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RESEARCH ARTICLE

Primary metabolites in root exudates are not affected by long-term soil warming in a temperate forest

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Abstract

1. Primary metabolites in root exudates are essential for plant nutrition and rhizosphere microbiome function, potentially responding sensitively to climate warming. However, the effects of long-term soil warming on exudate metabolites in forests remain unclear.
2. We investigated how long-term soil warming (>14 years, +4°C) in a temperate mountain forest in Austria affects the root exudation rates and profiles of primary metabolites in *Picea abies* (Norway spruce), the dominant tree species at the site and explored how strongly root exudate rates are controlled by root tissue metabolism. We used targeted metabolite quantification to measure three major groups of primary metabolites—sugars, amino acids and organic acids—in root exudates and tissues.
3. Root exudation rates and profiles of primary metabolites showed no response to long-term soil warming, though sampling date had significant effects. Primary metabolites in root tissues and in root exudates showed largely overlapping compositional patterns, and their concentrations were strongly positively correlated, suggesting that root tissue metabolism plays a central role in controlling exudate composition and rate.
4. Our findings show that woody plant roots maintained metabolically stable exudation profiles under long-term soil warming, as supported by the absence of warming effects on exudates across years and seasons, despite independent measurements at the same site reporting pronounced increases in fine root growth and respiration. Together, these results indicate a warming-induced reallocation of root carbon away from exudation and towards structural and metabolic investment, highlighting the complexity and prioritization of carbon use strategies under climate change.

KEYWORDS
long-term soil warming, primary metabolites, root exudates, root tissue, temperate forest

1 | INTRODUCTION

Plants allocate 20%–50% of recent photosynthetic carbon (C) belowground and 5%–10% to the soil through root exudates (Brunn et al., 2022, 2025; Pausch & Kuzyakov, 2018; Robert et al., 2025). Root exudates contain a complex array of primary and secondary metabolites, such as amino acids, organic acids, sugars, flavonoids and terpenoids (Badri & Vivanco, 2009; Robert et al., 2025; Williams & de Vries, 2020). These compounds are not only a C source for microbial growth and proliferation, stimulating microbial decomposition of soil organic matter (Panchal et al., 2022; Pausch & Kuzyakov, 2018), but are also considered to serve as plant defensive compounds, as physical and chemical signals and to control interactions with other organisms (Badri & Vivanco, 2009; Panchal et al., 2022). The rates and profiles (refers to the detailed composition and concentration of various essential metabolites present within a specific sample) of root exudates vary depending on many factors, such as plant species (Proctor & He, 2017), root morphology (Williams et al., 2022), abiotic factors (i.e. temperature and moisture) (Berini et al., 2018; Williams & de Vries, 2020) and biotic factors (i.e. insect infestation or mycorrhizal colonization) (Lv et al., 2023; Meier et al., 2013; Robert et al., 2025). Among these factors, temperature is of utmost concern because global air temperatures rise continuously and rising temperatures often being accompanied by other stressors like soil moisture deficits (Berini et al., 2018; Reich et al., 2018). For instance, warming adversely affects plant photosynthesis by reducing soil moisture (Reich et al., 2018), which may in turn modify plant metabolite levels (Berini et al., 2018). These metabolic changes not only directly impact the biochemical interactions between plants and soil microbes but may also lead to unforeseen outcomes, reducing the plants beneficial role in mitigating climate change, specifically through reduced fixation and storage of atmospheric CO_2 in plant biomass and transfer into soils (Badri & Vivanco, 2009; Panchal et al., 2022).

Over recent decades, studies have extensively documented the impact of warming on root exudation rates, yet findings remain inconsistent (Heinzle et al., 2023; Leuschner et al., 2022; Wang et al., 2021; Xiong et al., 2020; Yin et al., 2013). Previous studies have reported contrasting responses of root exudation to warming: in some forests, exudation rates increased by 55%–78% under experimental warming or at warmer sites along altitudinal gradients (Leuschner et al., 2022; Yang et al., 2025; Yin et al., 2013), whereas other studies documented 15%–22% reductions or no significant changes in exudation under warming (Heinzle et al., 2023; Xiong et al., 2020). Such discrepancies may arise from differences in soil nitrogen (N) availability, plant species, plant age and experimental duration (Heinzle et al., 2023; Wang et al., 2021; Xiong et al., 2020; Yin et al., 2013), but our long-term soil warming experiment, which minimizes variation in species composition and nutrient inputs, provides a unique opportunity to disentangle these factors. Importantly, most previous research has focused on bulk root C exudation rates (Brunn et al., 2022; Yin et al., 2013), which masks the functional roles of specific compounds (Walker et al., 2023). Yet, primary metabolites—including organic acids, sugars and amino acids—are generally considered to represent a major fraction of root exudates and play crucial roles in fuelling microbial activity, nutrient mobilization and plant–soil feedbacks (Canarini et al., 2019; Gargallo-Garriga et al., 2018; Sandnes et al., 2005; Wilson et al., 2021). Profiling these metabolites at compound level therefore enables a mechanistic understanding of how root exudation mediates rhizosphere interactions, particularly under warming scenarios (Robert et al., 2025; Yang et al., 2025). Despite their ecological importance, however, only few studies have quantified primary metabolites in tree root exudates due to their low concentrations (e.g. organic acids in the low μM range; Ryan et al., 2001; Sandnes et al., 2005) and methodological challenges (Wang et al., 2018; Zhou et al., 2019). Although these compounds are difficult to measure, recent advances in targeted metabolomics now allow reliable identification and quantification of these compounds in root exudates, providing new opportunities to assess plant metabolic responses. Such approaches have provided valuable insights into plant metabolic responses to environmental change (Escolà Casas & Matamoros, 2021; Sardans et al., 2020). However, no long-term (>10 years) field warming experiment in forests has yet addressed how primary metabolite profiles in root exudates respond to warming, representing a major knowledge gap. Here, we combine a unique long-term soil warming platform with targeted metabolomics to investigate these responses in situ.

Previous studies have revealed that root morphological traits, such as specific root length, root tissue density and root diameter are viable predictors of root exudate profiles and of root exudation rates (Rathore et al., 2023; Williams et al., 2022; Yang et al., 2025). Exudation generally increases with higher specific root length and surface area but declines with greater diameter, as shown in perennial grasses, beech and conifers (Akatsuki & Makita, 2020; Degenhardt et al., 1998; Upadhyay et al., 2022). This follows the root economics spectrum along the trade-off between 'fast' acquisitive roots (thin diameter, high specific root length/surface area, high respiration and N concentration, short lifespan) and 'slow' conservative roots (thicker diameter, low metabolic activity, greater tissue density, longer lifespan) (Weemstra et al., 2016). By regulating the root–soil interface and carbon allocation strategies, these morphological root traits also shape metabolite profiles, with fine roots of small diameter preferentially releasing soluble sugars, amino acids and organic acids (Sun et al., 2021). Warming alters root morphology and physiology (Jiang et al., 2022a; Kwatcho-Kengdo et al., 2022), for example by increasing specific root length, surface area and tip density in temperate forests (Kwatcho-Kengdo et al., 2022). Root exudation occurs across the root surface and is tightly constrained by surface area related traits, such as specific root area (SRA); hence morphology–exudation linkages are expected to persist under warming, even if their strength and direction could shift with altered root physiology. However, how strong primary metabolite profiles of root exudation are linked to root morphology under long-term warming is still unknown.

Root exudation has also been linked to plant nutrition, in terms of exudate quantity and quality (Canarini et al., 2019; Robert

et al., 2025). Root exudation rates and exudate composition (e.g. sugars, organic acids, amino acids) play an essential role in mobilizing N and phosphorus (P) under low-nutrient conditions. Sugars and amino acids stimulate rhizosphere microbial activity and turnover, enhancing mineralization of soil organic matter and mobilizing N for plant uptake through the priming effects (Badri & Vivanco, 2009; Canarini et al., 2019; Zhalnina et al., 2018). Conversely, the exudation of organic acids such as citric acid mobilizes P through acidification and complexing iron thereby chemically liberating bound iron (Fe) and P (Badri & Vivanco, 2009; Jiang et al., 2022b; Tawaraya et al., 2018). These rhizosphere processes feedback on plant nutrition, enabling more efficient nutrient uptake. Warming can directly or indirectly influence soil nutrient availability, such as the bioavailability of N or P (Bai et al., 2013; Tian et al., 2023b), which may in turn alter the rates and profiles of root exudates (Jiang et al., 2022b; Tawaraya et al., 2018). For example, under P deficiency, the exudation of γ-aminobutyric acid and of carbohydrates was stimulated in maize plants (Carvalhais et al., 2010). Given their central role in nutrient mobilization and plant–soil feedbacks, primary metabolites are critical targets for understanding how root exudation responds to long-term warming.

