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ORIGINAL PAPER

Soil extracellular polymeric substances and microbial biomass react differently to field induced drought stress in contrasting cropping systems at different wheat developmental stages

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Abstract

Drought events are becoming more severe and recurrent over Europe. Changes in temperature and rain patterns can affect soil nutrient mobility and availability, modulating the biomass and activity of soil microbial communities. Here, we investigated the effects of drought on extracellular polymeric substances (EPS) and microbial biomass carbon (MBC) and nitrogen (MBN) in differently managed cropping systems. An on-field drought simulation experiment using rain-out shelters was conducted as part of a long-term field experiment cultivated with winter wheat, comparing cropping systems with contrasting fertilization strategies and crop protection measures: A biodynamic system and a mixed conventional system with no pesticide application, and a purely minerally fertilized conventional system, with conventional pesticide use. The implemented drought lasted for three months, starting at plant tillering stage and ending at ripening stage. No watering was performed on the drought treatment during that period. Soils were sampled at stem elongation, flowering, and ripening. EPS-carbohydrates and EPS-proteins significantly increased by approximately 20% due to induced drought but remained roughly constant from stem elongation to ripening under drought. Mean EPS-carbohydrates to EPS-proteins ratio was 1.9. MBC and MBN remained largely unaffected by drought. The ratio of both EPS fractions to microbial biomass was lowest in the biodynamic system and highest in the minerally fertilized conventional system, indicating that rhizodeposits and mucilage were predominantly diverted into microbial biomass, rather than into microbial EPS. This might be an important reason for the higher soil fertility of the biodynamic system.

Keywords EPS · Carbohydrates · Protein · Mucilage · Rhizodeposition · Soil microbial biomass

Introduction

Soil microbial biomass is the active fraction of soil organic matter (SOM) (Jenkinson et al. 1988), as microorganisms perform most enzymatic processes in soils (Burns et al. 2013). Soil microorganisms thrive in the hotspots of substrate availability (Banfield et al. 2018; Kuzyakov and Blagodatskaya 2015): in the detritussphere (plant litter and harvest residues), in the drilosphere (organic matter processed by earthworms), and especially in the rhizosphere (rhizodeposits and root residues). During growth, soil microbes not only convert substrate to biomass, but they also excrete non-biomass metabolites such as extracellular polymeric substances (EPS) and extracellular enzymes (Joergensen and Wichern 2018). Redmile-Gordon et al. (2014) described an approach for the extraction of soil EPS

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with cation exchange resin (CER), which separates biomass and non-biomass metabolites (Bérard et al. 2020).

EPS perform multiple functions in soil, and are formed by proteins, carbohydrates, lipids, nucleic acids and other humic substances (More et al. 2014). The presence of EPS in general, can provide advantages such as cell adhesion to surfaces (Flemming and Wingender 2010), cell and enzyme protection (Flemming et al. 2023; Op De Beeck et al. 2021; Whitfield et al. 2015) against external stressors (Whitfield et al. 2015), such as desiccation (Ilyas et al. 2020), heavy metals (Li et al. 2022; Redmile-Gordon and Chen 2017), salinity (Awad et al. 2012), likely also protecting dormant microorganisms when substrates are depleted. The functions that EPS perform can, however, be highly dependent on their proportion of proteins and carbohydrates. Proteinaceous EPS were found to promote soil particle aggregation (Krause et al. 2019) and previous studies (Li et al. 2022) have reported the carbohydrate fraction of EPS to increase as a reaction to heavy metal contamination.

All crops transfer assimilates into the soil by rhizodeposition (Wichern et al. 2008), a process that embraces all compounds released by living root cells, such as ions, gases, volatile organic components, mucilage, lysates, and secretions as well as decaying fine root fragments (Uren 2001; Wichern et al. 2008). During initial plant development, rhizodeposition mainly consists of root exudates, whereas after flowering, rhizodeposits were found to increasingly consist of the more recalcitrant remains from decaying fine root particles during maturation (Arcand et al. 2013; Gavito et al. 2001; Hupe et al. 2019). Root exudates are easily available carbon sources rapidly decomposed by rhizosphere microorganisms (Ahmed et al. 2018a), and can be transformed into microbial EPS. Mucilage can be defined as a plant-excreted colloidal EPS (Staudinger et al. 2022), which functions as a protective lubricant for the root tip against physical damage during growth through the soil (McKenzie et al. 2013). They keep the soil near the roots wet and hydraulically well-conductive, favouring water retention and facilitating the water flow from dry soil towards the root surface (Ahmed et al. 2014).

