



Root exudation links root traits to soil functioning in agroecosystems

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Abstract

Background and aims Root exudation is a key process for plant nutrient acquisition, but the controls on root exudation and its relationship to soil C and N processes in agroecosystems are unclear. We hypothesized that root exudation rates would be related to root morphological traits, N fertilization, and soil moisture. We also anticipated that root exudation would be correlated with bulk soil enzyme activity.

Methods Root exudation, root traits, and bulk soil extracellular enzyme activity were assessed in maize (*Zea mays* L.), soybean (*Glycine max* (L.) Merr.),

biomass sorghum (*Sorghum bicolor* (L.) Moench), giant miscanthus (*Miscanthus × giganteus*), and switchgrass (*Panicum virgatum* L.). Measurements were taken in situ during two growing seasons with contrasting precipitation regimes, and N fertilization rate was varied in sorghum during one year.

Results Specific root exudation (per unit root surface area) was negatively related to root diameter and was generally higher in annuals than perennials. Sorghum N fertilization did not affect root exudation rates, and soil moisture regime had no effect on annual root exudation rates within maize, sorghum, and miscanthus. Specific root exudation was negatively related to bulk soil C- and N-degrading soil enzyme activities.

Conclusion Intrinsic plant characteristics appeared more important than environmental variables in

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controlling in situ root exudation rates. The relationships between root diameter, root exudation, and soil C and N processes link root morphological traits to soil functions and demonstrate the potential trade-offs among plant nutrient acquisition strategies in agroecosystems.

Keywords Root economic space · Root exudation · Root traits · Soil carbon · Soil enzymes · Soil nutrients

Introduction

Plant root exudation is increasingly recognized as a major strategy for plant resource acquisition (Wen et al. 2022) and stress tolerance (Chai and Schachtman 2022). The release of root exudates into the rhizosphere serves several key plant functions including enhancing soil nutrient availability (Jilling et al. 2021; Ma et al. 2022), facilitating water uptake (Carminati et al. 2016), and promoting symbiotic soil microbes (Vives-Peris et al. 2020). As a significant flux of C from plants to soils, root exudation can also promote soil organic C (SOC) sequestration through soil aggregation and formation of mineral-associated organic matter (Panchal et al. 2022). Since the potential benefits of root exudation for plant and soil health have become more evident, interest in utilizing root exudates to enhance the functions of agroecosystems has increased (Preece and Peñuelas 2020; Sun et al. 2021; Panchal et al. 2022; York et al. 2022). Yet, the controls on root exudation and relationships to other soil processes have not been fully elucidated, thus hampering our ability to predict how agroecosystem management affects soil C and N cycling.

Mounting evidence indicates that root exudation is a key component of the plant resource acquisition strategy and is therefore linked to other root traits within the root economic space framework (Wen et al. 2022). However, compared to root exudation, root morphological traits such as diameter, specific root length (SRL), and root tissue density (RTD) have been more widely studied and thus currently represent the core components of the root economic space (Bergmann et al. 2020; Wen et al. 2022). Root trait suites vary within the root economic space depending upon plant resource acquisition strategy, which changes primarily by degree of plant-mycorrhizal

collaboration (Bergmann et al. 2020; Wen et al. 2022). Species with thicker, lower SRL roots tend to have greater mycorrhizal colonization and are therefore classified on the “outsourcing” side of the collaboration gradient, whereas species with thinner diameter roots that maximize resource uptake fall within the “do-it-yourself” category (Bergmann et al. 2020). Several studies in forests have reported that specific root exudation is negatively related to root diameter (Jiang et al. 2022; Yin et al. 2023) or positively related to SRL (Meier et al. 2020; Wang et al. 2021), both of which suggest that root exudation may be negatively correlated with the degree of mycorrhizal colonization and more closely associated with the do-it-yourself strategy of thin-root species. However, we currently lack a full understanding of the relationships between root exudation rates and other root traits, particularly in agroecosystems, which encompass both annual and perennial species.

As a central component of the plant resource acquisition strategy, root exudation plays a key role in N appropriation from soils (Coskun et al. 2017; Wen et al. 2022). Root exudates may enhance N availability by priming N mineralization of soil organic matter (SOM), mobilizing N from mineral particles, promoting free-living N-fixing bacteria, or reducing N losses by releasing biological nitrification inhibitor compounds (Coskun et al. 2017; Jilling et al. 2021; Sun et al. 2021). In theory, as plants become N-limited and leaf growth declines, the surplus above-ground C resulting from continued photosynthesis is allocated belowground where it can be used for one or more nutrient acquisition strategy (Prescott et al. 2020). Therefore, N limitation is expected to result in increased C allocation to root exudation. Although there is empirical evidence from forests to support this (Xiong et al. 2020; Meier et al. 2020), several ex situ studies of agricultural plants have contrarily reported lower root exudation in low-N versus high-N soils (Zhu et al. 2016; He et al. 2021; Smercina et al. 2021). Root dynamics vary significantly between field- and pot-based experiments (Hupe et al. 2019), and thus a better understanding of the effect of N fertilization on in situ root exudation is needed to determine how plant C allocation changes in response to N management in agroecosystems.

Root exudates also play a role in maintaining plant water status by providing a bridge to help maintain hydraulic connectivity between roots and soil

(Carminati et al. 2016) and by promoting the activity of beneficial rhizosphere microbiota (Williams and de Vries 2020). When plants become water-limited, a greater proportion of C is usually allocated belowground (Eziz et al. 2017; Kou et al. 2022), and root exudate chemical composition shifts (Williams and de Vries 2020), but the effect on total root exudation is less clear. Analogous to N limitation, mild-to-moderate water limitation is theorized to shift surplus aboveground C to belowground, which may result in increased root exudation (Prescott et al. 2020). Empirical studies have generally supported this framework (Preece and Peñuelas 2016), but the response of root exudation to water limitation also depends in part on species traits and soil nutrient availability (Williams and de Vries 2020; Preece et al. 2021). As such, further work to understand the effects of soil water availability on root exudation in agroecosystems is needed.