Although the role of C supply to roots in mediating the rates of C release via root exudates is widely acknowledged (Brunn et al., 2022, 2025; Dilkes et al., 2004; Farrar & Jones, 2000), there's a gap in understanding how the concentration and dynamics of primary metabolites in root tissues relate to the flux rates of root exudates, particularly under climate warming. Resolving this gap is essential for linking plant C allocation with rhizosphere processes that regulate nutrient cycling and soil C turnover. Though the specific underlying transporters are yet to be characterized the exudation of primary metabolites in fine roots is likely mediated by relatively specific efflux carriers, which enable the passive efflux of (groups of) primary metabolites (Canarini et al., 2019; Robert et al., 2025). Primary metabolite exudation is therefore controlled by the inside-outside pointing concentration gradient driving the facilitated diffusion process and is potentially regulated through up- and downregulation of the gene expression of those transporters (Canarini et al., 2019; Robert et al., 2025). The carriers thereby confer a high level of regulation and of specificity to the root exudation process, as metabolites lacking carrier systems will not turn up in the root exudates in substantial quantities, given that cellular bio-membranes are largely impermeable to hydrophilic polar metabolites. Thus, not all metabolites present in root tissues are released into exudates, resulting in only a partial overlap between the two metabolomes. However, the relationship between the profiles of primary metabolites in root tissues and in root exudates is still poorly known (Canarini et al., 2016; Robert et al., 2025; Weinhold et al., 2021), particularly under changing climate conditions, and this represents an important field for further scientific investigation.

Here, utilizing the infrastructure of the Achenkirch soil warming experiment, we investigated how prolonged soil warming (>10 years) influences the quantity and profiles of primary metabolites in tree root exudates in situ. In the Achenkirch forest long-term warming increased fine root biomass by 18% and fine root growth by 128% (Kwatcho-Kengdo et al., 2022), while microbial communities showed strong signs of (increased) P limitation in warmed soil (Shi et al., 2023; Tian et al., 2023b). We therefore hypothesized that (1) soil warming increases the rates of primary metabolites in root exudates due to enhanced root activity and growth, with a particular stimulation of organic acid release to mobilize limiting inorganic phosphorus. We therefore expected that warming will change the composition of root exudates, shifting towards a higher share of organic acids. We also expected (2) that warminginduced modifications of root morphology, such as higher specific root length and surface area, to accompany the increase of primary metabolite exudation rates, reflecting the role of morphology in regulating rhizosphere C fluxes. Finally, we hypothesized (3) that root exudate profiles would differ from root tissue metabolomes due to selective export mechanisms, but that for metabolites present in both compartments, exudation rates would be positively correlated with tissue concentrations, consistent with diffusiondriven transport.

2 | MATERIALS AND METHODS

2.1 | Study location and experimental design

This study was conducted in a temperate mountain forest located in the Northern Limestone Alps of Achenkirch, Austria (47°34′50″ N, 11°38′21″ E) at an elevation of 910 m a.s.l. This >120-year-old forest primarily consists of spruce trees (*Picea abies*, approximately 80%), interspersed with European beech (*Fagus sylvatica*, about 20%). The soils are characterized by patches of Chromic Cambisols, varying between 30 and 60 cm in depth and shallower Rendzic Leptosols, measuring between 10 and 30 cm in depth. Dolomite bedrock underlies the forest, and the prevailing humus form is Mull. Due to the carbonate bedrock, the soil has a nearly neutral pH. The site receives an average annual rainfall of 1563 mm and has an average annual air temperature of 6.8°C (both from 1988 to 2017). Annual wet N deposition is approximately 15 kg ha^{-1}.

The soil warming facility was laid out in a paired block design consisting of 12 plots, each measuring 2 m × 2 m. These plots were grouped into six blocks, with three blocks established in 2004 and three in 2007, resulting in a total of six pairs. Each pair of plots within a block was subjected to one of two treatments: ambient temperature serving as the control and soil warming by 4°C above ambient. To achieve the warming effect, soil warming cables (Etherma, Salzburg, Austria) were installed at a depth of 3 cm, spaced 7.5 cm apart, in the warming plots. Similarly, dummy cables were inserted in the control plots to replicate the physical disturbance caused by the installation of active cables. During the snow-free period, which typically spans from April to November, the soil temperature in the warmed plots was maintained at a consistent 4°C above that of the control plots. During winter the soil warming was stalled to omit unintended effects by snow melt. The soil temperature at 5 cm depth increased by 4.1°C ± 0.1°C across all sampling periods (Heinzle et al., 2023).

Baseline soil C and nutrient properties of the control and warmed plots at this site have been characterized in detail previously (Tian et al., 2023a, 2023b) and are summarized in Table S1. In brief, long-term soil warming reduced total soil P but increased dissolved organic C, dissolved organic N and NH_4^+. In contrast, total soil C, total soil N, NO_3^- and Olsen inorganic P remained largely unchanged. These background differences in soil C and nutrient status between control and warmed plots provide the biogeochemical context for interpreting the root exudation responses examined in this study. No specific permits were required for the fieldwork or soil sampling at the long-term soil warming experimental site.

2.2 | Root exudate and root tissue collection

Root exudates were collected during May, August and October 2020, as well as in April, July and October 2021 using a dedicated root cuvette system designed for in situ root exudate collection (Phillips et al., 2008), as modified and described in Heinzle et al. (2023). Briefly, in each subplot two terminal fine root branches (5–10 cm long, consisting of first to third order fine roots <2 mm diameter) were identified and traced in the top 10 cm of soil, and cleaned by detaching soil particles, by rinsing with ultrapure (Milli-Q) water and by treatment with 0.5 mM $CaCl_2$ for an hour. Then the live (attached) root branches were placed in 30 mL glass syringes equipped with a three-way Luer lock and filled with acid-washed glass beads (1 mm diameter). After sealing the syringes with Parafilm, each one was flushed twice with 15 mL 0.5 mM $CaCl_2$ solution (total 30 mL) to remove residual metabolites and then filled with 15 mL $CaCl_2$ as the exudation medium. The syringes were placed at ~5 cm soil depth between heating or dummy cables. For each plot, two replicate root exudate samples and one blank control were collected, yielding 24 exudate samples and 12 blanks per campaign (36 samples in total). After 24 h of in situ incubation, the exudation medium was collected by suction, followed by two additional flushes with 15 mL $CaCl_2$ solution each. This procedure yielded a final exudate volume of ~38–42 mL per sample. The sample solution and the flushing solutions were then combined, passed through Chromafil CA-20/25-sterile cellulose acetate filters (0.20 μm pore size) and stored at −20°C. Roots were cut at the location where they entered the syringe and carefully rinsed from glass beads, after the exudate sampling was completed. A subsample of the same root branch adjacent to the studied segment was collected for genetic analysis of tree species affiliation, showing that across all five sampling times more than 95% of fine root samples were assigned to Norway spruce, with the remainder exhibiting mixed spruce–beech signals or being unidentifiable (Heinzle et al., 2023). The root segments were cooled at +4°C until they were scanned for root morphological traits and subsequent freeze-drying for root metabolite analysis (Heinzle et al., 2023; Liu et al., 2024). Therefore, root morphology and root C exudation (Heinzle et al., 2023), root metabolite content (Liu et al., 2024) and root primary metabolite exudation were measured all on the same root samples. For metabolite analysis, 10 mL of each exudate sample was freeze-dried and subsequently redissolved in 1 mL of Milli-Q water to concentrate the analytes above the instrumental detection limit.

2.3 | Root morphological trait analyses

The root segments were scanned for root morphological traits including root length, surface area and volume using WinRHIZO 2004b (Regents Instruments, Inc., Quebec, Canada). After scanning, the roots were freeze-dried to establish dry mass. Metrics like SRL, SRA and root tissue density (RTD) were derived from the ratio of root dry mass to its length, area and volume (Heinzle et al., 2023). As WinRHIZO is known to have limited accuracy for estimating root volume (Seethepalli et al., 2021), results involving root volume and derived RTD should be interpreted with caution.

2.4 | Metabolite extraction from root tissue

We performed paired root tissue metabolite determinations for all those individual roots that were studied for root exudation. Root tissue metabolites were extracted and determined and their response to warming and season tested recently from the same experiment (Liu et al., 2024). In brief, for metabolite extraction from freeze-dried root tissues, samples were pulverized in a ball mill and 20–40 mg of fine powder (<10 μm) was subjected to extraction in 2 mL vials using 1.5 mL of methanol/chloroform/MQ mixture (12:3:5 v/v/v) at 70°C for 30 min with intermittent vortexing. Cooled samples were centrifuged at 10,000 *g* for 5 min, and 800 μL supernatant was pipetted into a new vial. Phase separation was achieved by adding 800 μL MQ and 250 μL chloroform and mixing thoroughly, followed by centrifugation to partition chloroform and aqueous phases. The aqueous phase was further purified by transferring 1.2 mL into a new vial, adding 500 μL chloroform, mixing and centrifuging to separate phases. The final aqueous phase (1.0 mL) was vacuum-dried (Speed Vac, Savant; Thermo Fisher) and redissolved in 1.0 mL MQ water for metabolite analysis.

