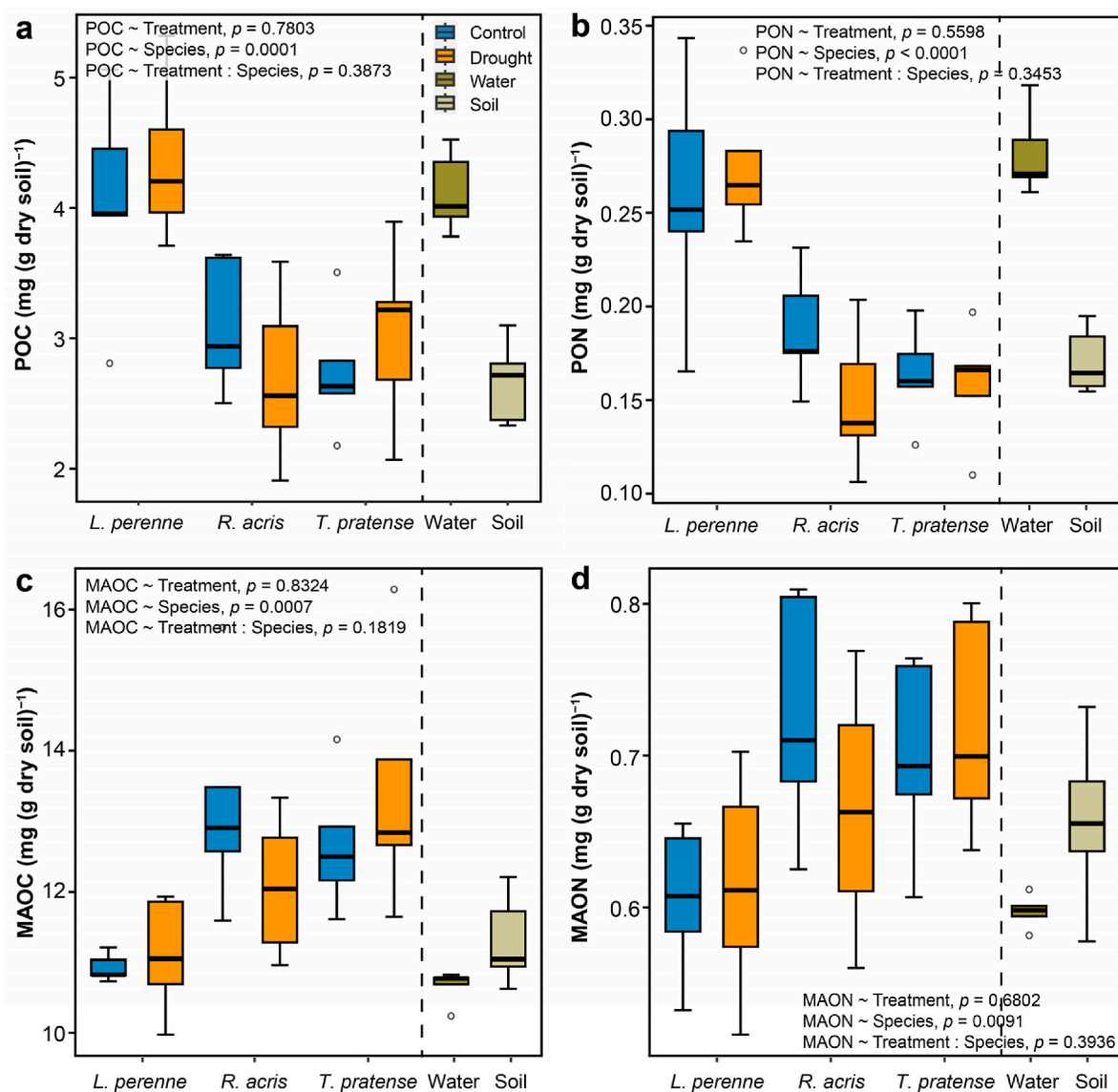


Fig. 1. Changes in POC (a), PON (b), MAOC (c) and MAON (d) induced by root exudate addition from *L. perenne*, *R. acris* and *T. pratense* that experienced drought vs. control conditions, compared to only water addition (Water) and no addition (Soil). Blue and orange indicate exudates from control and drought plants; dark and light green colors represent the water addition (Water) and no addition (Soil) treatments. The central line in the boxes marks the median value of the data, and the upper and lower bounds of boxes represent the first and third quartiles, respectively. The lines above and below boxes represent the maximum and minimum values. Dots placed past the line edges are outliers. (n = 5). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

the RespiCond system using packages Matrix (Bates and Maechler, 2009), lme4 (Bates et al., 2015) and lmerTest (Kuznetsova et al., 2017) of R. Graphs visualizing treatment effects on C and N pools, soil nutrients and root exudate-induced respiration were plotted using the package ggplot2 (Wickham, 2016). When a factor or an interaction was considered significant, we ran a post-hoc analysis with the emmeans function in the emmeans package, applying Bonferroni correction (Lenth, 2024).