Since climate change is currently threatening food production with the increased risk of intense droughts (Easterling et al. 2007; Wheeler and von Braun 2013), investigating the effect of drought on plant-microbe interactions is essential and can help in the development of mitigation strategies. The transfer of assimilates to roots increases under drought as indicated by decreased shoot-to-root ratios (Bacher et al. 2022; Kleikamp and Joergensen 2006) but reduces the microbial turnover due to lowering the demand for energy maintenance (Joergensen and Wichern 2018), leading to increased MBC to SOC ratios (Insam et al. 1989). Drought often increases the formation of mucilage (Nazari et al. 2023) or plant-derived EPS (Le Gall et al. 2021) without lowering microbial decomposition (Ahmed et al. 2017, 2018a), which potentially increases the soil microbial biomass during a cropping season.

For different plant species over longer periods of time, and under different management practices, varying plant-microbe interactions can occur. Wheat (*Triticum aestivum*) is one of the most important staple crops cultivated in Europe (Kahiluoto et al. 2019). The resilience of wheat has been studied regarding the composition and growth rates of the related microbial community in reaction to drought (Bardgett et al. 2008; Breitkreuz et al. 2021). However, this has not been studied for microbial and plant-originated EPS exudation and their changes during plant developmental stages. The influence of different cropping systems, from more conservative to intensive systems might also affect EPS accumulation levels, as a result of the direct changes in soil organic carbon accumulation (SOC) and microbial biomass carbon (MBC) and nitrogen (MBN). The application of farmyard manure has been particularly shown to increase the above-mentioned parameters (Joergensen et al. 2010; Krause et al. 2022) and might modulate EPS excretion levels in reaction to drought stress.

In order to investigate the coupled effects of drought and cropping system on EPS production and soil microbial biomass at different wheat developmental stages, a drought simulation experiment was conducted on the DOK (*Dynamisch*, *Organisch*, *Konventionell*) long-term trial in Switzerland, an investigation embedded into the Bio-Diversa + Project MICROSERVICES, which investigates drought effects on the microbiome and their associated soil functions under winter wheat in Europe. Soil sampling was performed at three developmental stages of winter wheat, e.g., stem elongation, flowering, and ripening, from three different cropping systems: biodynamic system (BIODYN), solely fertilized with composted farmyard manure and slurry, a conventional system (CONFYM) receiving a combination of farmyard manure and mineral fertilizers, and a conventional system (CONMIN) only minerally fertilized. We addressed the following hypotheses: (1) simulated drought promotes the formation of carbohydrate dominated EPS, and secondly, (2) increases in EPS accumulation will be higher at plant stages with greater root growth and exudation (i.e. flowering), and (3) cropping systems fully or partly fertilized with farmyard manure will present higher amounts of microbial biomass and therefore more EPS, especially under drought.

Materials and methods

Site description and soil processing

Soil samples were collected in the DOK long-term trial in Therwil in Switzerland (47°30'9.48"N, 7°32'22.02"E), which compares different organic and conventional cropping systems since 1978 (Krause et al. 2022; Mäder et al. 2002). The trial site is located at 300 m above sea level. The mean annual temperature is currently 11 °C and the mean annual precipitation is 840 mm. The loamy soil was classified as Haplic Luvisol developed on alluvial loess deposits (IUSS Working Group WRB 2022; Leifeld et al. 2009). The field experiment is designed as a randomized split-split plot with four field replicates for each cropping system.

The three cropping systems investigated in the current experiment were BIODYN, CONFYM and CONMIN. The BIODYN system was fertilized with composted farmyard manure and slurry, and received additional biodynamic preparations (Horn manure and Horn silica) (Table S1) during the experimental period. Weeding was done mechanically, and no chemical pesticides were applied. The other two systems (CONFYM and CONMIN) were managed conventionally and treated with herbicides, fungicides, insecticides, and synthetic plant growth regulators. Fertilization type and amounts, as well as information on pesticide and plant regulator applications are available in Table S1, and further described in detail by Kost et al. (2024). Rain-out shelters were installed in November 2021 on one side of the plot in each of the abovementioned cropping systems. Winter wheat (*Triticum aestivum* var. Wiwa) was sown in mid-October on the other side, where a rainfed control was established. As specified by Kost et al. (2024), the control plots received around 193 mm of rainfall from mid-November 2021 to April 2022. The sheltered plots were irrigated during that period, with a total of 55 mm of rainfall equivalent. From 1 April to July 14, 2022, the sheltered plots were completely deprived from irrigation and rainfall. This resulted in a total reduction in precipitation, of 72% during winter and a complete withdrawal from April to mid-July, targeting a soil water content of around 10%. Soil moisture and temperature, as well as air humidity were measured in drought-induced and control plots, together with plant growth and nutrient contents (potassium, magnesium and phosphorus), which were all investigated and reported by Kost et al. (2024).

Three soil cores per plot were taken at 0–15 cm depth using a soil corer (diameter of 5 cm) at three different plant stages (stem elongation, flowering, and ripening) and pooled. All samples were sieved to 2 mm and stored at 4 ºC until analysis. For analysis of SOC and total N, samples were dried for 24 h at 105 °C, milled using a swing mill (Retsch, Haan, Germany), and measured using a Vario MAX (Elementar, Hanau, Germany) elemental analyser. SOC was determined as total C minus carbonate C, which was gas-volumetrically determined after the addition of 10% HCl to the soil using a calcimeter. Gravimetric water content (GWC) of soil samples was determined by drying them for 24 h at 105 °C (DIN EN ISO 2014).