2.5 | Root exudate and root tissue metabolite analysis

2.5.1 | Standards and reagents

Exudate and root tissue compounds were identified and quantified using authentic standards, based on retention time and mass spectral convergence. All organic acid, amino acid and sugar standards were purchased from Sigma-Aldrich (St. Louis, MO). Organic acids included acetic acid, *cis*-aconitic acid, citric acid, formic acid, fumaric acid, maleic acid, malic acid, malonic acid, oxaloacetic acid, oxalic acid, succinic acid, tartaric acid and vanillic acid; amino acids

included α-alanine, arginine, asparagine, aspartic acid, glutamine, glutamic acid, glycine, isoleucine, leucine, lysine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, β-alanine and γ-aminobutyric acid; and sugars included arabinose, galacturonic acid, glucosamine, fucose, mannose, rhamnose, ribose, deoxyribose, maltose, glucose, raffinose, sucrose and fructose. For organic acids, individual stock solutions were prepared at 1 g L^{-1} and pooled to generate a mixed standard with 100 mg L^{-1} of each analyte. A serial dilution ranging from 5 to 100 mg L^{-1} was prepared for calibration and method validation. For amino acids and sugars, stock solutions were prepared at a concentration of 20 mM and subsequently combined to yield a mixed standard containing 1 mM of each analyte. Calibration curves were established using a serial dilution ranging from 20 nM to 25 μM, which was also employed for method validation. Metabolite concentrations in the root tissue and root exudate samples—estimated via peak areas—were quantified against these calibration curves.

2.5.2 | Amino acids

Amino acids were analysed using an Ultimate 3000 UPLC (Thermo Fisher Scientific Dionex, Sunnyvale, CA, USA) after derivatization with AQC reagent (AccQ-Tag Ultra Derivatization Kit; Waters Corp., Milford, USA). The UPLC system was coupled to a Q Exactive Orbitrap mass spectrometer with an ESI source run in the positive ion mode (Thermo Fisher Scientific, Inc., USA). For the derivatization process, 10 μL sample was added to 70 μL borate buffer in a 1.5 mL polypropylene tube, followed by 20 μL AQC reagent. The mixture was vortexed and heated for 10 min at 55°C. Once derivatized, the samples underwent chromatographic analysis on a 2.1 × 100 mm, 1.7 μm C18 reverse-phase column (AccQ-TagTM ULTRA; Waters, USA) that was maintained at 55°C, and the injection volume was 1 μL. The eluents utilized were 0.1% formic acid in Milli-Q water (A) and 100% acetonitrile (B), with a flow rate of 0.4 mL min^{-1}, following a binary gradient program: 0–0.5 min, 0.1% B; 0.5–2.5 min, 0.1%–5.0% B; 2.5–8 min, 5.0%–20% B; 8–8.25 min, 20% B; 8.25–11 min, 20%–10% B; 11–11.2 min, 10%–0.1% B; 11.2–15 min, 0.1% B. Analysis conditions of the Orbitrap mass spectrometer are listed below: spray voltage at 3 kV, capillary temperature at 320°C, resolution at 70,000 and mass scan range of 50–750 m/z.

2.5.3 | Organic acids

Organic acids underwent direct analysis (no pre-column derivatization) on a Dionex ICS-5000 ion chromatography system (Thermo Fisher Scientific Dionex, Sunnyvale, CA, USA) coupled to a Q Exactive Orbitrap mass spectrometer (Thermo Fisher Scientific, Inc., USA). Using a Dionex IonPac AS11-HC analytical column (2 × 250 mm) paired with an AS11-HC Guard Column (2 × 250 mm), the samples were separated at a flow rate of 0.3 mL min^{-1} and a column temperature of 30°C. Samples (10 μL injection volume) were separated according to the following gradient: 0–0.2 min, 1 mM KOH; 0.2–17 min, 1–85 mM; 17–20 min, 85 mM KOH; 20–21 min, 85–1 mM KOH and 21–30 min, 1 mM KOH. The gradient was produced online using a EGM4 eluent generator. During analysis, the Orbitrap was operated in negative ESI mode with parameters set to: spray voltage of 3 kV, capillary temperature of 320°C, resolution of 70,000 and a mass scan range between 50 and 750 m/z. Due to its mass being below the Orbitrap's detection limit (<50 m/z), formic acid (monoisotopic mass 44.99765 g/mol) was exclusively quantified using conductivity detection by ICS-5000 and the Chromeleon 7.2 software. In contrast, the remaining organic acids were quantified using the mass spectrometric signals with Freestyle 1.7 software by Thermo Scientific.

2.5.4 | Sugars

Sugars were analysed via pre-column derivatization with 1-phenyl-3-methyl-5-pyrazolone (PMP; Sigma-Aldrich) using the Ultimate 3000 UPLC system coupled to a Q Exactive Orbitrap mass spectrometer (Thermo Scientific) (Salas et al., 2023). Fresh 0.5 M PMP solution in methanol was prepared daily and 100 μL aliquots of samples or standards were mixed with 100 μL PMP solution and 200 μL ammonia (25%–30% ammonium hydroxide solution; Sigma-Aldrich). The mixture was then vortexed briefly and subjected to a 20-min reaction at 70°C in a water bath. After cooling, it was neutralized with 200 μL 100% formic acid and subsequently filtered to be ready for LC–MS analysis. The chromatographic separation was conducted on a 2.1 × 100 mm AccQ-TagTM ULTRA column (Waters, USA). The mobile phases used for the sugar separation were 20 mM ammonium acetate in water (solvent A) and acetonitrile (solvent B). The gradient was as follows: 0–0.5 min, 13% B; 0.5–2 min, 13%–16% B; 2–15 min, 16%–21% B; 15–16 min, 21%–60% B; 16–17 min, 60%–90% B; 17–20 min, 90%–13% B; 20–25 min, 13% B. The total analysis time was 25 min per injection, with a constant flow rate of 0.3 mL min^{-1}. An injection volume of 2 μL and a column temperature of 35°C were used. For analysis, the Orbitrap mass spectrometer was set to positive ESI mode, with further parameters being 3.5 kV spray voltage, 300°C capillary temperature and 50–1000 m/z mass scan range.

Fructose, sucrose and raffinose do not respond well to PMP derivatization due to being non-reducing sugars or having a less reactive keto group, or because of their unique molecular structure causing steric hindrance. To tackle this, we utilized the Dionex ICS-5000 ion chromatography system (Dionex, Thermo Scientific) for direct sugar separation using a CarboPac PA20 analytical column (4 × 250 mm i.d.), paired with a CarboPac PA20 guard column (4 × 50 mm i.d.). For sugar detection, we used pulsed amperometric detection with a gold electrode and a palladium hydrogen (PdH) reference electrode. The elution was performed at a flow rate of 0.3 mL min^{-1}, with a column temperature of 30°C, an injection volume of 10 μL and a KOH gradient as follows: 0–15 min, 20 mM KOH; 15–20 min, 20 mM to 100 mM KOH; 20–25 min, 100 mM KOH; 25–30 min, 100 to 20 mM KOH.

2.5.5 | Calculation of root exudation rates

The absolute concentrations of metabolites were corrected by subtracting the values obtained from the blank controls. Specifically, the net exudation rates of primary metabolites were calculated based on blank-corrected concentrations. The exudation rates of primary metabolites from root systems were quantified using three distinct metrics: mass-normalized ($\mu g\,g^{-1}$ root biomass day^{-1}), area-specific ($ng\,cm^{-2}$ root area day^{-1}) and length-specific ($ng\,cm^{-1}$ root length day^{-1}) fluxes. These rates were calculated by multiplying the measured metabolite concentrations ($ng\,L^{-1}$) in the exudate collection solution by the sampled solution volume collected using the syringe-based system. Subsequently, the total amount of primary metabolites released from the roots within each cuvette was normalized to the corresponding fine root biomass, root area and root length to yield mass-normalized, area-specific and length-specific exudation rates, respectively.

2.6 | Statistical analysis

The statistical analyses were performed using R software 4.2.3 version. Logarithmic or square root transformations were applied to metabolite concentration data to ensure homoscedasticity and normality when necessary. Linear mixed-effects models using the 'lme4' package were run to assess the effects of sampling time and warming treatment, considering warming and sampling date as fixed factors and block and warming duration as random factors. We used Tukey post hoc tests to explore group-specific differences when interactions showed statistical significance ($p < 0.05$). To address the false discovery rate (FDR) associated with multiple comparisons, we applied the Benjamini–Hochberg (BH) correction to the raw *p*-values using the R function '*p.adjust* ()'.