Plants typically invest 5% to 21% of total fixed C to root exudation (Wen et al. 2022), and thus root exudation offers a promising trait-based approach to facilitate SOC sequestration (Panchal et al. 2022; York et al. 2022). However, the flux of root exudate C into the rhizosphere does not translate directly into SOC storage. Root C exudation can prime the mineralization of native SOM in the rhizosphere (Han et al. 2020), thereby reducing (e.g., Cheng 2009; Liang et al. 2018) or even reversing (e.g., Henneron et al. 2020) the net C sequestration potential of the exuded C in the rhizosphere. This potential “paradox” of soil C sequestration is thought to result from microbial N mining of native SOM (Dijkstra et al. 2021), whereby soil microbes use root exudate C to increase extracellular enzyme production, mineralize N from SOM, and thereby mitigate N limitation (Brzostek et al. 2013; Jilling et al. 2021). On the other hand, root exudation can promote the formation of soil macroaggregates (Baumert et al. 2018) and mineral associated organic matter (Sokol et al. 2019), both of which physically protect SOM and thus decrease the potential for enzymatic SOM degradation in the bulk soil (Dungait et al. 2012). The effect of root exudation on net changes in SOC thus depends in part on the balance between its effect on SOM protection versus priming.

Our objectives were to quantify rates of root exudation in agroecosystems, elucidate the apparent controls of exudation, and to assess the relationship between root exudation and soil C and N processes.

In line with the root economic space framework, we hypothesized that root exudation rates would be related to root morphological traits, including root diameter and SRL. We also hypothesized that root C exudation rates would be greater with no N fertilization and lower seasonal soil moisture availability, in accordance with the surplus aboveground C framework. To test our hypotheses, we measured a suite of root traits, root C exudation, and soil C- and N-degrading extracellular enzyme activities across six agroecosystems during two growing seasons with contrasting precipitation regimes.

Materials and methods

Study site

The study took place at the University of Illinois Energy Farm in Urbana, IL USA (40.0659, -88.1933) in 2018 and 2019. The 30-yr (1991–2020) mean annual temperature and precipitation are 11.4 °C and 1038 mm, respectively (NOAA 2022; station USC00118740). During the growing season (May–September), the mean temperature is 21.3 °C and average total precipitation is 527 mm (NOAA 2022). Soils at the site are Mollisols dominated by Dana silt loams (fine-silty, mixed, superactive, mesic Oxyaquic Argiudoll), Flanagan silt loams (fine, smectitic, mesic Aquic Argiudoll), and Drummer silty clay loams (fine-silty, mixed, superactive, mesic Typic Enfoaquoll) (Soil Survey Staff 2022).

This study leveraged ongoing research infrastructure, and therefore the experimental design differed between 2018 and 2019. In 2018, maize (*Zea mays* L.), biomass sorghum (*Sorghum bicolor* (L.) Moench), giant miscanthus (*Miscanthus × giganteus*), and switchgrass (*Panicum virgatum* L.) were studied in four randomized replicate plots (122 m × 27.5 m) each within a long-term biofuels experiment established in 2008 (Anderson-Teixeira et al. 2013; Moore et al. 2020, 2021). The switchgrass crop was discontinued after 2018 and therefore was not sampled in 2019. In 2019, maize, sorghum, miscanthus, and soybean (*Glycine max* (L.) Merr.) were studied in four replicate plots each. As 2019 was the soybean phase of the maize and sorghum plots, soybean was studied in the plots that had been planted to sorghum in 2018, and alternate plots at the same site were utilized for maize

and sorghum in 2019. Maize was studied in plots (122 m × 27.5 m) that had historically been maize-soybean rotations, while a sorghum N-rate experiment (0 vs. 112 kg N ha⁻¹; 12 m × 6 m plots in a randomized design) was leveraged to examine the effect of soil fertility on sorghum root exudation (Schetter et al. 2022; Burnham et al. 2022). Other than the sorghum plots that did not receive N (sorghum -N), all crops in both years received typical N fertilizer application rates and followed standard agricultural management practices (Table S1). Miscanthus and switchgrass are perennial plants, while the other species are annuals.

Root exudation

To provide access to roots, two soil pits measuring approximately 30 cm × 30 cm × 30 cm were excavated in each plot around the time of plant emergence in each study year. The soil pits were placed near the edge of the plot to provide access while eliminating potential trampling of plants and soil. The inner soil pit wall was targeted 15–30 cm from the base of a plant. The inner wall of each soil pit was scraped flat, and a 30 cm × 30 cm × 0.25 cm polypropylene (PP) sheet was pinned in place. The PP sheet served to minimize evaporative water loss from the inner soil wall and to provide a flat, vertical surface to facilitate root growth (Fig. S1). When not in use, the soil pits were covered with plywood panels to provide thermal insulation and to prevent light from entering the belowground environment.