Microbial biomass

MBC and MBN were determined by fumigation extraction (Brookes et al. 1985; Vance et al. 1987). A pre-extraction step was performed to remove root debris and avoid fumigation of root cells instead of MB cells by shaking 30 g of soil in 70 mL of 0.05 M K_2SO_4 for 30 min at 200 rev min^{-1} (Mueller et al. 1992). The samples were then centrifuged for 10 min at 4000 g and filtered. The centrifugation pellet was mixed homogeneously and 15 g were fumigated with $CHCl_3$ for 24 h at 25 ºC in the dark. After the removal of the $CHCl_3$, the fumigated soil was extracted with 60 mL 0.05 M K_2SO_4 (Potthoff et al. 2003). At the same time as fumigation started, 15 g of the pre-extracted soils were mixed with 0.05 M K_2SO_4 and extracted as non-fumigated samples. Organic C and total N in the extracts were analysed using a Multi N/C 2100 S analyser (Analytik Jena, Germany). MBC was calculated as E_C / k_{EC}, where EC = (organic C extracted from fumigated soils) – (organic C extracted from non-fumigated soils) and k_{EC} = 0.45 (Wu et al. 1990). MBN was calculated as E_N / k_{EN}, where E_N = (total N extracted from fumigated soils) - (total N extracted from non-fumigated soils) and k_{EN} = 0.54 (Brookes et al. 1985).

Extracellular polymeric substances

EPS was extracted using cation exchange resin (CER) as described by Redmile-Gordon et al. (2014), however, with the omission of the pre-extraction step (Bublitz et al. 2023). Phosphate buffered saline (PBS) was used as extraction buffer, prepared with 2 mM $Na_3PO_4 \times 12\ H_2O$ (760 mg L^{-1}), 4 mM $NaH_2PO_4 \times H_2O$ (552 mg L^{-1}), 9 mM NaCl (526 mg L^{-1}), and 1 mM KCl (74.6 mg L^{-1}) and adjusted to pH 7. The amount of CER used for each soil sample was determined according to Frølund et al. (1996) as suggested by Redmile-Gordon et al. (2014):

$$1\ \text{g SOC} = 177.8\ \text{g CER}$$

Moist soil (2.5 g dry weight equivalent) was weighed into 50 mL centrifuge tubes, and CER was added along with 25 mL of chilled PBS. The mixture was placed in a shaker at 2 cycles per second for two hours in the dark at 4 °C. Samples

were centrifuged at 4200 g for 20 min, and the supernatant was decanted and stored at -20 °C.

Total protein was photometrically quantified in 0.5 mL aliquots of the EPS extracts according to Lowry et al. (1951) as modified by Redmile-Gordon et al. (2013) for soil extracts, which contain potentially confounding polyphenolic compounds. Samples were compared to standards of bovine serum albumin (BSA, Sigma A7906). Non-proteinaceous chromogens in soil extracts were corrected by (i) measuring the absorbance of a second set of samples using Lowry reagents without $CuSO_4$, and (ii) applying the mathematical correction given by Frølund et al. (1996):

$$\mathrm{Abs}_{\mathrm{protein}} = 1.25\,(\mathrm{Abs}_{\mathrm{A}} - \mathrm{Abs}_{\mathrm{B}})$$

where Abs_A is the absorbance obtained for soil extracts using Lowry reagents, Abs_B is the absorbance supplied by the set analysed using Lowry reagents without $CuSO_4$, and $Abs_{protein}$ is the theoretical absorbance of proteins. All photometric measurements were carried out using a microplate reader (FLUOstar Omega, BMG Labtech, Ortenberg, Germany).

Total carbohydrates were determined according to Mopper and Gindler (1973), adapted by Joergensen et al. (1996), by reducing Cu^{2+} to Cu^{+} in the ends of mono- and disaccharides. Prior to carbohydrate quantification, EPS extracts were hydrolysed for 10 min at a temperature of 100 °C in an autoclave, as proposed by Bublitz et al. (2023). The reduction of hydrolysed polysaccharides was photometrically detected at a wavelength of 562 nm (Smith et al. 1985), after the reaction of carbohydrates with a reagent consisting of an aqueous buffer (4% Na_2CO_3, 4% $[(NaPO_3)_6]$ and 0.2% aspartic acid solution), 3 mL bicinchoninic acid (BCA) (Sigma D8284–5G) (40 g L^{-1}) and 0.45 mL of a $CuSO_4 \times 5$ H_2O solution (63 g L^{-1}). Total carbohydrates were quantified by adding 2 mL BCA reagent to 0.5 mL neutralized hydrolysates in a test tube, placing it into a heating cabinet at 60 °C for 120 min, and reading it colorimetrically at 562 nm, using a microplate reader.