To determine differences in primary metabolite profiles in root exudates between control and warming treatments, we employed PERMANOVA (through the ADONIS procedure) based on the Bray–Curtis distance. Differences in metabolite profiles were visualized using non-metric multidimensional scaling (NMDS), and Spearman correlations revealed compound associations to NMDS1 and NMDS2 axis scores. These analyses leveraged the 'vegan' and 'corrplot' R packages. We used the scores of the first two components (NMDS1 and NMDS2) as summarized root exudate profile proxies for subsequent analyses. Using the 'envfit' function with 999 permutations, sampling time and treatment were fitted as centroids, and soil moisture, temperature, root diameter, root surface area, SRL, RTD and SRA were fitted as vectors onto the NMDS ordination plot to examine how root exudate profiles were related to these variables. Venn diagrams were constructed using the 'VennDiagram' package to visualize shared and unique metabolites between root tissue and root exudates. Principal component analysis (PCA) was performed to investigate the metabolite profiles of root tissues compared to root exudates. Additionally, regressions were run to relate the natural logarithms of the average metabolite values in root exudates to those in root tissues within each metabolite group (amino acids, organic acids and sugars).

We analysed 33 common primary metabolites (amino acids, organic acids and sugars) in both root exudates and fine root tissues, pooling all samples across seasons and treatments. Hierarchical clustering was applied separately to each matrix using Euclidean distance and Ward's method (ward.D2). Dendrograms were generated using the hclust() function in R and visualized with the 'dendextend' package. To compare clustering structures, we constructed a mirror dendrogram and calculated the entanglement index and cophenetic correlation coefficient to quantify topological similarity between the two trees.

Finally, utilizing the 'LEAPS' package in R, we conducted regression subset selection to identify the subset of predictor variables that best explains the response variable (root exudate profiles) in a regression model. We applied an all-subset regression based on a branch-and-bound algorithm to ascertain the optimal model elucidating root exudate profiles, as gauged by the maximum R^2 value. After this model selection, we rigorously assessed the influence of distinct predictors on root exudation patterns, ensuring the absence of confounding effects from other incorporated variables. Our chosen predictors encompassed both abiotic factors, such as soil moisture and temperature and biotic properties such as root morphological attributes, namely specific root length, root tissue density, specific root area and diameter.

3 | RESULTS

3.1 | Warming effects on the rates and profiles of root exudates

In the control treatment, amino acids, organic acids and sugars constituted 7.7%, 47.2% and 45.1% of the primary metabolites in root exudates based on the fine root biomass, respectively. These proportions remained consistent in the warming treatment (Figure 1d). Similarly, based on root surface area (Figure 1e) and root length (Figure 1f), there were no discernible differences between the control and warming plots. The linear mixed-effects models showed that warming had no significant effect on the root exudation rates of primary metabolite groups (i.e. total organic acids [TOA]; total amino acids [TAA;] total sugars [TS]; and total primary metabolites [TPM]) based on fine root biomass (Figure 1a; Table S2), root surface area (Figure 1b; Table S2) and root length (Figure 1c; Table S2), but sampling date had significant effects (Table S2). The exudation rates of TPM and TS per fine root biomass were lowest in spring 2021 and peaked in summer 2021 (Figure 1a). Exudation of TAA was lowest in summer 2020 and reached the highest values in autumn 2021, while TOA exudation was lowest in summer 2021 and highest in autumn 2020. Notably, compared to totals, that is group-wise rates, root exudation rates of single compounds, such as glycine, proline, fumaric acid and ribose, were increased in warmed plots (Figure S1a–c; Table S3).

Non-parametric dissimilarity analyses following PERMANOVA (Adonis) (Table 1) revealed significant differences in primary metabolite profiles of root exudates across sampling dates ($F = 56.563$, $p = 0.001$), but not between warmed and control treatments ($F = 1.231$, $p = 0.277$, Table 1). Although warming enhanced the

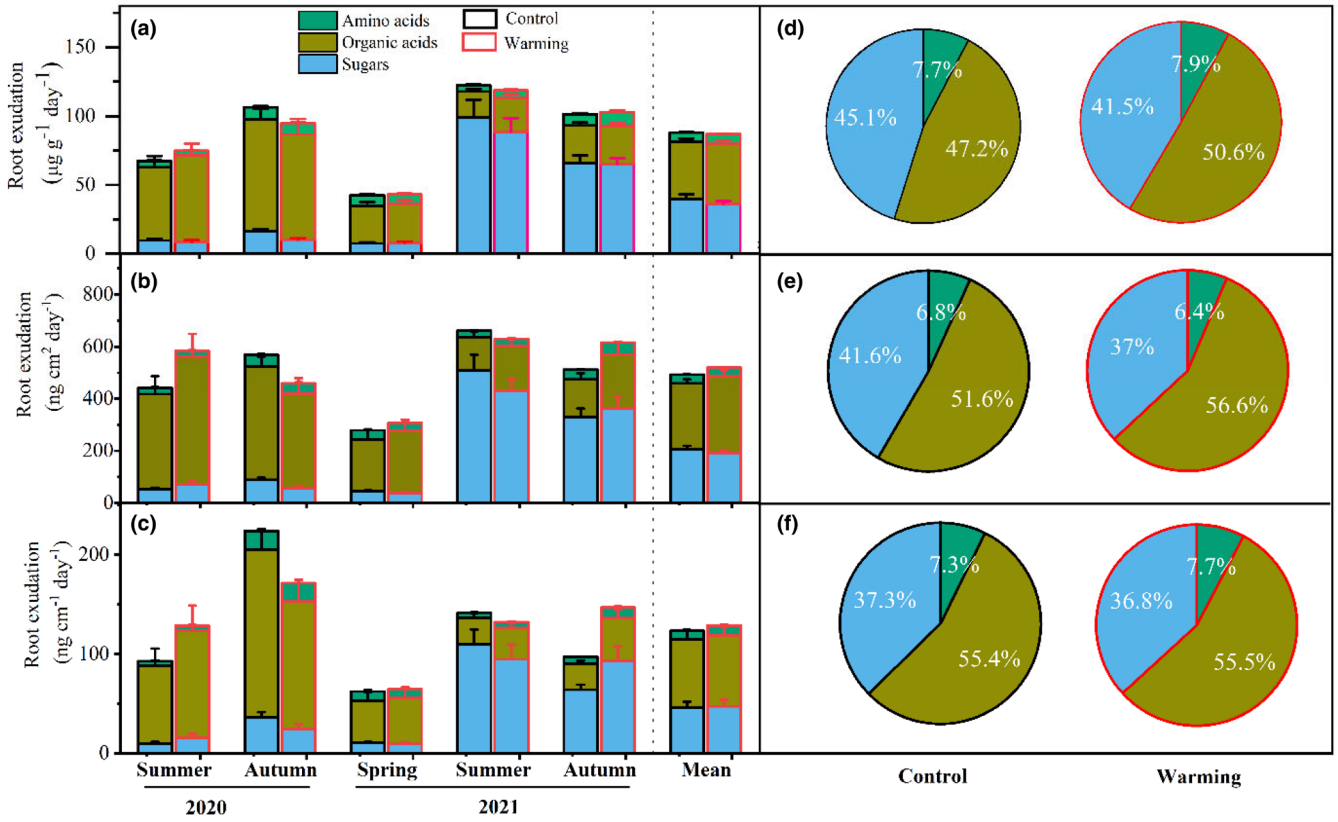

FIGURE 1 Effect of warming and season on composition fine root exudation rates base on the specific fine root biomass (a), root surface area (b) and root length (c), and the proportion (d–f) of root exudates by major groups of primary metabolites. Values in brackets are the relative percentage of each group. Error bars represent standard errors of the means ($n=6$). Pie charts show the relative proportions of sugars, amino acids and organic acids within the quantified metabolite pool detected by our targeted method, illustrating treatment-related shifts rather than absolute exudate composition.

TABLE 1 Significance tests of the influences of warming and sampling date on root exudate profiles.

	PERMANOVA tests			
Source	df	R^2	Pseudo *F*	*p*
Warming	1	0.003	1.231	0.277
Sampling date	4	0.659	56.563	**0.001**
Warming × sampling date	4	0.017	1.469	0.110
Residual	110	0.321		
Total	119	1.000		

Note: All statistical analyses were tested against $\alpha=0.05$, and statistically significant results are highlighted in bold. PERMANOVA tests were performed (Adonis) on the basis of Bray–Curtis distances.

exudation of a few individual metabolites (Figure S1), these changes were not systematic at the group level. NMDS analysis corroborated these findings (Figure 2a, Stress = 0.0802). NMDS 1 delineated metabolite distinctions between 2021 and 2022, while NMDS 2 highlighted the differentiation of metabolite profiles between summer, autumn and spring seasons (Figure 2a). Most metabolites, and most notably amino acids and organic acids, were negatively correlated with NMDS1, whereas sugars showed a positive association. Conversely, on NMDS2, amino acids exhibited a positive correlation, while both organic acids and sugars were negatively associated (Figure 2b).