We used QIIME2 for quality control (Bolyen et al., 2019), feature tabulation and taxonomic classification of both 16S and ITS reads. Singletons were removed from raw 16S and ITS data, and data were normalized by dividing the counts of each taxon by the total read count, producing relative abundances, using the package tidyverse (Wickham et al., 2019) to perform Non-metric Multi-Dimensional Scaling (NMDS) based on Bray-Curtis distances using the packages vegan (Oksanen et al., 2022), goeveg (Von Lampe and Schellenberg, 2024), ggplot2 (Wickham, 2016). Permutational multivariate analysis of variance (PERMANOVA) was used to detect differences in bacterial and fungal community composition in response to Treatment and Species, with the row and the column of sample cups in the RespiCond as independent random effects, using the adonis2 function in the vegan package (Oksanen et al., 2024). Alpha diversity indices were analysed using the package phyloseq (McMurdie and Holmes, 2013).

Indicator species analysis (ISA) was done to identify species of bacteria at family level and fungi at ASV level that were indicative of each Treatment and each Species using the Multipatt function in the indispes package (De Cáceres and Legendre, 2009; William Reville, 2024). Indicator microbial species were selected based on their relative abundance within each Species and Treatment group using species-site group association function (Tichy and Chytrý, 2006; De Cáceres et al., 2010), with a significance level of less than 0.05. The indicator species were ranked based on the Indicator Value. To explore relationships with soil properties, we then generated heatmaps to visualize Pearson correlations between the relative relative abundances of indicator species and soil C and N pools using the corr.test function in the psych package (William Reville, 2024) and pheatmap function in the pheatmap package (Kolde, 2019).