Water-soluble root exudates were collected in July and August of each year to capture the mid and late growing season, respectively (July 18 and August 22, 2018; July 19 and August 30, 2019). Root exudates were collected following the methods of Phillips et al. (2008) with some modifications for the soil and plant types in our study. Samples were collected from two representative intact roots and from one root-free blank in each plot, with the blank serving to correct for background C in the samples. After removing the PP sheet, the terminal end of each intact root was gently pried free from the soil wall using a dissecting needle and forceps until approximately 10–15 cm of root length was exposed for exudate collection. Care was taken to prevent damage to the root tissues and to maintain coupling of the root to higher-order roots during excavation. The root was washed free of soil

with deionized (DI) water. Once clean, the terminal end of the root was placed into the barrel of a 30 mL PP syringe with a three-way polycarbonate valve affixed to the bottom. The barrel was held upright, loaded with 1 mm borosilicate glass beads, and filled with a 1 mM CaCl₂ solution until the beads were completely submerged. A second 30 mL PP syringe was connected to the three-way valve via a 20 cm long, 1.6 mm inner diameter polyvinyl chloride tube. To flush the collection system, the three-way valve was opened, the plunger of the second syringe was pulled outward to transfer the CaCl₂ solution out of the root-containing syringe barrel, and the CaCl₂ solution was discarded. The three-way valve was closed, fresh CaCl₂ solution was added to cover the beads in root-containing syringe barrel, and parafilm was affixed to the top. The syringe barrel was pinned to the wall in an upright position, and the soil pit was covered with the plywood panel.

The intact roots were left to incubate in the field for 24 h, after which the CaCl₂ solution containing the exudates was collected. The secondary syringe was used to pull CaCl₂ solution from the root-containing syringe barrel, and the solution was then transferred into a PP vial. The syringe barrel containing the root was flushed twice with fresh CaCl₂ solution to remove any remaining exudates. The root was cut at the top edge of the syringe barrel, rinsed with DI water, and placed into a plastic bag. The root and sample solution were frozen at -20 °C until further processing. After thawing, the sample solution volume was measured and filtered through a 0.22 μm polyvinylidene fluoride syringe filter. Total organic carbon (TOC) in the solution was quantified using the non-purgeable organic C method on a Shimadzu TOC-L-CSH (Shimadzu Corp., Kyoto, Japan). The roots were thawed, scanned using an Epson V850 photo scanner (Epson America Inc., Long Beach, CA, USA) at 600 DPI in film mode, and root morphological traits were quantified using IJ_Rhizo (Pierret et al. 2013). Roots were then dried at 60 °C and weighed.

Root biomass and traits

To scale root exudation to an area-basis and quantify field-scale root morphological traits, belowground biomass (i.e., roots and rhizomes) was sampled to 30 cm in eight locations per plot using a 4.76 cm inner diameter slide hammer corer (AMS Inc., American

Falls, ID, USA). In the row crops, half the samples were taken in the rows and half were taken between rows. Belowground biomass samples for annual crops were collected following both exudate collection campaigns within each year, whereas samples for perennial crops were collected annually between the two exudate campaigns. The samples were frozen at $-20\text{ }^{\circ}\text{C}$ until further processing.

After thawing, the belowground biomass samples were serially sieved to 4 mm to separate plant material from soil, and the remaining soil was checked for plant fragments that passed through the sieve. In the perennial crops, roots were separated from rhizomes. Roots were soaked in a 1% sodium hexametaphosphate solution for approximately 15 min to aid with soil removal and then gently washed with water over a 500 μm sieve until all soil was removed. Roots were scanned and processed for morphological traits using the procedure described above. After drying at $60\text{ }^{\circ}\text{C}$, root samples were weighed, pulverized using a tissue homogenizer, and analyzed for C and N on a Costech 4010 CHNSO Elemental Analyzer (Costech Analytical Technologies, Valencia, CA USA).

Soil analyses

To assess components of bulk soil biological activity, eight bulk soil samples were collected to 10 cm deep with a push probe and composited from each plot concomitant with the root exudate collection campaigns. In row crops, half the samples were taken in row while the other half were taken between rows. The soils were sieved to 2 mm, a subsample of the soil was dried at $105\text{ }^{\circ}\text{C}$ to determine gravimetric moisture (g water 100 g^{-1} dry soil), and the remaining soil was stored at $-20\text{ }^{\circ}\text{C}$ until further processing. We sampled bulk soil instead of rhizosphere soil because we were interested in the potential effects of root exudation on SOC stocks. The rhizosphere only accounts for a small portion of total SOC (e.g., Zhu et al. 2020), and bulk soil and rhizosphere processes are highly correlated (Finzi et al. 2015).

To quantify the variation in potential bulk soil mineralization rates, a suite of soil hydrolytic and oxidative enzymes activities was assayed on field-moist soil subsamples (1.5 g dry weight equivalent) using fluorometric (4-methylumbelliferone substrate) and colorimetric (l -3,4-dihydroxyphenylalanine substrate) microplate spectrophotometer

methods, respectively (German et al. 2011). The hydrolytic enzymes included cellobiohydrolase (CBH), β -glucosidase (BG), α -glucosidase (AG), β -xylosidase (BX), N-acetyl- β -d-glucosaminidase (NAG), and acid phosphatase (AP), while the oxidative enzymes included phenol oxidase (PO) and peroxidase (PX). A 100 mmol maleate buffer adjusted to 6.5 pH was used for the assays. Colorimetric plates were read at 460 nm, and fluorometric plates were excited and read at 365 nm and 450 nm, respectively. Enzyme activities were measured at saturating substrate concentrations and therefore represent potential activity.

Soil microbial biomass C (MBC) was assayed using the direct chloroform extraction method (Gregorich et al. 1990) on field-moist subsamples (7.5 g dry weight equivalent). Extracted C from chloroformed and non-chloroformed subsamples was quantified via the non-purgeable organic C method on a Shimadzu TOC-L-CSH. An extraction efficiency of 0.17 was used to convert organic C to MBC (Gregorich et al. 1990).