3.2 | Drivers of variation in root exudate profiles

The variable most strongly related to the first two ordination axes was soil temperature ($R^2=0.583$, $p=0.001$, Axis 2), followed by root surface area ($R^2=0.412$, $p=0.001$, Axis 1), root diameter ($R^2=0.204$, $p=0.001$, Axis 1) and specific root length ($R^2=0.151$, $p=0.001$, Axis 1) (Figure 2a). Root surface area and specific root length were positively related to Axis 1, while root diameter showed a negative relationship with Axis 1. For Axis 2, soil temperature was strongly negatively correlated with root exudate profiles. Using NMDS1 scores as response variables, the best model selection pinpointed four predictors accounting for 47% of the variation, with root surface area being significant and specific root length and root diameter being marginally significant. For NMDS2, three predictors explained 59% variation, with both soil temperature and root diameter being significant and specific root length being marginally significant (Table 2). These results were consistent with linear regression analysis (Figure S2).

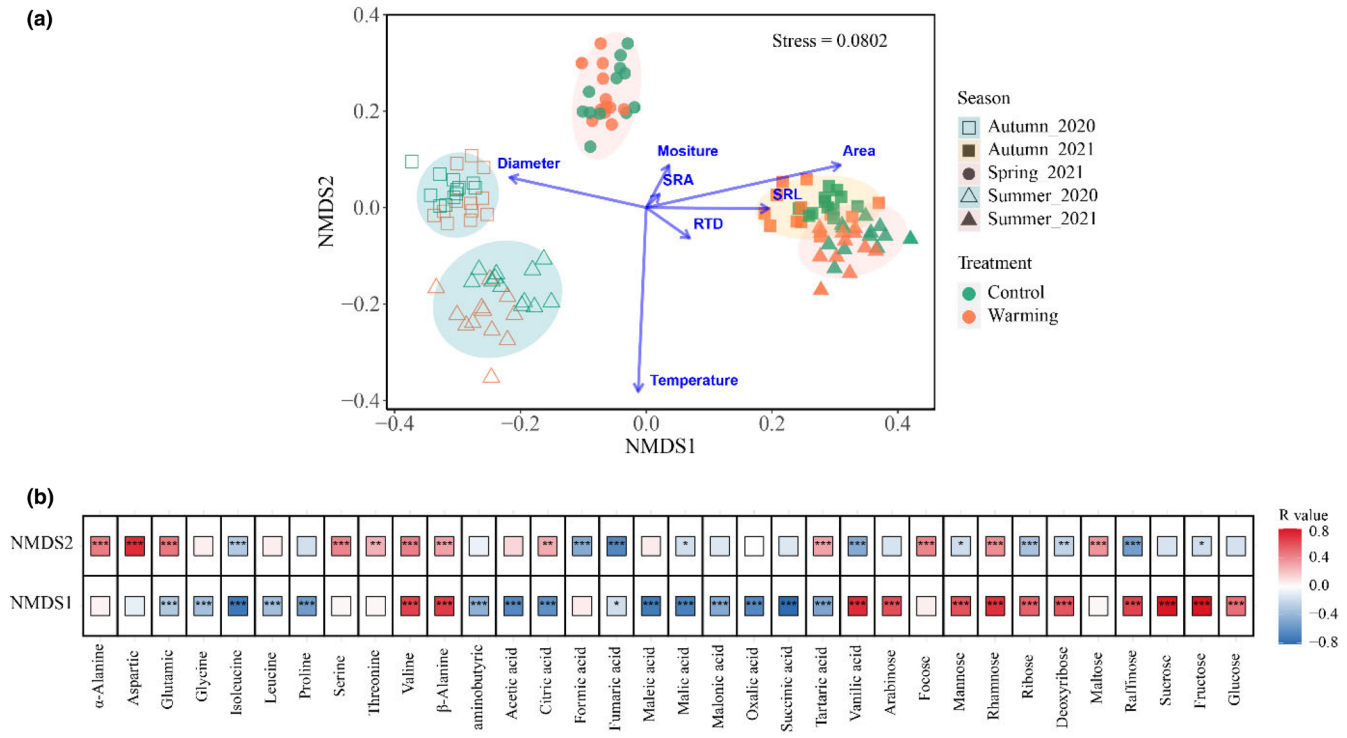

FIGURE 2 Non-metric multidimensional scaling (NMDS) (a) plots detailing the influence of warming and season on primary metabolites profiles of root exudates and spearman's rank correlation matrix of the root exudates metabolites compounds and NMDS scores (b). The direction and length of the vectors of environment factors or root morphology trait are computed by Bray–Curtis distances the 'envfit()' function in the vegan package. The scale from −1 (blue) to 1 (red) with a median of 0 (white). Significance levels are indicated relative to NA: ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$. RSA, root surface area; RTD, root tissue density; SRA, specific root area; SRL, specific root length.

TABLE 2 Best predictors for explaining variation of root exudate composition.

Predictors	NMDS1 scores	NMDS2 scores
Adjusted R^2	0.25	0.59
p	4.533e-07	<2.2e-16
Soil moisture		
Soil temperature	✓	✓**
Root diameter	✓***	✓*
SRL	✓*	✓°
SRA	✓*	
RTD	✓*	

Note: Tick marks indicate predictors selected in the best model. The effect of selected predictors on exudate composition was tested in the R program using linear models (function *lm*). Significance levels: °0.1; *0.05; **0.01,*** 0.001.

Abbreviations: RTD, root tissue density; SRA, specific root area; SRL, specific root length.

3.3 | Comparison of metabolite profiles in root tissues and root exudates

We quantified 34 primary metabolites in the root exudates, comprising 12 amino acids, 11 organic acids and 11 sugars, across five sampling dates in both warmed and control treatments. More metabolites were found in root tissues than in root exudates, with counts of 44 metabolites in root tissues and 34 in root exudates, respectively (Figure 3a). All metabolites detected in root exudates were also present in root tissues, except for maleic acid. Conversely, 11 unique compounds were identified exclusively in root tissues. Principal component analysis (PCA) distinctly segregated the metabolite profiles of root tissues and root exudates, whether considering only shared compounds (Figure 3b) or the entire metabolite profiles (Figure 3c). Amino acids and organic acids were the primary contributors to the separation along PC1, while sugars (specifically arabinose) and organic acids (such as acetic acid) drove the distinction along PC2, for both shared and all metabolite profiles. When metabolites were categorized by compound group, all groups showed differences between root tissues and root exudates (Figure S3).

Significant positive correlations were identified between metabolite levels in root tissues and in root exudates: for all compounds together (Figure 4a), for organic acids (Figure 4c) and for sugars (Figure 4d), with amino acids displaying only a marginally positive correlation (Figure 4b). However, when considering different treatments, the positive correlations for all compounds and sugars persisted, whereas organic acids and amino acids showed no significant correlation between tissue and exudate (Figure S4).

Hierarchical clustering revealed distinct compositional structures between root exudates and tissues (Figure 5). In exudates,