Statistical analysis

Statistical data evaluation was carried out with R version 4.3.1 (R Core Team 2023). Sample replicates for parameters such as (1) GWC, (2) EPS-proteins, (3) EPS-carbohydrates, (4) MBC, (5) MBN, (6) MB-C/N, (7) EPS-carbohydrates / MBC, and (8) EPS-proteins / MBN were averaged and examined using a two-factor ANOVA for the effect of cropping system and water regime on the data, as well as the interaction between them. As the same parameters were also quantified at different timepoints, resulting in repeated measurements that violate the independence assumption, the variable "plant stage" was analysed separately using a mixed linear model (package lme4 and lmerTest).

Before performing the Two-way ANOVA, outliers of each parameter were checked with the Dixon test (package Outliers). The values of the above-mentioned parameters were used as dependent variables, whereas water regime (drought-induced, rainfed control) and cropping system (BIODYN, CONFYM, and CONMIN) were used as independent categorical variables. Variance homogeneity of the residuals was checked using Levene and Fligner-Killeen tests (Conover et al. 1981), and normality was tested using the Shapiro-Wilk test.

In case residuals of variables did not meet the assumptions of homogeneity and normality, they were subjected to data transformation using the package bestNormalize (Peterson and Cavanaugh 2020) (Figs. S2 and S3). If assumptions were still not met, such as for SOC and total N, data were analysed with the non-parametric test aligned rank transform ANOVA (artANOVA) (Wobbrock et al. 2011), using the ARTool R package (Elkin et al. 2021) for the effect of water regime and cropping system, applying plant stage as grouping term. To check specifically the significant differences between cropping systems within each water regime treatment, a one-way ANOVA was additionally performed. One test was performed for drought-induced plot and another for the rainfed control plot, using cropping system as an independent categorical variable, followed by the Tukey HSD (honestly significant difference) post-hoc test to check differences between groups. When normality and variance homogeneity assumptions were not met, a non-parametric Kruskal-Wallis test was performed, followed by the Dunn's test with Bonferroni correction for p-values, for differences between groups.

For the effect of plant stage on each numerical variable, a general mixed linear model for all data was performed using the "lme4" package in R (Bates et al. 2015), with restricted maximum likelihood (REML), using plant stage and water regime as fixed effects, and cropping system as a random variable. The significance of fixed effects was verified using the Kenward-Roger approximation for degrees of freedom (Kenward and Roger 1997). Pairwise comparisons of estimated marginal means (EMMeans) with multiple comparison adjustments (Tukey method) was used as a post-hoc test for each plant stage, using the emmeans R package (Lenth 2017). A Pearson correlation was performed between MBC and MBN, and EPS-carbohydrates as well as EPS-proteins and GWC.

Table 1 Soil gravimetric water content mean values per plant stage

Plant stage	Gravimetric water content (% dry soil)	
	Rainfed control	Drought-induced
Stem elongation	33 a	14 a
Flowering	19 b	10 b
Ripening	28 a	8 b
CV (± %)	49	34

CV = mean coefficient of variation between replicate plots (n = 4), letters represent significant differences between the plant stages (P < 0.05, Emmeans); probability values of mixed linear model: plant stage = not significant (NS), water regime × plant stage = NS; probability values of the 2-way ANOVA: cropping system < 0.05, water regime < 0.01, water regime × system = NS

Results

Soil properties

Soil organic C and total N contents were not affected by water regime and plant stage (Table S2), indicating homogeneity of the experimental plots. The cropping systems differed in mean SOC and total N contents in an increasing order CONMIN < CONFYM < BIODYN. Soil gravimetric water content was significantly reduced in the drought-induced treatment (Table 1) and declined from 14% at the beginning of the drought period to 8% at wheat ripening, however, its interaction with cropping system was not significant.

EPS extracts

EPS-carbohydrates were significantly affected by water regime and the interaction between water regime × plant stage (Table 2). EPS-carbohydrates varied around a mean of 130 µg g^{-1} soil in the drought-induced treatment without being affected by plant stage over the season (Fig. 1a). In contrast, EPS carbohydrates increased significantly from 90 µg g^{-1} soil at stem elongation to 120 µg g^{-1} soil at ripening in the rainfed control. The difference in EPS-carbohydrate content between drought-induced and rainfed control treatment was largest at the initial stem elongation stage and declined during the growing season. Cropping system had significant but inconsistent effects at flowering and ripening only in the rainfed control (Fig. 1b).