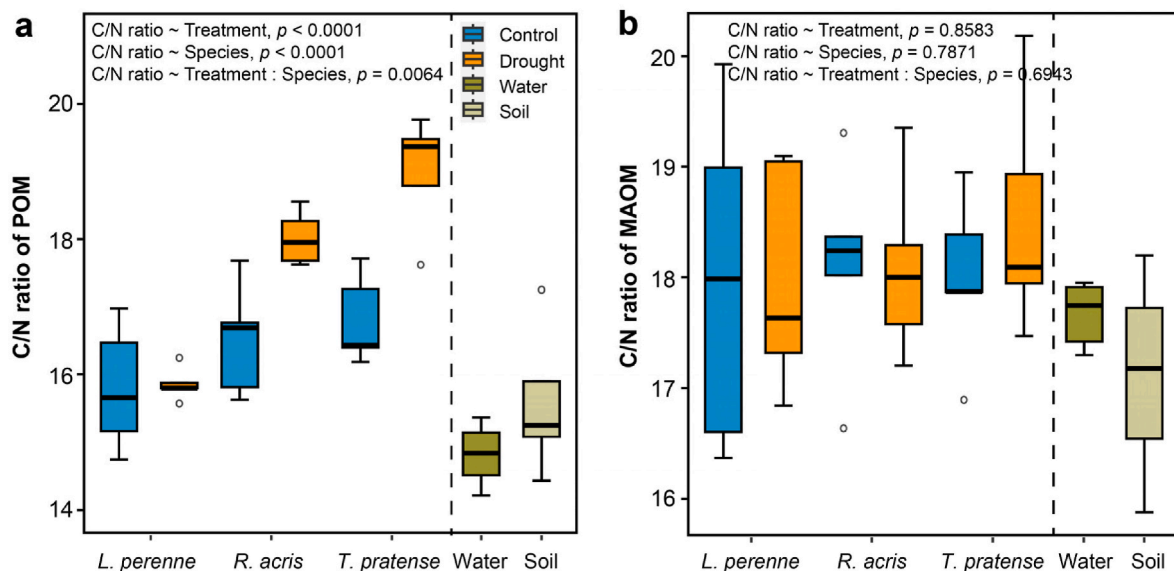


Fig. 2. Changes in C/Ns of POM (a) and MAOM (b) induced by root exudate addition from *L. perenne*, *R. acris* and *T. pratense* that experienced drought vs. control conditions, compared to only water addition (Water) and no addition (Soil). Blue and orange indicate exudates from control and drought plants; dark and light green colors represent the water addition (Water) and no addition (Soil) treatments. The height of boxes reflects the volatility of properties. The central line in boxes marks the median value of the data, and the upper and lower bounds of boxes represent first and third quartiles, respectively. The lines above and below boxes represent the maximum and minimum values. Dots placed past the line edges are outliers. (n = 5). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

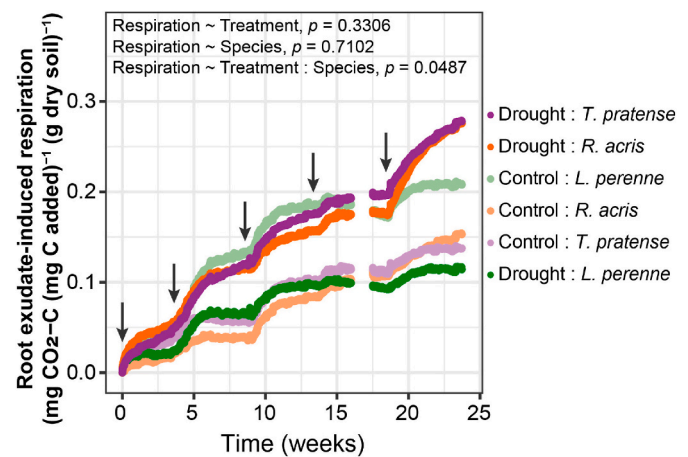


Fig. 3. Root exudate-induced respiration for *L. perenne*, *R. acris* and *T. pratense* species subjected to control and drought treatments. Dots represent individual observations. Green, orange and purple represent root exudates collected from *L. perenne*, *R. acris* and *T. pratense* species. Light and dark colors represent root exudates collected from control and drought plants. Arrows indicate the time points of root exudate applications. The gap is caused by a data recording failure from December 25, 2022 to January 2, 2023. (n = 5). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3. Results

3.1. Effects of drought-affected root exudates on POM and MAOM across different plant species

The root exudates of different plant species influenced POM and MAOM, but drought had no effect (Fig. 1). *R. acris* and *T. pratense* root exudates decreased particulate organic carbon (POC) contents (Main effect of Species, $F_{2, 24} = 13.8$, $p = 0.0001$; Fig. 1a) and particulate organic nitrogen (PON) contents (Main effect of Species, $F_{2, 24} = 20.9$, $p < 0.0001$; Fig. 1b), but increased mineral-associated organic carbon

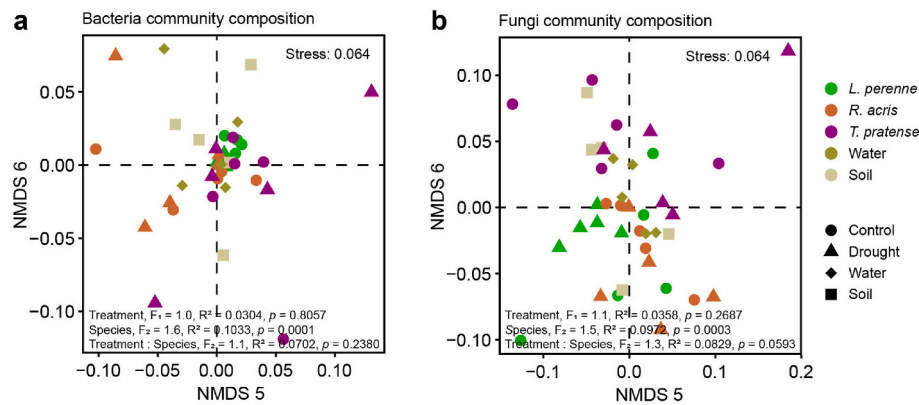


Fig. 4. NMDS plots of bacterial (a) and fungal (b) community composition as affected by drought treatment and root exudates of *L. perenne*, *R. acris* and *T. pratense* species, water addition (Water) and no addition (Soil). Dots represent individual observations.