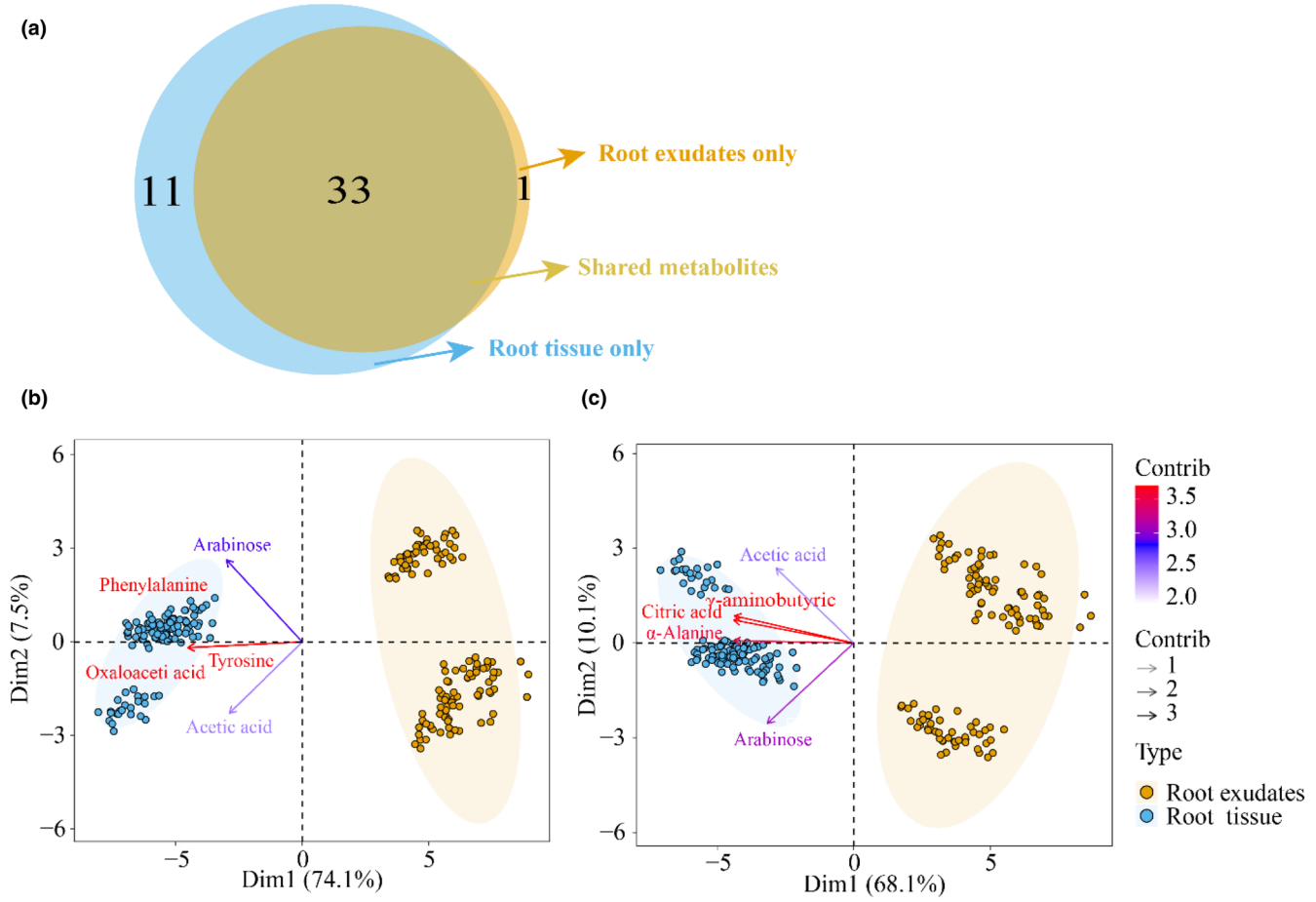

FIGURE 3 Venn diagram showing the numbers of shared and unique primary metabolites identified in root tissue and root exudates (a), with labels indicating 'Root tissue only', 'Root exudates only' and 'Shared metabolites' for clarity. Principal component analysis (PCA) biplots of the first two significant components are shown for the common metabolites (b) and for all metabolites (c) in root tissue (blue) and root exudates (yellow).

sugars such as glucose, fructose and mannose clustered closely, suggesting coordinated exudation, whereas these metabolites were more dispersed in tissue profiles. A mirror dendrogram linking shared metabolites yielded an entanglement index of 0.427 and a cophenetic correlation of 0.37, indicating moderate divergence in hierarchical structure between the two compartments.

4 | DISCUSSION

4.1 | Response of primary metabolites in root exudates to soil warming

In partial support of our first hypothesis, we found that soil warming enhanced the release of a few individual metabolites, but these increases were insufficient to modify overall exudation rates or the composition of primary metabolites (Figure 1; Figure S1). Previous studies at our experimental site have shown that plant roots and soil microbes differ in the macronutrients constraining their function. Specifically, root P concentrations did not decrease under warming indicating plant P sufficiency (Kwatcho-Kengdo et al., 2022), whereas microbes exhibited severe co-limitation by C and P (Shi et al., 2023). This difference likely explains the absence of a root P limitation response, which in vascular plants is generally expressed as enhanced root organic acid exudation under conditions of warming and P limitation. In contrast, though plant roots can also produce extracellular phosphatases, the strong increase in soil phosphatases in warmed soil was likely originating from soil microbes, as a response to strong microbial P limitation which also showed up in declines in microbial growth in warmed soils and decreased microbial biomass P (Tian et al., 2023a). Differential nutrient constraints of plants and microbes in the same ecosystem have been proposed for high latitude systems and beyond (Čapek et al., 2018), with far-reaching biogeochemical implications for global change effects, including warming responses of vegetation and soil microbes.

However, aside of organic acids we also found no warming response in exudation of for other classes of root primary metabolites, that is in sugars and amino acids. This clearly contrasts the expectation in our first hypothesis that faster root growth and higher root respiration in warmed plots (Kwatcho-Kengdo et al., 2022, 2023; Schindlbacher et al., 2009) will be paralleled by generally higher rates of root metabolism and higher primary metabolite contents. We

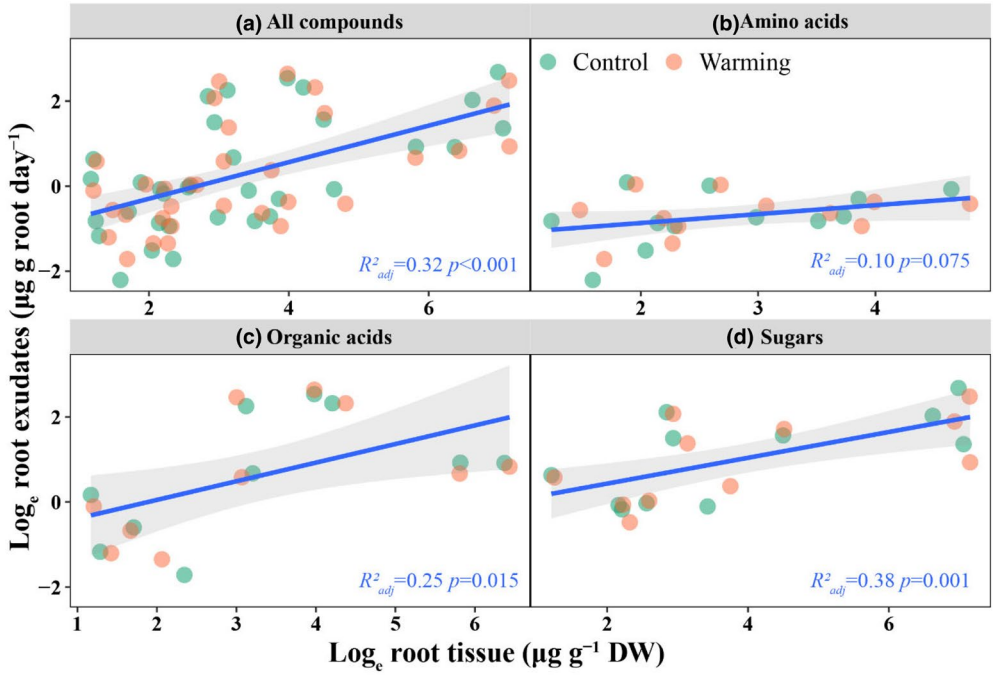

FIGURE 4 Relationship between root biomass metabolite concentration with root exudate metabolite concentrations for different metablite classes: (a) all compounds; (b) amino acids; (c) organic acids; (d) sugars. Values are expressed as natural logarithm of root biomass concentrations (grams per dry matter) and root exudates rates (gram per gram of dry root in per day). Each point represents the average value of each investigated metabolite. R^2 and equation are reported when relationships were significant.