EPS-proteins exhibited significant water regime and water regime × plant stage effects (Table 2). In addition, EPS-proteins were significantly affected by plant stage, being highest at flowering and lowest at ripening, particularly in the rainfed control (Fig. 1c). EPS-proteins varied around a mean of 73 µg g^{-1} soil in the drought-induced treatment compared to the rainfed control, with 61 µg g^{-1} soil. The difference in EPS-protein content between drought-induced and control treatment was negligible at

Table 2 Average extracellular polymeric substances (EPS) carbohydrates and proteins, microbial biomass carbon (MBC), nitrogen (MBN), MB-C/N, EPS-carbohydrates/MBC and EPS-proteins/MBN ratios; probability values and effect sizes η_p^2 of a two-way ANOVA, using cropping system and water regime as factors, and a mixed linear model using cropping system as random variable

	EPS-carbohydrates		EPS-proteins		MBC		MBN		MB-C/*N*		EPS-carbohydrates/MBC		EPS-proteins/MBN	
	(µg g^{-1} soil)													
Plant stage														
Stem elongation	113 a		66 b		369 a		48 b		7.8 a		0.33		1.50 a	
Flowering	111 a		76 a		392 a		62 a		6.4 c		0.31		1.30 ab	
Ripening	124 a		59 c		413 a		61 a		6.9 b		0.33		1.10 b	
Probability values of the mixed linear model														
PS	NS		< 0.01		0.09		< 0.01		< 0.01		NS		< 0.01	
WR × PS	< 0.01		< 0.05		0.06		0.05		NS		< 0.01		< 0.01	
Probability values and effect sizes [η_p^2] of the 2-way ANOVA														
CS	NS	[NS]	NS	[0.06]	< 0.01	[NS]	< 0.01	[NS]	NS	[NS]	< 0.01	[NS]	0.05	[0.09]
WR	< 0.01	[NS]	NS	[< 0.01]	NS	[< 0.01]	NS	[< 0.01]	NS	[0.06]	< 0.01	[0.10]	0.06	[< 0.01]
WR × CS	NS	[0.10]	NS	[0.04]	NS	[< 0.01]	NS	[0.01]	NS	[0.07]	0.05	[NS]	NS	[< 0.01]
CV (± %)	23		24		30		33		10		35		40	

WR = water regime; PS = plant stage; CS = cropping system; CV = mean coefficient of variation between replicate plots (n = 4), letters represent significant differences between the plant stages (P < 0.05, Tukey-HSD)