(MAOC) contents (Main effect of Species, $F_{2, 24} = 10.1$, $p = 0.0007$; Fig. 1c) and mineral-associated organic nitrogen (MAON) contents (Main effect of Species, $F_{2, 22.9} = 5.7$, $p = 0.0091$; Fig. 1d) in comparison to the water addition treatment. The effect of *L. perenne* exudates on the contents of POC ($df = 33.8$, $p = 0.9995$), PON ($df = 33.8$, $p = 0.9590$), MAOC ($df = 34.1$, $p = 0.9663$) and MAON ($df = 34.1$, $p = 0.9978$) did not differ from the water addition treatment.

3.2. Effects of drought-affected root exudates on C/N ratios of POM and MAOM across different plant species

Compared to the water addition only, the addition of drought-affected root exudates from *R. acris* and *T. pratense* increased the C/N ratio of POM (Treatment: Species interaction, $F_{2, 21.4} = 6.5$, $p = 0.0064$; Fig. 2a). The C/N ratio of MAOM was not affected by drought or species (Fig. 2b).

3.3. Effects of drought and plant species on root exudate-induced respiration

Drought-affected root exudates altered cumulative soil microbial respiration depending on the plant species (Treatment: Species interaction, $F_{2, 23.5} = 3.4$, $p = 0.0487$; Fig. 3). Droughted *R. acris* and *T. pratense* root exudates triggered higher respiration than those from control plants, while *L. perenne* exudates reduced respiration compared to exudates from control plants.

3.4. Impacts of root exudate addition on bacterial and fungal community composition, and correlations with soil properties

The bacterial and fungal community composition was affected by root exudate addition from the different plant species, but it was not affected by whether those root exudates came from droughted plants (Fig. 4a and b). Bacterial diversity and richness were highest with exudates from *L. perenne*, while evenness was highest with *T. pratense* exudates, while fungal evenness was reduced with droughted *T. pratense* exudates (Supplementary Table 4).

We identified indicator species for both the response of bacterial communities and fungal communities to root exudate addition of the different plant species, as well as to root exudates from droughted vs. control plants. Root exudates from the different plant species stimulated fungal indicator species predominantly from the phylum *Ascomycota*, while the bacterial indicator species varied and belonged to different bacterial phyla (Fig. 5a and b; Supplementary Table 2). *L. perenne* stimulated bacterial species belonging to the phyla *Verrucomicrobiota*, *Planctomycetota* and *Firmicutes*. For *R. acris* and *T. pratense*, the bacterial indicator species were similar, both including the phyla *Actinobacteriota*.

Additionally, *T. pratense* uniquely stimulated certain bacterial taxa, such as *Amb-16S-1323* and *Rhizobiales_unclassified*, belonging to the phyla *Proteobacteria* (both also belonging to class *Alphaproteobacteria* and order *Rhizobiales*). The relative abundances of microbial indicator species stimulated by *L. perenne*, *R. acris* and *T. pratense* root exudates were correlated with POM and MAOM properties differently. Specifically, the relative abundance of indicator species for *L. perenne* root exudates was positively correlated with POM contents and negatively correlated with the C/N ratios of POM and MAOM, while the indicator species for *R. acris* and *T. pratense* exudates were negatively correlated with POM contents and positively correlated with the C/N ratios of POM and MAOM.

Root exudates from the control and drought plants stimulated fungal indicator species both mainly dominated by the phyla *Ascomycota* and *Basidiomycota*, but root exudates stimulated distinct bacterial indicator species in the control vs. drought plants (Fig. 5c and d; Supplementary Table 4). For exudates from control plants, indicator bacterial taxa belonged to the phyla *Proteobacteria*, *Firmicutes* and *Acidobacteriota*, which were negatively correlated with the C/N ratio of POM. In contrast, droughted root exudates stimulated bacteria corresponding to *Desulfobacterota* and *Firmicutes*, which were positively correlated with the C/N ratio of POM.

4. Discussion

We set out to test how root exudates from three common grassland species affect the distribution of SOM across fractions of different stability, and whether this is affected by drought. We hypothesized that *L. perenne* root exudates increase POM contents, while *R. acris* and *T. pratense* root exudates accumulate MAOM, and that root exudates from droughted plants reduce the contents of POM but increase the contents of MAOM. We also hypothesized that root exudates from droughted plants would increase soil respiration, especially for *T. pratense* and *R. acris*. We found that *R. acris* and *T. pratense* root exudates resulted in a loss of the less stable POM fraction, and in a concomitant increase in the more stable MAOM fraction. While drought did not alter the effect of root exudates on the total POM and MAOM pools, root exudates from droughted *R. acris* and *T. pratense* plants increased microbial respiration and the C/N ratio of POM. These findings suggest that exudates from droughted plants stimulate microbial growth and N demand, leading to microbial N mining of POM, or that microbial C-use efficiency decreases in response to these exudates, resulting in the preferential consumption and respiration of C-rich exudates. Our study indicates that different plant species, via altered root exudation and cascading effects on microbial community composition and activity, affect the distribution and stabilization of SOM.