Calculations

Exudate samples were corrected for background C by subtracting the C concentration of the blank samples within each plot and measurement date. For a small number of samples, the blank C values exceeded the sample C values, in which case exudate C was set to zero. Exudate C mass (mg C) was calculated as the product of volume (L) and concentration of C (mg C L^{-1}) in the exudate samples. Root area-specific exudation (mg C m^{-2} root d^{-1}) was determined by dividing the exudate C mass by the surface area of the incubated root (m^2 root) and the incubation period (1 d). Field-scale root surface area (m^2 root m^{-2} area), as measured on roots from the belowground biomass cores, was used to convert the root area-specific exudation rates to area-scaled daily exudate C fluxes (mg C m^{-2} area d^{-1}).

Statistical analyses

Datasets were first analyzed separately by year due to the differences in crop types between 2018 and 2019. The effects of crop and date on below- and aboveground variables were assessed using generalized least squares regression models with allowance

for heteroscedasticity using the ‘gls’ procedure (Pinheiro et al. 2022). The marginal effects of crop, date, and their interaction were determined using the Type III sum of squares. For statistically significant factors or interactions, group means were separated using the ‘emmeans’ procedure, and statistical differences among groups was assessed after adjusting for multiple comparisons using the multivariate *t* method (Lenth 2022). For the three crops that were present during both study years (maize, miscanthus, and sorghum+N), interannual differences (2018 vs. 2019) were tested using an analogous approach.

To visualize suites of root traits and bulk soil biological activity, two-dimensional ordinations were constructed using principal component (PC) analysis with the ‘rda’ procedure (Oksanen et al. 2022). The root trait ordinations included specific root C exudation rate (ExC), root diameter (Dia), specific root length (SRL), root nitrogen content (N), and root tissue density (RTD), and the bulk soil biological activity ordinations included soil extracellular enzyme activities and MBC. Permutational multivariate analysis of variance (PERMANOVA) with Euclidian distance and 100,000 permutations was performed with ‘adonis.II’ to assess the effects of crop and dates on root trait and soil biological activity variables (Hervé 2022).

Relationships between specific root exudation rates and root traits or soil biological activity factors were assessed using Spearman’s rank correlation coefficients (r_s), which were calculated using ‘rcorr’ (Harrell 2022). Data analyses were performed in R 4.2.2 (R Core Team 2022), and the statistical significance level was set at $\alpha=0.05$.

Results

Weather and soil moisture

The 2018 growing season was relatively wet and hot, whereas 2019 represented a relatively dry and moderately warm growing season. Growing season (May through September) temperatures were 1.7 °C and 0.7 °C above average in 2018 and 2019, respectively (NOAA 2022). Precipitation was 15% above average during the 2018 growing season, whereas precipitation was 14% below average in the 2019 growing season (NOAA 2022).

The precipitation differences between years translated in greater gravimetric soil moisture in 2018 ($p<0.03$), averaging 21.3% in 2018 compared to 11.9% in 2019 (Table 1). Annual crops (maize, sorghum, and soybean) trended toward lower soil moisture than perennials (miscanthus and switchgrass) except in August 2019. In 2018, there was no significant difference in soil moisture between sampling dates ($p=0.43$). In 2019, soil moisture was significantly lower in August than July ($p<0.01$), but the magnitude of difference varied by crop ($p=0.006$).

Specific and area-scaled root exudation

Specific root exudation ranged from 7 to 258 mg C m⁻² root d⁻¹ among crops and dates. Specific root exudation rates were typically greater in annual than perennial crops (Fig. 1a), were not different between sorghum -N and sorghum+N ($p>0.94$), and did not vary between the wet and dry years ($p=0.49$) across the three cropping systems present during both years

Table 1 Gravimetric soil moisture within each cropping system during sampling campaigns in July and August of 2018 and 2019

Year	Date	System	Soil moisture (g water 100 g ⁻¹ dry soil)
2018	July	Miscanthus	20.5 (1.3) AB
		Switchgrass	22.8 (0.6) A
		Maize	16.8 (0.5) B
		Sorghum+N	16.5 (1.4) AB
	August	Miscanthus	22.4 (2) AB
		Switchgrass	25.6 (1.1) A
		Maize	21.1 (1.5) B
		Sorghum+N	24.9 (2.3) AB
2019	July	Miscanthus	16.8 (0.8) A
		Soybean	11.8 (1.1) B
		Maize	11.8 (0.1) B
		Sorghum+N	12.1 (0.5) B
		Sorghum -N	12.8 (0.7) B
	August	Miscanthus	11.0 (0.6)
		Soybean	10.2 (0.5)
		Maize	10.1 (0.7)
		Sorghum+N	11.7 (0.7)
		Sorghum -N	11.1 (1.3)

Values are means with standard errors. Letters indicate differences among cropping systems within each sampling date ($p<0.05$) after adjusting for multiple comparisons