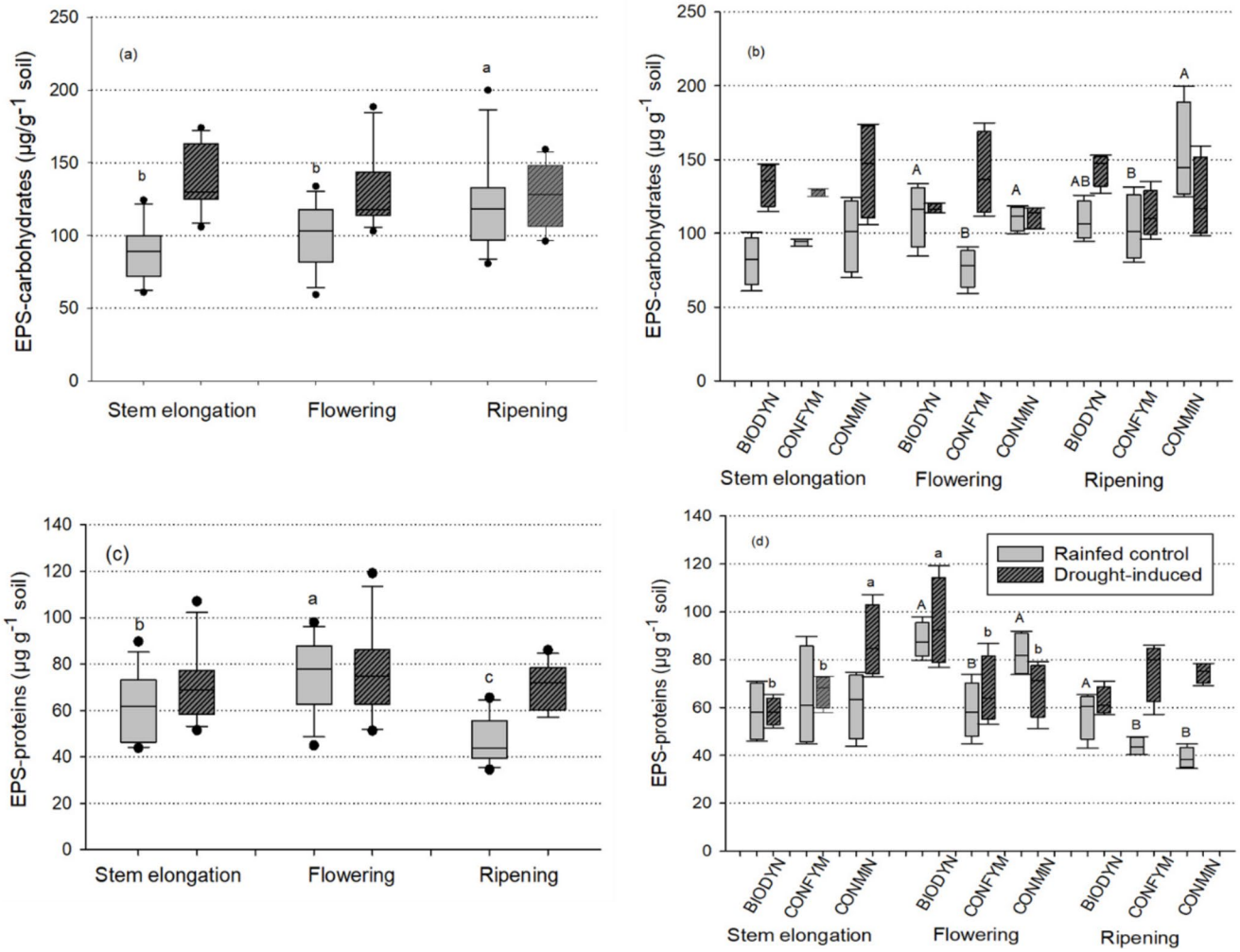

Fig. 1 Soil extracellular polymeric substances (EPS) carbohydrates (**a**, **b**) and proteins (**c**, **d**) under rainfed control and drought-induced conditions at stem elongation, flowering, and ripening stages of wheat combined across all cropping systems (**a**, **c**) and split by biodynamic (BIODYN), mixed conventional (CONFYM) and mineral conventional (CONMIN) cropping systems (**b**, **d**). Lowercase letters represent differences between groups in the rainfed control plots and uppercase letters in drought-induced plots ($P<0.05$, Tukey HSD)

flowering and largest at the ripening stage. Cropping system had significant but inconsistent effects at flowering and ripening only in the control treatment and at stem elongation in the drought-induced treatment (Fig. 1d).

EPS-carbohydrates and EPS-protein showed both a significant negative relationship (r = -0.41, $P<0.01$) with the GWC. The ratios of EPS-carbohydrates / EPS-proteins varied around 1.9 throughout the sampling period. This ratio would result into a mean C/N quotient of 8.3 ((EPS-carbohydrate-C + EPS-protein-C) / EPS-protein-N; carbohydrates × 0.40 = carbohydrate-C, proteins × 0.45 = protein-C, protein-C / 3.125 = protein-N), which is slightly above the mean biomass C/N ratio of 7.0 and moderately below the mean SOC/total N ratio of 10.3.

Microbial biomass

MBC differed significantly between the cropping systems in the order of CONMIN = CONFYM < BIODYN (Fig. 2b). However, MBC was not affected by plant stage or water regime (Table 2; Fig. 2a). MBN was closely related to MBC with $r=0.94$ ($P<0.01$) and showed an average MB-C/N ratio of 7.0 (Table 2, Fig. S2). However, MBN exhibited a maximum at flowering, particularly in the drought-induced treatment (Fig. 2c). Effects of the cropping system were identical to those on MBC (Fig. 2d). MBC was significantly related to SOC with $r=0.75$ ($P<0.01$) and MBN to total N with $r=0.64$ ($P<0.01$).