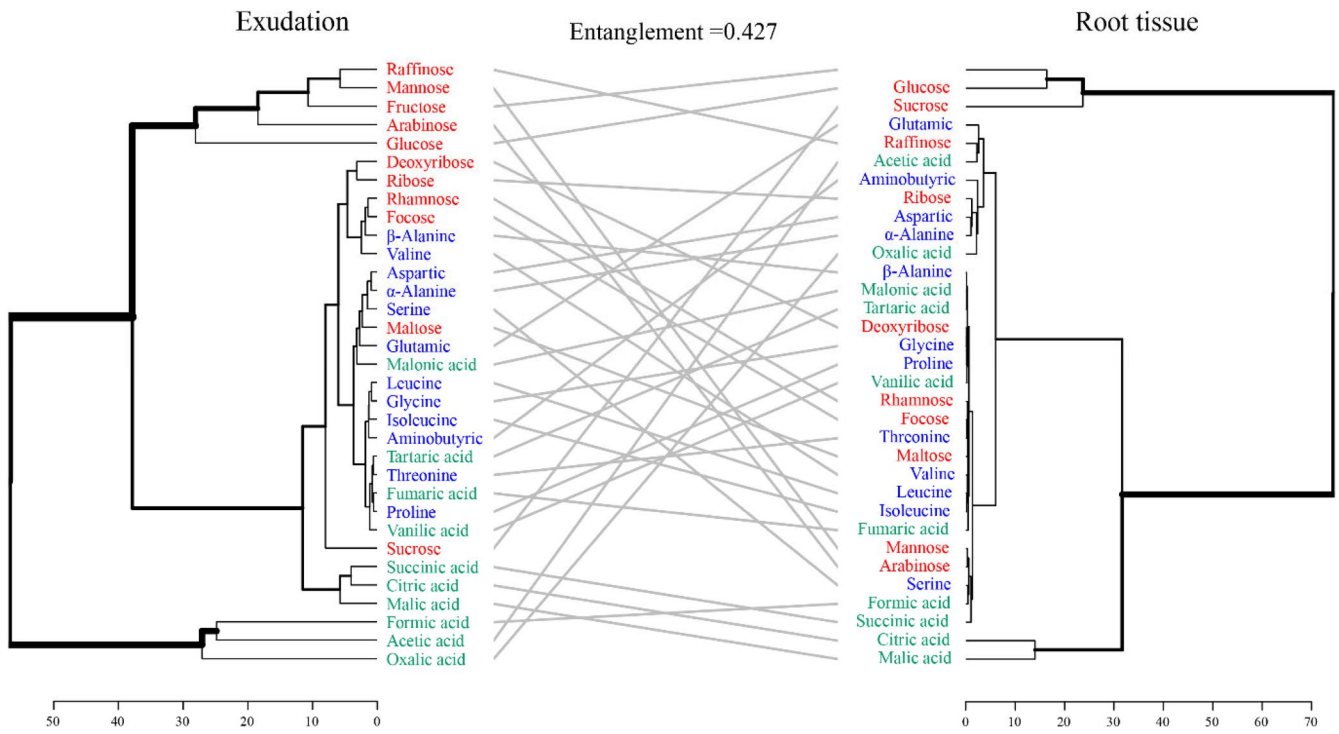

FIGURE 5 Mirror dendrogram comparing hierarchical clustering of root exudate and root tissue metabolites. Left and right panels show Ward's hierarchical clustering of standardized concentrations of primary metabolites in root exudates and root tissues, respectively. Metabolites are coloured by class: Blue (amino acids), green (organic acids), red (sugars). Grey lines link identical metabolites across trees.

hypothesized that higher primary metabolite contents of warmed roots would then trigger higher rates of root exudation of those metabolites. Although warming increased root amino acids and sugars (Liu et al., 2024), this did not translate into greater exudation rates of these compounds. Root C exudation showed no long-term warming effect but was sensitive to short-term warming (Heinzle et al., 2023), and

primary metabolite exudation likewise showed no response to long-term warming (this study). Despite pronounced stimulation of fine root growth which increased by 128% (Kwatcho-Kengdo et al., 2022) and fine root respiration rising by 50%–90% in warmed soils (Schindlbacher et al., 2025), the additional belowground C appeared to be allocated mainly to growth and maintenance respiration rather than to exudation, possibly reflecting tight physiological regulation of exudation and acclimation of root metabolism under chronic warming.

Aside from altered root C allocation the lack of warming response of root exudation may be explained by concomitant but inverse effects of soil warming on soil moisture dynamics, soil nutrient availability and root morphology. Soil moisture plays a pivotal role in modulating primary metabolite profiles and root exudates rates. For instance, plant net C allocation belowground and into root exudates tended to increase under drought (Brunn et al., 2022), which would likely be accompanied by increased root exudation of primary metabolites. In another study plant root exudates were dominated by secondary metabolites under drought and shifted to primary metabolites during drought recovery (Gargallo-Garriga et al., 2018). Soil warming is generally expected to reduce soil moisture levels, possibly leading to drier conditions, while our findings showed no substantial change between control and warming treatments due to the frequent occurrence of rainfall in the study region (Heinzle et al., 2023). The stability in soil moisture at the study site might partially explain why the root exudation rates of primary metabolites were unaffected by warming. It is noteworthy, however, that while the overall exudation rates of primary metabolites remained unchanged, specific compounds such as proline and glycine showed increased exudation rates (Figure S1). These findings suggest that Norway spruce may employ nuanced strategies to adapt their exudate profiles in response to climatic warming.

Soil nutrient availability is another key factor influencing the rates and the profiles of root exudates. Numerous studies have shown that plants can promote the accessibility of soil mineral nutrients, specifically by secreting organic acids to solubilize P (Canarini et al., 2019; Lambers et al., 2006), by releasing biological nitrification inhibitors to optimize the utilization of N (Subbarao et al., 2007), or by generally upregulating the exudation of primary metabolites to trigger a rhizosphere priming effects to mobilize soil N (Dijkstra et al., 2013). Our recent research at the same site revealed that prolonged soil warming led to a reduction in soil N and P content (Tian et al., 2023a, 2023b). This was expected to result in an increase in primary metabolites in root exudates. However, our findings suggest that root exudates might not be as responsive to variations in soil nutrient availability as previously believed. The consistent levels of primary metabolites in root exudates, despite the potential nutrient depletion, imply that factors other than nutrient availability may play a more significant role in controlling the root exudation process. Alternatively, plants might adjust their physiological traits to adapt to the changing soil nutrient dynamics. In the Achenkirch experiment plant root systems adjusted N and P uptake to a level that root N and P contents were not altered or did not decrease (Kwatcho-Kengdo et al., 2022). This indicates that plants largely circumvented P limitation and were highly competitive in the acquisition of this resource while microbes showed strong signs of P limitation. This plant adjustment hypothesis is supported by our earlier study indicating that soil warming lead to an increase in root biomass, and in root production and turnover (Kwatcho-Kengdo et al., 2022, 2023), indicating that one major warming response of higher plants was to increase belowground C allocation to relieve belowground resource constraints. These changes could secondarily increase root surface area and the total flux of primary metabolites from root exudates to soil on ground surface area basis (though not per root biomass), thereby further helping to mitigate the limitations imposed by soil nutrient scarcity.

Although we expected warming to strengthen the link between root morphology and exudation by promoting more acquisitive fine root traits (e.g. greater SRL and higher SRA) and thereby stimulating root exudation to support nutrient uptake, our results did not support this expectation. Root traits such as SRL, SRA and diameter remained important predictors of metabolite profiles across seasons and years (Table 2), long-term warming did not consistently modify these traits and therefore did not enhance their explanatory power for exudation dynamics. For instance, although a 29% increase in SRL was reported in 2019 (Kwatcho-Kengdo et al., 2022), no significant differences in SRL, specific fine root area or root tissue density between the control and warming treatments in 2020 and 2021 (Heinzle et al., 2023). The lack of support for our hypothesis may reflect complex underlying mechanisms rather than its invalidity. For instance, significant interannual variation in SRL was observed, and these morphological shifts coincided with differences in exudate metabolite profiles across years. This suggests that root morphology remains an important predictor of exudation dynamics, even if warming did not directly alter those traits during the sampling years. Additionally, warming increased ectomycorrhizal colonization at the site (Kwatcho-Kengdo et al., 2022), a factor known to reduce SRL through the development of thicker, shorter root tips (Sun et al., 2010) and to influence exudate composition (Lv et al., 2023). These opposing effects—enhanced mycorrhizal-driven exudation versus reduced SRL—may have cancelled each other out, resulting in the observed lack of warming effects on exudation. Thus, although our data do not confirm the initial hypothesis under warming, they highlight the multifaceted interactions between root morphology, mycorrhizal associations and root exudation that merit further investigation.

4.2 | Seasonal and interannual variation of primary metabolites in root exudates

Seasonal dynamics of root C exudation rates have been documented in previous studies (Gao et al., 2024; Heinzle et al., 2023; Xiong et al., 2020). Surprisingly, much less research has been directed towards understanding the seasonal dynamics of root exudate profiles, particularly of primary metabolites (Xiong et al., 2023). It has been shown that root exudation rates show strong seasonal plasticity, typically exhibiting higher rates in spring and autumn (Gao et al., 2024; Heinzle et al., 2023; Jakoby et al., 2020; Zhao et al., 2023). However, our results do not support this result, as we

showed that the exudation rates of primary metabolites were relatively low in spring and tended to increase in summer and autumn (Figure 1). These findings highlight that root C exudation (Heinzle et al., 2023) and primary metabolite exudation (this study), measured on the same roots, are not congruent and that the seasonal patterns of primary metabolites and of total C in root exudates can be asynchronous. This is attributed to the lower contribution of primary metabolites to root exudate C in spring (approximately 12% in both treatments, data not shown) compared to summer and autumn (around 40% in both treatments, data not shown), as observed in our study. This suggests that during certain seasons there is a greater allocation of C in root exudates to other compounds than the primary metabolites analysed here, such as lipids and secondary metabolites. For example, in a subtropical tree plantation a higher proportion of lipids and salicylic acid metabolites were present in winter as part of prominent defence strategies, whereas in summer, more carbohydrates were exuded by roots priming the biogeochemical activity of the soil microbes (Chen et al., 2023).

Asynchrony of exudation rates of primary metabolites and of total C in root exudates further suggests that the profile of primary metabolites in root exudates changes with season (intra-annually) and across years (inter-annually). This was confirmed by non-parametric dissimilarity analyses and NMDS (Figure 2; Table 1). Both, the linear regression and the prediction models showed that the changes in root exudate profiles with season were well predicted by soil temperature (Figure 2), and those between years by changes in root diameter and specific root length ($R^2 = 0.59$, $p < 0.001$, Table 2). This aligns with the findings of Proctor and He (2017) and Rathore et al. (2023) who demonstrated that root traits (such as specific root length and root diameter) are strong predictors of changes in the profiles of root exudate metabolites and reinforces hypothesis two. Fine root biomass, root diameter and specific root length reflect the adaptive nature of root morphology to meet altered belowground resource requirements of plants, implying that the plant's growth strategy across seasons and years might be an important factor influencing the profiles of root exudates.