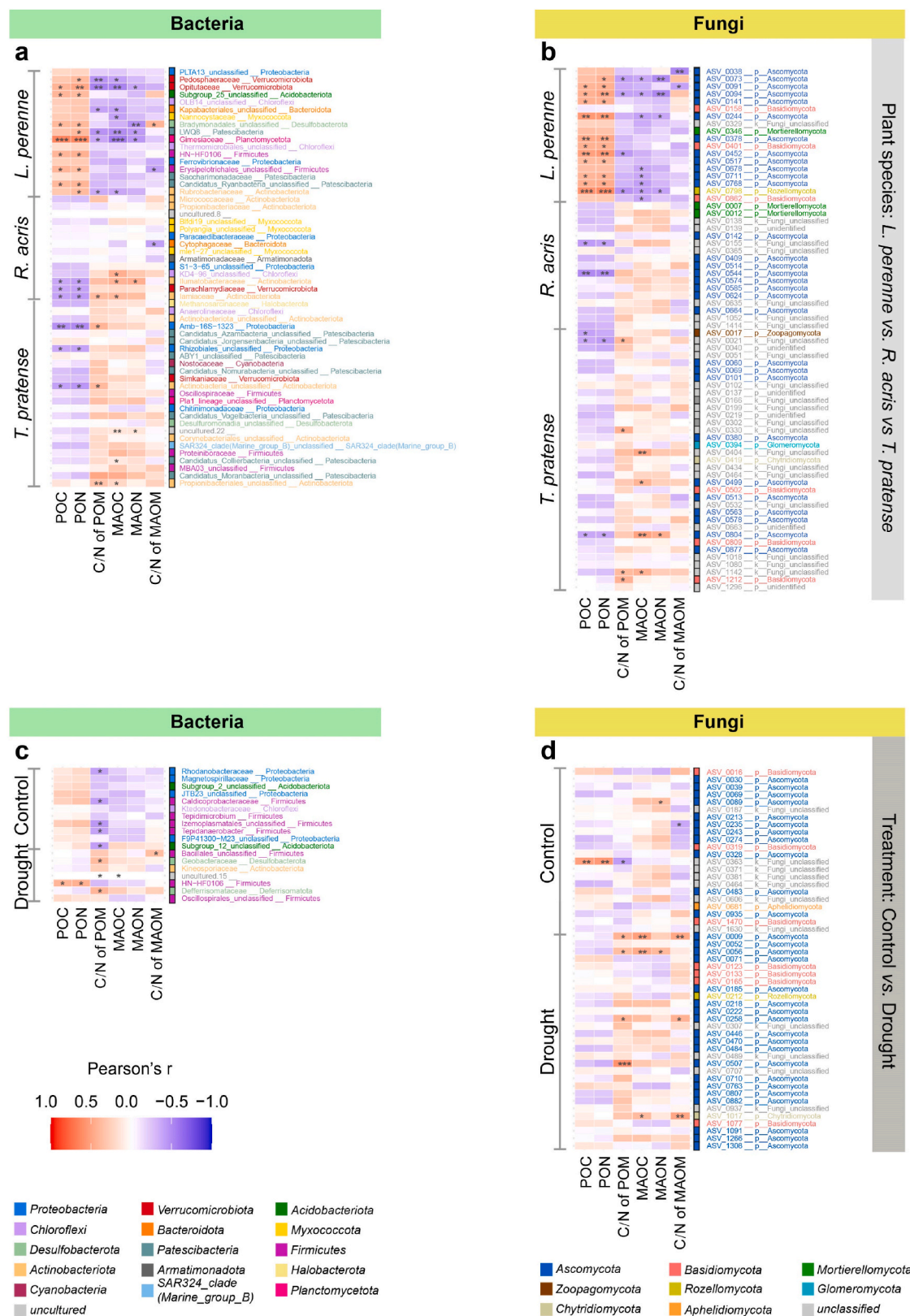


Fig. 5. Heatmaps of the correlations between bacterial (a and c) and fungal (b and d) indicator species for root exudates from different plant species (a and b) and for root exudates from control and drought plants (c and d). Indicator species names are colored by phylum. The color within cells represents Pearson correlation coefficients (r values), with red and blue colors representing positive and negative correlations, respectively. Stars within cells represent statistical significance (*: $p < 0.05$; **: $p < 0.001$; ***: $p < 0.0001$). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