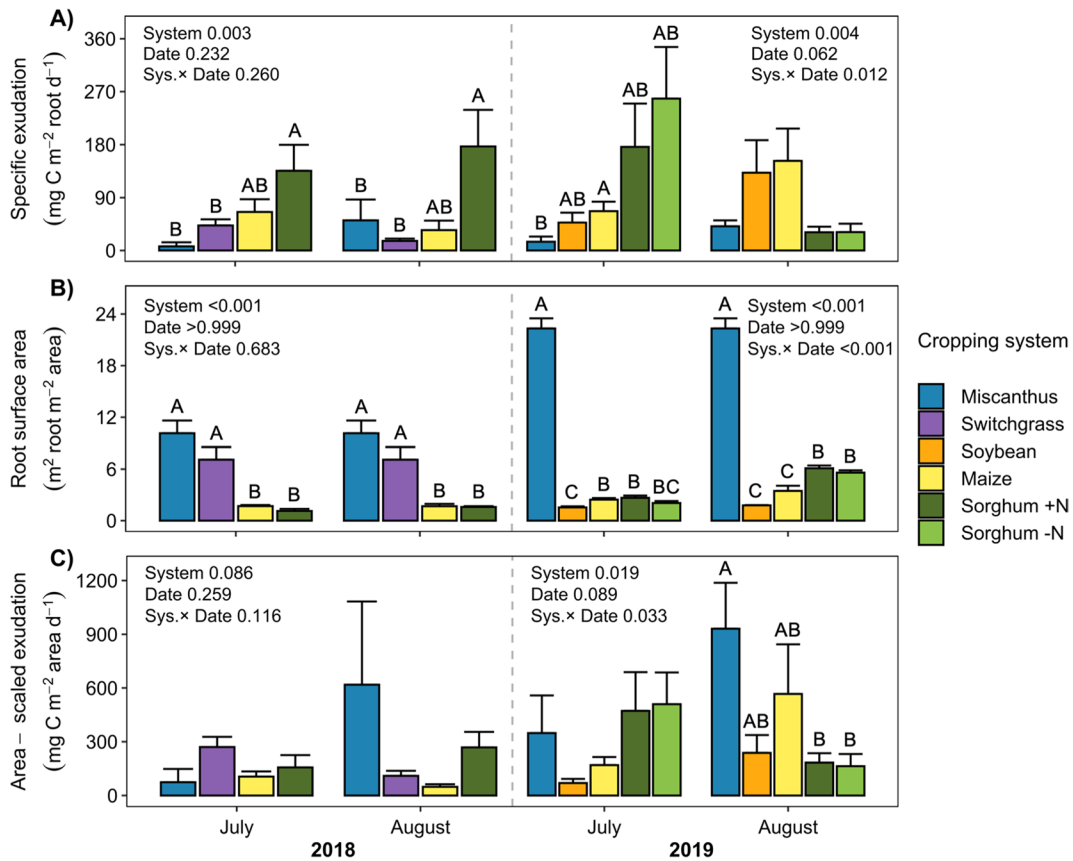


Fig. 1 Specific root exudation (A), root surface area to 30 cm (B), and daily area-scaled root exudation rates to 30 cm (C) for bioenergy cropping systems in July and August of 2018 and 2019. Error bars show one standard error. *P*-values for the effect of cropping system, date, and their interaction are shown

in each panel. Letters above error bars indicate differences between cropping systems within each date (system×date $p < 0.05$) or within each year (system×date $p \geq 0.05$) after adjusting for multiple comparisons

(miscanthus, maize, and sorghum +N). In 2018, sorghum +N specific exudation was greater than miscanthus and switchgrass on both measurement dates ($p < 0.03$). In July 2019, maize specific exudation was greater than miscanthus ($p < 0.05$), but there were no significant differences between crops in August 2019. Mass-based specific exudation rates (Table S2) were strongly correlated with surface area-based specific exudation rates (Spearman's rank correlation coefficient $r_s = 0.96$, $p < 0.001$).

Root surface area to 30-cm depth was greater in perennials crops compared to annuals (Fig. 1b) and did not differ between sorghum -N and sorghum +N ($p > 0.40$). However, root surface area was greater in the drier year than the wet year ($p < 0.001$) within the cropping systems that were present in both

years, although the interannual difference varied by system ($p < 0.001$). In 2018, root surface area was approximately six times greater in miscanthus and switchgrass compared to maize and sorghum. In 2019, miscanthus had upwards of three times greater root surface area than all annual crops, and there were date-dependent differences among the annual crops ($p < 0.001$).

Area-scaled daily root exudation to 30 cm was similar among crops, except in August 2019, when miscanthus had higher rates than both sorghum treatments ($p < 0.05$; Fig. 1c). Area-scaled daily exudation did not vary between sorghum N treatments ($p > 0.99$) or between the wet and dry years ($p = 0.23$) within the cropping systems that were studied in both years.

Root traits and soil biological activity

Across all species, specific root exudation was negatively correlated with root diameter in 2018 ($r_s = -0.49$, $p < 0.01$; Table 2) and 2019 ($r_s = -0.38$, $p < 0.05$). In 2019, specific exudation was also negatively correlated with RTD ($r_s = -0.46$, $p < 0.01$) and positively correlated with SRL ($r_s = 0.51$, $p < 0.001$).

The suite of root traits varied significantly among crops ($p < 0.001$) and dates ($p < 0.04$) in both years, with perennial species tending to have greater root diameter and lower specific root exudation than annuals (Fig. 2). In a principal components ordination, plant root traits separated along a primary axis (PC1) that spanned from narrow diameter, low specific exudation to high SRL, high exudation. The secondary axis (PC2) explained variability in RTD and tissue N content. In 2018, sorghum and maize root trait suites were largely separated by differences in N content and SRL (Fig. 2a). In 2019, soybean was separated from the other crops primarily along PC2, which was driven primarily by high root N content in soybean

Table 2 Spearman's rank correlation coefficients (r_s) indicating the strength and direction of the relationships between specific root exudation and root traits (Fig. 2) or soil biological activity indicators (Fig. 4)