On the other hand, soil temperature was a strong interannual predictor of the root exudate profile (Figure 2; Table 2). This relationship is likely due to the close relationship between plant photosynthesis and the release of root exudates. For example, the quantity of exudates produced is strongly influenced by solar radiation received the day before sample collection (Tixier et al., 2023). Soil and air temperature covary strongly, and photoperiod and air temperature are strongly linked during the plant growing season, strengthening the above relationship, and plant phenology and with this likely exudate profile changes dramatically with season, from spring through summer to autumn.

4.3 | Selectivity and drivers of the root exudation process

Our third hypothesis was largely supported: root exudate metabolomes differed significantly from root tissue metabolomes, and exudation rates of sugars and organic acids were positively correlated with their tissue concentrations, consistent with diffusion-driven transport, although amino acids showed weaker or absent correlations likely due to re-uptake (Figure 4). Root exudation of primary metabolites is primarily mediated by facilitated passive diffusion through specific transporter proteins, driven by concentration gradients from root cytoplasm to the soil environment (Canarini et al., 2019). While energetically passive, the process is selective, governed by the presence and specificity of efflux transporters (Canarini et al., 2019; Robert et al., 2025; Sharma et al., 2023). In our study, 33 of 43 detected root tissue metabolites were also found in exudates, indicating that the absence of appropriate transporters likely limits the efflux of certain compounds. Despite this overlap, relative abundance profiles differed significantly (Figure 3), suggesting additional regulation beyond metabolite availability. Similar results were obtained for rice plants (*Oryza sativa* cv. Haenuki) which showed that ~70% of the metabolites in the root tissue were detected in the root exudates of N- or P-treated plants (Tawaraya et al., 2018) and in the tree species *Cinnamomum camphora* (L.) J. Presl. (camphor, Lauraceae) in a biodiversity experiment in the subtropical region of China (Weinhold et al., 2021). We found strong positive correlations between tissue and exudate concentrations for sugars and organic acids, but not for amino acids (Figure 4). This also aligns with data in the study by Canarini et al. (2016) who found a strong correlation between measured root tip contents of primary metabolites and their release into root exudates (Canarini et al., 2019). The weaker relation between tissue and exudate contents of amino acids, compared to that of organic acids and sugars (Figure 4), may be attributed to the fact that many amino acids are actively reabsorbed by plant roots after exudation from roots or from the soil solution (Phillips et al., 2006). For instance, Phillips et al. (2006) reported that the amino acid uptake capacity of roots in *Lolium multiflorum* Lam., *Medicago truncatula* L. and *Zea mays* L. exceeded their efflux rate by 94%–374%. Reuptake would weaken the relationship between tissue and exudate contents of primary metabolites. Moreover, to investigate mechanistic underpinnings, we performed cluster analysis of metabolite-specific exudation rates (Figure 5). Patterns aligned with known transporter families—for example SWEETs for hexoses and sucrose, UMAMITs for amino acids and MATE/ALMTs for organic acids (Baker et al., 2012; Chen et al., 2015). Additional clusters suggested unidentified transporters for pentoses, disaccharides and small organic acids. These findings highlight that exudation is shaped not only by concentration gradients but also by transporter availability and selectivity. This may explain why some high-abundance tissue metabolites are absent in exudates, while others with modest concentrations are consistently exuded.

The 'push' and 'pull' hypotheses have been fundamental in clarifying the relationship of concentrations between root tissues and root exudates (Dilkes et al., 2004; Farrar et al., 2003; Farrar & Jones, 2000; Jakoby et al., 2020; Karst et al., 2017; Leuschner et al., 2022). The 'push' hypothesis suggests a direct transfer mechanism of increased photosynthate C allocated to

roots leading to higher root exudation (Dilkes et al., 2004; Karst et al., 2017). This hypothesis is in contrast with our previous studies at the same site, which highlighted that long-term soil warming increased C allocation to root growth (i.e. increased fine root biomass production and turnover) (Kwatcho-Kengdo et al., 2022, 2023) but did not affect C exudation rates from individual roots (Heinzle et al., 2023). This may be explained by the fact that root exudates can be rapidly adjusted to respond to transient environmental stresses, whereas modifications of root morphological and architectural traits typically occur over a longer time period. In contrast, the 'pull' hypothesis argues that root exudation is influenced by external biotic or abiotic factors, such as by ectomycorrhizal fungal colonization (Meier et al., 2013), which attracts and 'pulls' photosynthate C via exudates into the rhizosphere. Based on the 'pull' hypothesis and previous results demonstrating that long-term soil warming triggered an emerging soil P limitation at this site (Tian et al., 2023b), we expected that root exudation of organic acids will be increased to mobilize P bound abiotically, but our results did not support this hypothesis. Overall, the relationship of primary metabolite concentration in root tissues and root exudates was more complex than what the 'push' and 'pull' hypotheses can explain. Notably, another study indicated that the changes in concentration of metabolites in root tissue and root exudates are not related under N-deficient or P-deficient conditions (Tawaraya et al., 2018). This lack of correlation might be attributed to active re-uptake systems operating at the cellular or tissue level, which could alter metabolite distribution in response to nutrient stress. On the other hand, this might also be amplified by the weak relation between bulk fine root tissue metabolite contents and their contents at the sites of exudate release, the root tips and the fine root rhizodermis. These results collectively highlight the complexity of linking tissue metabolite pools with exudation rates and point to the need for a broader characterization of root exudate chemistry to capture the full spectrum of plant responses to environmental change.

This study focused on the targeted quantification of primary metabolites, providing ecologically meaningful insights into plant carbon allocation for potential microbial utilization and nutrient mining. However, this targeted approach captures a narrower range of the exudate metabolome compared with untargeted metabolomics, which can detect hundreds to thousands of features (Ritter et al., 2025). This limited coverage may partly explain the absence of pronounced warming effects in our dataset. Future work combining targeted and untargeted metabolomics on the same samples would allow a more comprehensive characterization of root exudates and may reveal additional warmingrelated shifts in exudation patterns.

5 | CONCLUSIONS

The analysis of long-term soil warming effects on the rates and profiles of primary metabolite exudation in mature spruce trees revealed a key relationship between root tissues and exudates. Our findings challenge the hypothesis that soil warming increases the exudation of primary metabolites, indicating a change in root C allocation towards growth. These results further suggest that root exudation responses to environmental changes are highly nuanced, likely involving complex physiological and morphological adjustments. The positive correlation between metabolites in root tissues and exudates, despite their differing profiles, indicates that root exudation is selective, driven by facilitated passive diffusion. These insights improve our mechanistic understanding of rhizodeposition and its interaction with soil microorganisms under climate warming.

AUTHOR CONTRIBUTIONS

Xiaofei Liu: Investigation; methodology; data curation; visualization; writing—review and editing. **Jakob Heinzle:** Investigation; methodology; writing—review and editing. **Ye Tian:** Investigation; writing—review and editing. **Erika Salas:** Methodology; writing—review and editing. **Werner Borken:** Conceptualization; investigation; writing—review and editing. **Andreas Schindlbacher:** Conceptualization; investigation; project administration; writing review and editing. **Wolfgang Wanek:** Conceptualization; data curation; investigation; methodology; supervision; visualization; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the Figshare Digital Repository: https://doi.org/10.6084/m9.figshare.27230943 (Liu et al., 2025).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1. Boxplot of the average concentration of amino acids (a), organic acids (b) and sugars (c) in root extracts in all five times samples.

Figure S2. Linear relationship between the soil environment (soil temperature and soil moisture), root morphology trait and the profiling of primary metabolites, expressed as NMDS1 (a–h) and NMDS2 (i–p) scores from NMDS in control and warming plot.

Figure S3. Biplot of the first two significant components of the PCA for the investigated metabolites for the root tissue (blue) and root exudates (yellow) by amino acids (a), organic acids (b) and sugars (c).

Figure S4. Relationship between root biomass metabolite concentration with root exudate metabolite concentrations in warming (orange) and control (green) treatment, for different metabolite classes: (a) all compounds; (b) amino acids; (c) organic acids; (d) sugars.

Table S1. Soil C and nutrient properties in the 0–10 cm layer control and warmed plots at the Achenkirch soil warming experiment.

Table S2. Linear mixed-effects models testing the effects of warming, sampling date and their interactions on the amino acids, organic acids, sugars and total primary metabolites in root exudates base on the specific fine root biomass ($\mu g\, g^{-1}\, day^{-1}$), root surface area ($ng\, cm^{-2}\, day^{-1}$) and root length ($ng\, cm^{-1}\, day^{-1}$).

Table S3. Linear mixed-effects models testing the effects of warming, sampling date and their interactions on the contents of single compounds in root exudates.

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