4.1. Root exudates of *R. acris* and *T. pratense* enhance POM decomposition and MAOM formation

The addition of root exudates from the forb *R. acris* and the legume *T. pratense*, but not those from the grass *L. perenne*, resulted in a loss of POM and an increase in MAOM. Root exudates are preferentially used by soil microbes, with the produced microbial residues contributing to the formation of relatively stable SOM (Kallenbach et al., 2016), but this increased microbial activity can also lead to the decomposition of SOM (Garcia-Pausas and Paterson, 2011; Huo et al., 2017; Sarker et al., 2019; Mo et al., 2021). Thus, our results suggest that *R. acris* and *T. pratense* root exudates stimulate the microbial decomposition of POM, which may lead to a concomitant increase in MAOM, likely through increased microbial turnover and the resulting production of microbial residues that are subsequently adsorbed (Cotrufo et al., 2013). These processes occur probably because root exudates stimulate microbial activity and convert degradable OM into microbial byproducts via iterative turnover of OM in POM (Kallenbach et al., 2016; Johnson et al., 2018; Sokol and Bradford, 2019; Sokol et al., 2022). Although we did not use isotopic labelling to trace the fate of the added exudates, these findings are in line with the priming effect where microbes fed by root exudates accelerate decomposition of native OM (Kuzyakov et al., 2000; Kuzyakov, 2002a; Keiluweit et al., 2015; Jilling et al., 2021). Our finding that *R. acris* and *T. pratense* root exudates, induced a loss of POM is in line with results from Hou et al. (2025) that suggested that forb and legume root exudates induce a stronger priming effect. *R. acris* and *T. pratense* release N-rich root exudates (Paynel and Cliquet, 2003; Fustec et al., 2010; Dietz et al., 2020; Williams et al., 2022) that may stimulate microbial turnover and microbial decomposition of POM in native soils. Although MAOM has traditionally been considered slower to form and more persistent than POM (Christensen, 2001; Lavallee et al., 2020), recent findings indicate that hydroxyl radicals generated during microbial exudate metabolism make MAOM more susceptible than POM (Liu et al., 2023), thereby accelerating its turnover and promoting further fast-cycling MAOM formation (Heckman et al., 2023; Jilling et al., 2025). In contrast to *R. acris* and *T. pratense*, *L. perenne* root exudates did not affect the quantities of POM and MAOM compared to the water addition treatment, potentially because *L. perenne* root exudates are dominated by simple sugars (e.g., glucose and fructose) and low levels of organic acids (Dietz et al., 2020; Williams et al., 2022) (also supported by root exudate composition data from an earlier study of the same species growing under the same conditions; Supplementary Table 3). Sugars provide a more bioavailable or metabolizable energy source (Renella et al., 2006), thereby suppressing SOM decomposition and contributing to the stability of the POM and MAOM pools (Wen et al., 2022). Therefore, our results demonstrate that root exudates can enhance POM decomposition and MAOM formation. Future work can employ isotopic labelling approaches to trace the fate of exudate-derived C in microbial communities and in POM and MAOM, and investigate the detailed chemical composition and functional traits of exudates in order to provide direct mechanistic evidence.

We also found that water addition led to higher POC and PON, but did not affect MAOC and MAON compared with the no addition soil group. This pattern is consistent with the effects of *L. perenne* root exudates. However, microbial respiration induced by water was lower than that triggered by *L. perenne* exudates (Supplementary Figs. 3 and 4). This indicates that water may increase POM formation but not drive the decomposition of more stable MAOM during the short incubation period. Likely, the modest increase in soil moisture may enhance microbial activity enough to mobilize and partially decomposes labile organic particles (e.g. POM). Despite lack of direct microbial biomass turnover measurement, this may not be sufficient to change extensive microbial turnover or produce enough microbial residues to alter the more stabilized MAOM pool (Borowik and Wyszowska, 2016; Cotrufo and Lavallee, 2022; Fig. 2).