Category	Variable	Year	
		2018	2019
Root traits	Dia	-0.49**	-0.38*
	SRL	0.33	0.51***
	RTD	0.30	-0.46**
	N	0.17	0.24
Soil enzymes	CBH	-0.05	-0.28
	BG	-0.02	-0.23
	AG	-0.33	0.13
	BX	-0.48**	-0.36*
	NAG	-0.57***	-0.28
	AP	-0.17	0.24
	PO	-0.19	-0.06
	PX	-0.11	-0.22
Microbial biomass	MBC	0.03	-0.35*

Dia root diameter, *SRL* specific root length, *RTD* root tissue density, *N* root nitrogen content, *CBH* cellobiohydrolase, *BG* β -glucosidase, *AG* α -glucosidase, *BX* β -xylosidase, *NAG* N-acetyl- β -d-glucosaminidase, *AP* acid phosphatase, *PO* phenol oxidase, *PX* peroxidase, and *MBC* microbial biomass C. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$

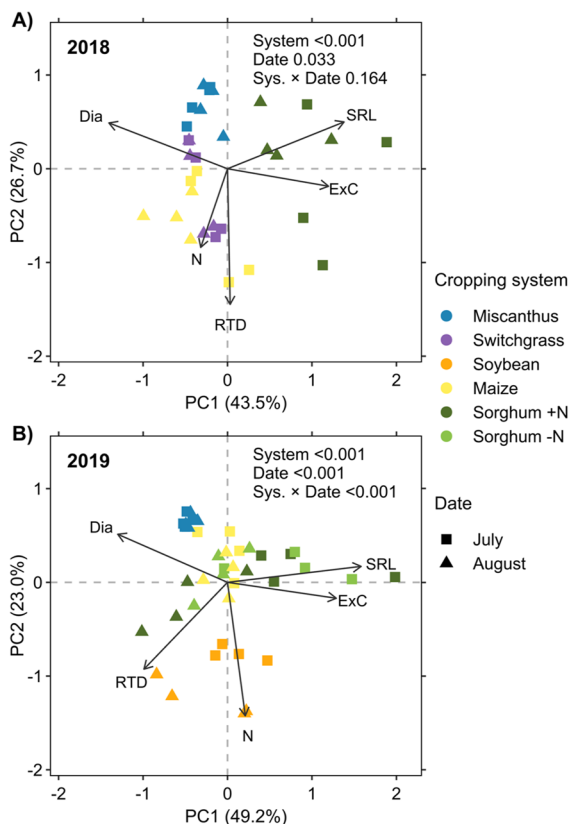


Fig. 2 Principal component (PC) ordinations for root traits in bioenergy cropping systems during 2018 (A) and 2019 (B). Filled markers show the PC scores for each replicate plot, and the arrows show the PC loadings for the root traits contained in the analysis. *P*-values show the PERMANOVA effects of cropping system, date, and their interaction. ExC=specific root C exudation rate, Dia=root diameter, N=root nitrogen content, RTD=root tissue density, SRL=specific root length

roots (Fig. 2b). In 2019, sorghum+N and sorghum -N root trait suites were similar, but from July to August, sorghum roots at both N levels shifted toward lower SRL and greater RTD (Fig. 2b).

Specific root exudation showed primarily negative relationships with bulk soil hydrolytic extracellular enzyme activities (Table 2). In both study years, specific root exudation was negatively related to BX activity ($p < 0.03$; Fig. 3a, c) and in 2018 specific root exudation was negatively correlated with NAG activity ($p < 0.001$; Fig. 3b). In 2019, root exudation was also negatively related to soil MBC ($p < 0.05$).

Soil biological activity suites varied among crops ($p < 0.03$) but were similar between dates ($p \geq 0.05$) within each year (Fig. 4). While there was significant

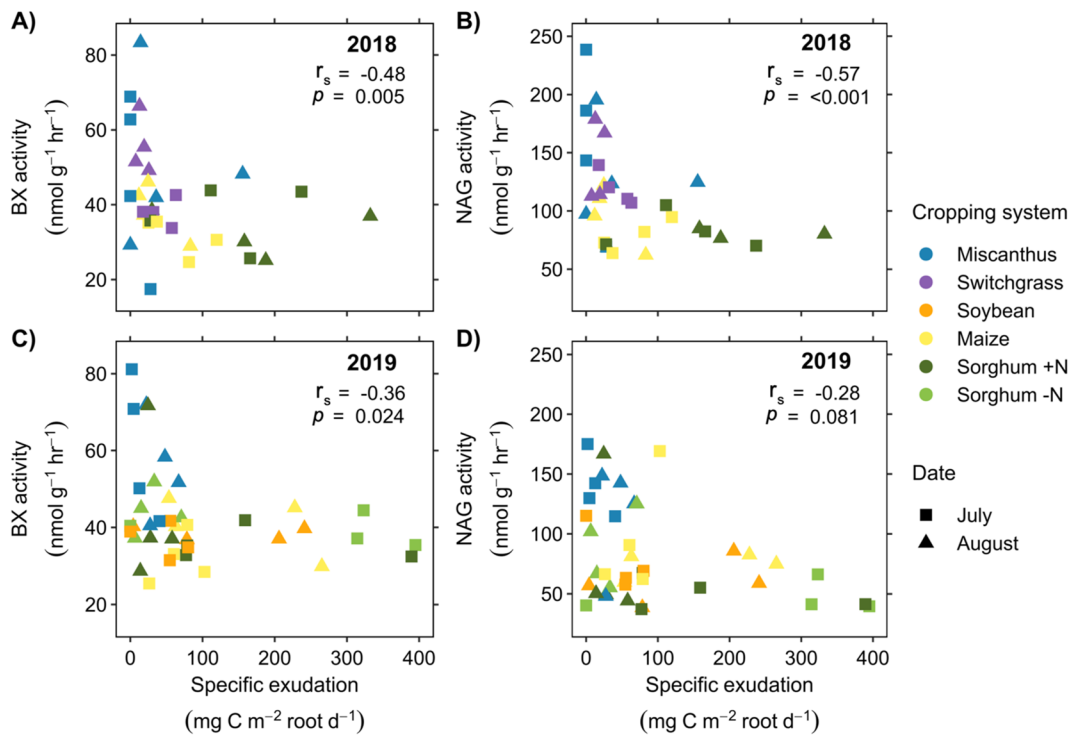


Fig. 3 Relationship between specific root exudation and potential β -xylosidase (BX) activity (A and C) and potential N-acetyl- β -d-glucosaminidase (NAG) activity (B and D) in