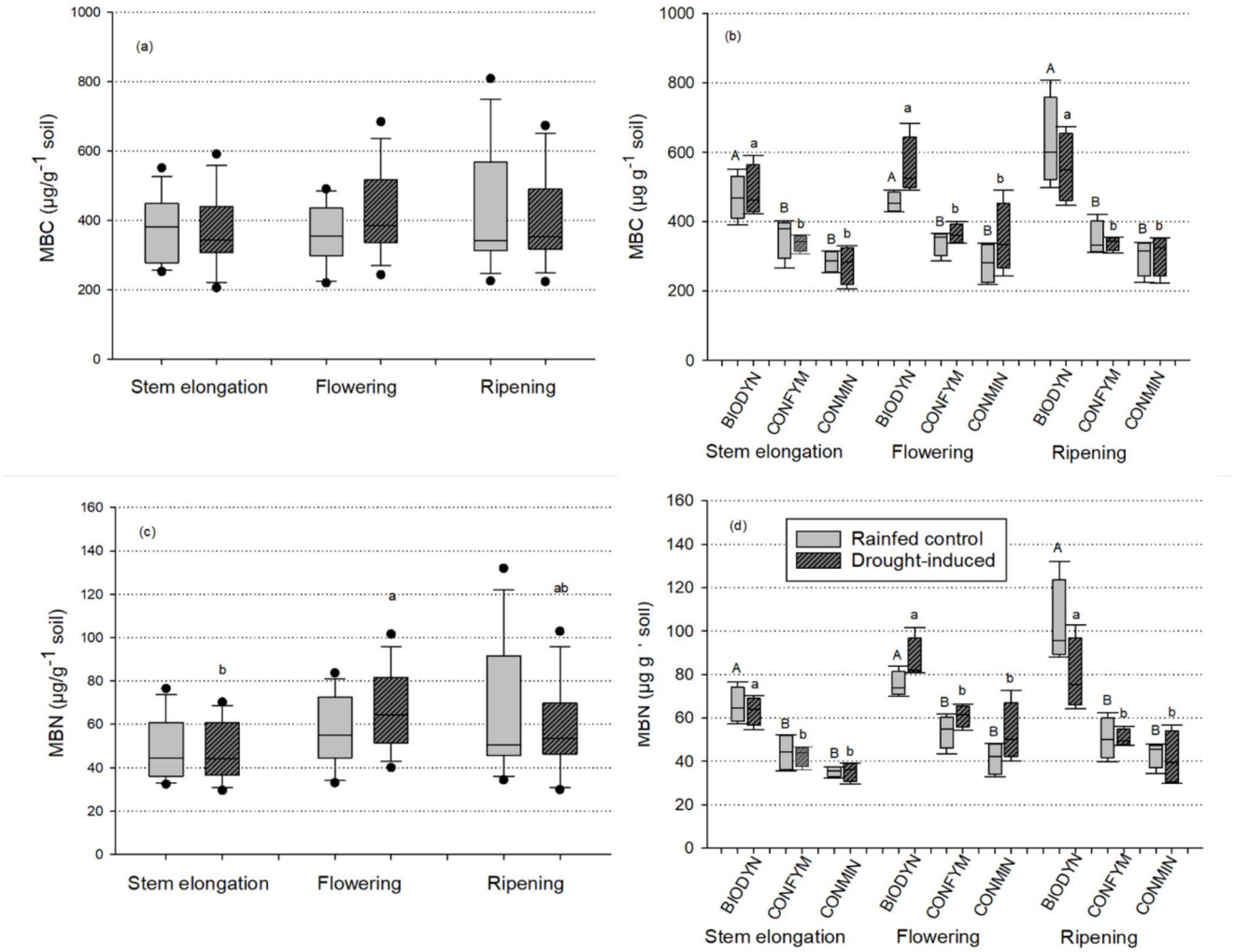

Fig. 2 Microbial biomass carbon (MBC) (**a**, **b**) and nitrogen (MBN) (**c**, **d**) under rainfed control and drought-induced conditions at stem elongation, flowering, and ripening stages of wheat combined across all cropping systems (**a**, **c**) and split by biodynamic (BIODYN), mixed conventional (CONFYM) and mineral conventional (CONMIN) cropping systems (**b**, **d**). Lowercase letters represent differences between groups in the rainfed control plots and uppercase letters in drought-induced plots ($P<0.05$, Tukey HSD)

Ratios of EPS extracts to microbial biomass

The ratio of EPS-carbohydrates / MBC presents a similar picture over the season as EPS-carbohydrates (Fig. 3a) and varied around a mean of 0.32. However, the EPS-carbohydrates / MBC ratio increased from 0.26 at stem elongation to 0.33 at ripening in the rainfed control, whereas the respective ratio under drought declined from 0.40 at stem elongation to 0.34 at ripening, leading to a significant water regime × plant stage interaction (Table 2). Particularly the cropping systems exhibited significant responses, leading to increasing EPS-carbohydrates / MBC ratios in the order BIODYN (0.23)<CONFYM (0.32)<CONMIN (0.43), neglecting the significant differences between drought-induced and control treatments at stem elongation (Fig. 3b).

The ratio of EPS-proteins / MBN generally declined from stem elongation to ripening (Table 2), particularly in the control treatment (Fig. 3c), and varied around a mean of 1.3. The cropping systems led to higher EPS-proteins / MBN ratios (Fig. 3d), in the order BIODYN (0.9)<CONFYM (1.3)<CONMIN (1.7) without significant water regime × plant stage interaction (Table 2).

Discussion

Drought and seasonal effects on soil EPS

EPS-proteins, and particularly EPS-carbohydrates were significantly increased by drought, which is in accordance with our hypothesis (1). Both EPS fractions were negatively