Root exudates from different plant species shifted bacterial

communities (Fig. 4; Fig. 5a and b), with *L. perenne* exudates stimulating the bacterial phyla *Verrucomicrobiota*, *Planctomycetota* and *Firmicutes*, *R. acris* stimulating *Actinobacteriota* and *T. pratense* stimulating *Actinobacteriota* and *Proteobacteria*. Within *Proteobacteria*, two taxa, *Amb-16s-1323* and *Rhizobiales_unclassified*, were identified, both belonging to the class *Alphaproteobacteria* and the order *Rhizobiales*. The relative abundances of bacterial indicators for *L. perenne* exudates were positively correlated to POM and negatively correlated with MAOM, while the relative abundances of indicators for *R. acris* and *T. pratense* exudates were negatively correlated to POM and positively correlated with MAOM. These findings suggest that root exudates drive patterns in rhizosphere microbial community assembly (Zhalnina et al., 2018) which may further affect POM and MAOM dynamics. The phylum *Actinobacteria* and the order *Rhizobiales* have the ability to degrade plant residues (Abdulla and El-Shatoury, 2007; Lewin et al., 2016; Bao et al., 2019). *Actinobacteria* are involved in the degradation of labile SOM molecules into persistent SOM molecules (Gao et al., 2025), while *Rhizobiales* have the ability to fix N (Jorquera et al., 2014) and participate in the breakdown of aromatic compounds and flavonoids (Barz, 1969; Barz et al., 1970; Rao et al., 1991; Pillai and Swarup, 2002) (supported by exudate compositional data from a previous experiment; Supplementary Table 3). Both bacteria contribute to producing microbial residues, such as cellular debris that are the main contributor of stable SOM (Wen et al., 2024). However, *Verrucomicrobiota*, *Firmicutes*, *Planctomycetota* as indicators of *L. perenne* exudates are primarily known for decomposing complex carbohydrates and polymers into stable MAOM (Berlemont and Martiny, 2015; López-Mondéjar et al., 2019, 2022).

4.2. Droughted root exudates from *R. acris* and *T. pratense* increase microbial respiration, with potential implications for POM

The impact of drought on the cumulative amount of microbial respiration triggered by root exudates varied with plant species, with exudates from *R. acris* and *T. pratense* plants that experienced drought increasing respiration, while root exudates from droughted *L. perenne* decreased respiration. These findings are in line with previous work by De Vries et al. (2019) and Hou et al. (2025) showing that root exudates from droughted plants trigger more microbial respiration across a range of species, particularly in forbs and legumes. Based on these findings, we expected that root exudates from droughted plants would prime the decomposition of SOM and result in a loss of POM in particular. However, we found that, despite triggering more microbial respiration, root exudates from droughted plants did not affect the quantities of POM and MAOM. Instead, root exudates from droughted *R. acris* and *T. pratense* led to an increase of the C/N ratio of POM. Combined with the concomitant increase in microbial respiration (Fig. 3), there are two potential mechanisms that may explain these findings. First, drought-altered exudates may cause microbial N limitation and thereby trigger the microbial mining of POM. This is supported by findings from an earlier study of the same species growing under the same conditions, in which we found a lower relative abundance of N compounds in *R. acris* and *T. pratense* exudates in response to drought (Supplementary Table 3). Specifically, this earlier exudate compositional analysis showed lower relative abundance of N-rich compounds (i.e. amino acids) and higher relative abundances of low-N compounds (i.e. organic acids (such as acetic acid, lactic acid and galactaric acid)) collected from droughted *R. acris* and *T. pratense* compared to the control plants (Supplementary Table 3). Previous studies have also reported that drought increases various drought stress-related secondary metabolites with low N content including strigolactones, flavonoids (such as biochanin A and formononetin) and abscisic acid (Nair et al., 1991; Weston and Mathesius, 2013; Singh et al., 2023). The higher root exudate C/N ratio in response to drought that these changes may further trigger a microbial stress response (Hoffland et al., 1992; Wang et al., 2000; Henry et al., 2007) and microbial mining of N from POM, potentially with an accelerated decomposition of POM (Craine et al., 2007; Chen

et al., 2014). Second, the elevated microbial respiration and higher POM C/N ratio but no detectable POM loss induced by the addition of exudates from the droughted forb and legume may also reflect a reduced microbial C use efficiency and a limited breakdown of complex POM. Microbes preferentially utilize labile exudates (Kuzakov, 2002b; Panchal et al., 2022) and the more accessible components of POM (Fischer et al., 2002; Leifeld and Kögel-Knabner, 2005). However, drought-induced shifts in root exudate composition, including higher exudate C/N ratio, can lead to microbes respiring a larger fraction of these C-rich exudates per unit resource assimilated (De Vries et al., 2019; Hou et al., 2025; Na et al., 2025) through lower microbial C use efficiency (Poeplau et al., 2016). Under such conditions, microbes are less likely to allocate additional resources toward the breakdown of more complex POM. To confirm these potential mechanisms, future experiments would need to employ the use of stable isotope tracing.