2018 and 2019. Spearman's rank correlation coefficient (r_s) and corresponding p -value are given in each panel

overlap among the crop types, the annual species tended to occupy different spaces than perennials in the soil biological activity ordinations. Soil biological activity suites separated along a primary axis (PC1) that corresponded strongly to most hydrolytic enzyme activities. In 2018, the secondary source of variation (PC2) corresponded strongly to oxidative enzymes PO and PX (Fig. 4a), but in 2019, the secondary source was most strongly related to MBC (Fig. 4b).

Discussion

We found that specific root exudation was related to root morphological traits, which varied systematically among crop types. At least for sorghum, differences in N fertilizer levels and for other species soil moisture regime did not affect root exudation rates during our study, suggesting that the phenotypic plasticity of root exudation rates may be low relative to the genotypic controls. Root exudation rates were negatively correlated to bulk soil hydrolytic enzyme activities,

which suggests a potential tradeoff between nutrient acquisition from rhizosphere versus bulk SOM, whereby plants with higher root exudation rates rely less on bulk SOM nutrients. Overall, the relationships between root exudation rates, root morphological traits, and bulk soil enzyme activity underscore the centrality of root exudation as an ecosystem process linking belowground plant traits to soil C and N processes.

Variation in root exudation

Root morphology explained significant variability in specific root exudation rates among plants differing in life history and functional groups, providing further support for root exudation as a central component of the plant belowground resource acquisition strategy (Wen et al. 2022). Unlike aboveground strategies that vary primarily along a fast-to-slow conservation gradient, belowground resources acquisition strategies vary primarily along a “do-it-yourself” to “mycorrhizal outsourcing” collaboration gradient (Bergmann

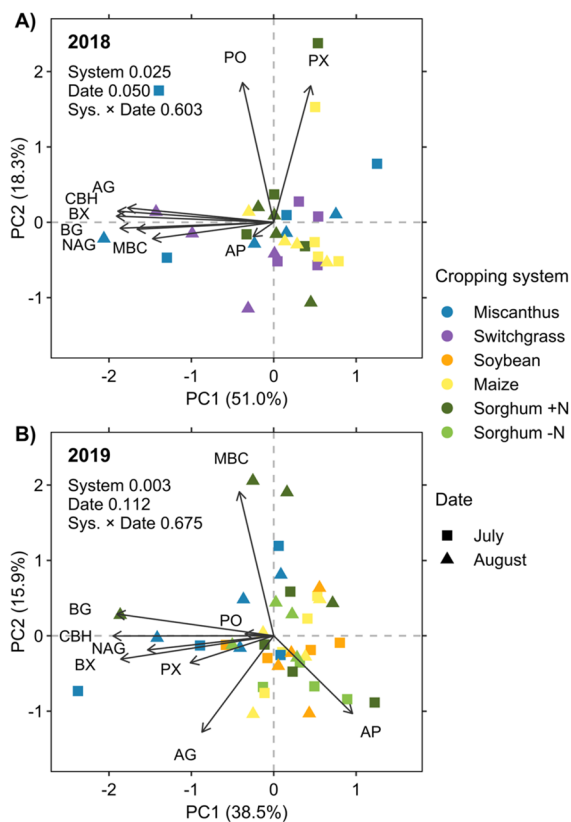


Fig. 4 Principal component (PC) ordinations for soil biological activity variables in bioenergy cropping systems during 2018 (A) and 2019 (B). Filled markers show the PC scores for each replicate plot, and arrows show the PC loadings for the soil variables in the analysis. The *P*-values for the PERMANOVA effects of cropping system, date, and their interaction are given in each panel. CBH=cellulohydrolase, BG= β -glucosidase, AG= α -glucosidase, BX= β -xylosidase, NAG=N-acetyl- β -d-glucosaminidase, AP=acid phosphatase, PO=phenol oxidase, PX=peroxidase, and MBC=microbial biomass

et al. 2020; Lachaise et al. 2022). Plants with thicker roots facilitate the formation of relationships with mycorrhizal fungi that provide soil-derived nutrients in exchange for plant-fixed C (Wen et al. 2019; Sweeney et al. 2021). In contrast, plants employing the do-it-yourself strategy typically produce thin diameter roots that maximize absorptive surface area for nutrient uptake (Bergmann et al. 2020; Wen et al. 2022). Although our study encompassed a limited number of species, our finding that specific root exudation was negatively related to root diameter has also been observed across other species (Jiang et al. 2022; Yin et al. 2023), broadly suggesting that

plants using the do-it-yourself strategy also invest C in root exudation to promote nutrient uptake from the rhizosphere. In this case, the collaboration gradient includes not only the tradeoff between root morphological traits that facilitate mycorrhizal colonization vs. direct nutrient uptake, but it also encompasses a tradeoff between plant C investment in mycorrhizae versus rhizosphere microbes.