In contrast to *R. acris* and *T. pratense* exudates, *L. perenne* exudates triggered less microbial respiration, and drought reduced microbial respiration compared to exudates from *L. perenne* control plants, without consequences for POM and MAOM. We could find very little information on the effect of drought on grass species root exudates, but results from our earlier study of root exudate composition response to drought suggests that *L. perenne* root exudates, dominated by sugars, particularly in droughted plants (Supplementary Table 3), would stimulate microbial uptake and metabolism of fresh C rather than rather than engagement in the decomposition of complex SOM. This aligns with the grass's adaptive strategy to drought, with roots shifting from an acquisitive to a more conservative strategy (De Vries et al., 2016; Reinelt et al., 2023).

While overall, root exudates from droughted plants did not shift bacterial and fungal community composition compared to root exudates from control plants (Fig. 4), there were clear bacterial indicator species associated with root exudates from drought and control plants (Fig. 5c). The bacterial taxa *Firmicutes* and *Desulfobacterota* were indicative for root exudates from droughted plants, while *Proteobacteria* (*Rhodanobacteraceae* also belonging to the class *Alphaproteobacteria*), *Firmicutes* and *Acidobacteriota* were indicative for root exudates from control plants. *Proteobacteria*, *Firmicutes* and *Acidobacteriota* are considered r-strategists (Li et al., 2021; Hu et al., 2023; Wang et al., 2024), while *Desulfobacterota* are considered as K-strategists (Kleindienst et al., 2017; Li et al., 2020; Hu et al., 2023). Thus, these indicator species associated with exudates from droughted plants may indicate a shift from r-to K-strategies, potentially through a shift to lower root exudate decomposability (Meyer, 1994; Fierer et al., 2007), which is in line with the two potential mechanisms that these exudates impose N limitation and stimulate microbial N mining from POM and/or reduce microbial C use efficiency. We only observed these potential mechanisms for *R. acris* and *T. pratense* exudates, and not for the grass *L. perenne*. A potential explanation for this is that grasses with their long thin roots represent the “do-it-yourself” strategy in the root economics space, while forbs and especially legumes invest more in microbial collaboration, including higher rates of root exudation, and these root exudates stimulating more microbial activity (Williams et al., 2022; Hou et al., 2025).

5. Conclusion

Taken together, our results show for the first time that root exudates from different plant species have implications for the dynamics of soil POM and MAOM pools. Exudates from a forb and a legume reduced POM contents while simultaneously promoting MAOM accumulation, whereas exudates from a grass did not alter these pools. This highlights that root exudates from diverse grassland species distinctly regulate the decomposition and stabilization of SOM, and that root exudates accelerate critical processes that transition short-term POM decomposition into long-term C storage as stable MAOM. Root exudates from droughted plants did not affect POM and MAOM pools but likely increased microbial mining for N within POM and/or decreased microbial C use efficiency. These findings offer mechanistic insights into the role of root

exudates in driving microbial activity and processes of loss and formation of POM and MAOM. This advances our understanding of the potential consequences of shifts in species abundances for POM and MAOM pools, as well as the effect of drought on these processes via altered stoichiometry. More research is needed on shifts in root exudate composition, the fate of exudate-derived C in SOM and validation across a broader range of plant species and soil types.

CRedit authorship contribution statement

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

Code availability

All code is publicly available on Figshare at <https://figshare.com/s/d562b399fee49f06ea6> (<https://doi.org/10.21942/uva.29195480>).

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.soilbio.2026.110125>.

Data availability

The DNA sequence data generated in this study have been deposited in the National Center for Biotechnology Information under BioProject accession number PRJNA1272829 and are publicly available upon publication. The experimental data generated in this study are publicly available on Figshare at <https://figshare.com/s/dc562b399fee49f06ea6> (<https://doi.org/10.21942/uva.29195480>).

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