Annual plants generally exhibited narrower diameter roots and subsequently higher specific root exudation rates than perennial plants, suggesting that specific exudation may be controlled to some extent by plant life history. Compared to annual species, perennials benefit more from long-term investment in mycorrhizal partners and therefore tend to have thicker diameter roots that facilitate mycorrhizal colonization (Wilson and Harnett 1998; Roumet et al. 2006). Thus, the observed difference in specific root exudation between annual and perennial species may be a result of the broader differences in nutrient acquisition strategies between the two life forms, with annual species relying more on root exudation than mycorrhizal symbiosis for nutrient uptake. As such, plant life history could provide a reasonable basis for broadly predicting specific root exudation rates. Given that root trait suites also vary predictably between monocot and eudicot species (Roumet et al. 2016), it is conceivable that specific root exudation also shows organization across these functional groups (Williams et al. 2022). However, more extensive and targeted work is needed to assess potential differences in root exudation between these broad plant groups.

We expected that N-limited sorghum plants would stimulate root exudation to promote plant N availability, but we observed no difference in specific root exudation or annual root exudation between the sorghum N treatments. One possible explanation is that the dry conditions during the N study (2019) limited the diffusion of fertilizer-derived N from the bulk soil into the rhizosphere, thus hindering the N application efficacy. In support of this, a concurrent study at our site showed that bulk soil ammonium and nitrate concentrations were similar between N fertilization levels in the wet year, but concentrations were consistently greater under N fertilization in the dry year (Burnham et al. 2022), suggesting that the added N was not plant accessible. It is also possible that there was an insufficient degree of N-limitation to invoke a root exudation response in the sorghum -N treatment,

as the field had historically received N fertilizer and an N-fixing soybean crop occupied the field the previous year. If N limitation was substantial, we may have expected greater root surface area (e.g., Peng et al. 2017) in unfertilized sorghum, but we did not observe such differences. Therefore, a greater degree of N limitation than occurred in our study may be necessary to induce changes in root exudation and other root properties in agroecosystems. In sum, our N fertilization component included only one species in one year, and therefore the results may not be representative of other species or other growing conditions.

In contrast to our hypothesis, we did not find evidence to indicate increased specific or area-scaled root exudation during the dry year. However, root surface area was greater during the dry year than the wet year, which supports the notion of surplus above-ground C allocation belowground resulting from water limitation (Prescott et al. 2020). We surmise that during the dry year, additional C may have been allocated belowground to hydrotropic root growth to extract water from wetter pockets of soil (Eapen et al. 2005). In general, sorghum is regarded as more drought tolerant than maize (Lamb et al. 2022), which may explain the more dramatic increase in root surface area in sorghum. Although miscanthus has relatively deep roots (Black et al. 2017), it is only considered to be moderately drought tolerant (Quinn et al. 2015) and therefore may have increased shallow root production to increase water extraction. While we did not observe a change in root exudation rates in response to soil moisture regime in either of the three crops, the response of root traits to water limitation is also known to vary widely among species (Lozano et al. 2020) and by drought duration (Zhang et al. 2019).

Bulk soil biological activity

We found generally negative relationships between specific root exudation and bulk soil hydrolytic enzyme activity. Specifically, BX and NAG were negatively correlated with root exudation in at least one of the two years. These two hydrolytic enzymes have different functional roles, with BX and NAG catalyzing the degradation of C-containing hemi-celluloses and C- and N-containing chitin, respectively (Alster et al. 2013). Thus, C and N mineralization from bulk SOM was lower in crops with high root exudation

rates compared to crops with low exudation rates, suggesting that the C gains from root exudate-driven physical protection may have been greater than C losses from root exudate SOM priming. While our finding does not directly demonstrate that root exudation increases SOC storage, it is consistent with results from a process-based model that accounts for rhizosphere C priming and protection mechanisms, which indicated that elevated root exudation increases both protected and total SOC at our site (Juice et al. 2022). Empirical studies using plant ^{13}C labeling or ^{13}C labeled root exudate substrates could help to further parse out the balance of priming versus physical protection (Blanc-Betes et al. 2023).

In a study of European Beech forests, a negative relationship between root exudation and bulk soil C- and N-degrading hydrolytic enzyme activity was attributed to greater fungal biomass under low root exudation (Meier et al. 2020). This explanation matches our finding of lower specific root exudation and higher enzyme activities in the perennial agroecosystems, which tend to harbor greater mycorrhizal and saprotrophic fungal biomass compared to annuals (Jesus et al. 2016) likely due in part to the chemical complexity of the perennial litter (Ridgeway et al. 2022). Therefore, we speculate that the negative relationship between root exudation and bulk soil hydrolytic enzyme activity may be emergent rather than causal, as higher enzyme activity presumably results from greater fungal abundance than from lower root exudation, per se. This hypothesis implies that root exudation and fungal abundance are negatively related, which aligns with the notion of a tradeoff between plant investment in root exudation versus fungal collaboration. Studies combining measurements of root exudation, mycorrhizal colonization, and soil biological activity are needed to comprehensively elucidate the tradeoffs between nutrient acquisition strategies.

Conclusions

In situ specific root exudation rates were negatively related to root diameter across several annual and perennial agroecosystems. Environmental factors such as N fertilization and seasonal soil moisture regime appeared to be less important than innate plant characteristics in determining root exudation rates in the two years of our study. The relationships among root

morphology, root exudation, and soil enzyme activity highlight the role of root exudation as an integral component of ecosystem C and N cycling and illustrate the potential tradeoffs in belowground resource allocation strategies among agricultural plant species.

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Data availability Data supporting this study are provided in the Supplementary Information. Additional datasets are available from the corresponding author upon reasonable request.

Declarations

Competing interests The authors declare no conflict of interest.

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