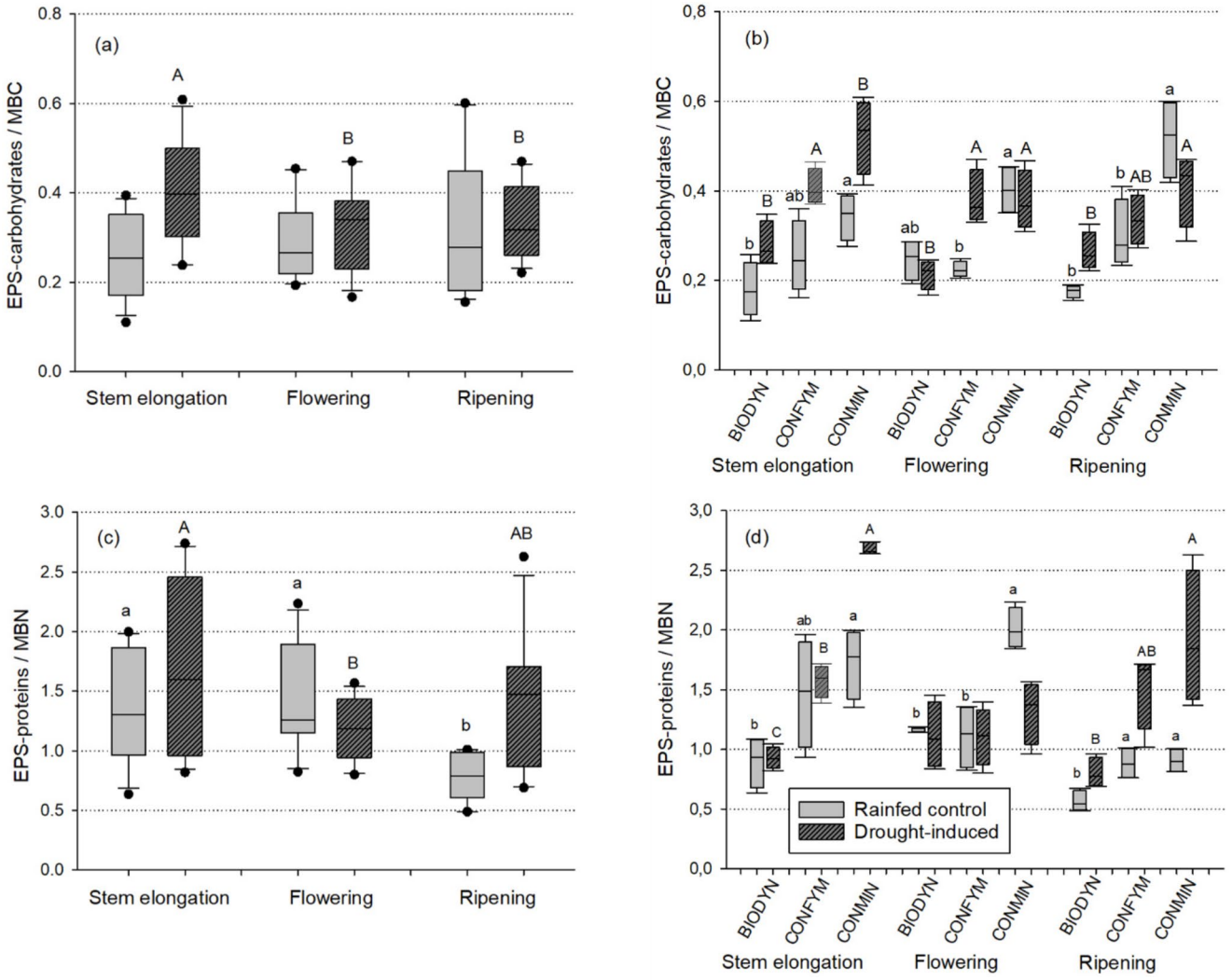

Fig. 3 Ratios of EPS carbohydrates / MBC (**a**, **b**) and EPS proteins / MBN (**c**, **d**) under rainfed control and drought-induced conditions at stem elongation, flowering, and ripening stages of wheat combined across all cropping systems (**a**, **c**) and split by biodynamic (BIODYN), mixed conventional (CONFYM) and mineral conventional (CONMIN) cropping systems (**b**, **d**). Lowercase letters represent differences between groups in the rainfed control plots and uppercase letters in drought-induced plots ($P < 0.05$, Tukey HSD)

correlated with soil moisture but remained roughly constant from stem elongation to ripening in the drought-induced treatment. This suggests that the main differences in EPS content between the drought-induced and control treatments developed between the start of drought-induction at the beginning of April and the first sampling at stem elongation at the end of April.

The largest difference in EPS-carbohydrate between drought-induced and control treatment was observed at stem elongation. This might indicate that young wheat plants under drought conditions invested more in carbohydrate dominated mucilage in the drought-induced than in the control treatment. Root C allocation has been shown by Swinnen et al. (1994) to reach its maximum at wheat tillering. Mucilage-like EPS are exuded by plants to prevent root tissue dehydration and store water (Ahmed et al. 2014; Chenu 1993; Tosif et al. 2021). Plant-originated EPS are then rapidly used by soil microorganisms, as an energy source and habitat (Ahmed et al. 2018a, b; Mary et al. 1993), with the advantage of reducing cellular energetic investments (Nazari 2021; Nazari et al. 2022).

Contrastingly to the calculated C/N ratios (8.3) of EPS-carbohydrates to EPS-proteins, the C/N ratio of wheat rhizodeposits can reach much higher values (Hirte et al. 2018). This might indicate that the whole EPS fractions extracted from stem elongation to ripening are likely plant originated mucilage which was fully transformed into microbial EPS. However, there is a need for a clear separation of EPS

originating from plants or microbes, which might be possible in the future with the analysis of biomarkers in the CER extracts, for example amino sugars and neutral sugars as microbial biomarkers (Salas et al. 2023) or hydroxyproline as plant biomarkers (Salas et al. 2024). This demonstrates the importance of discussing the processes involved in EPS production from soil C sources to microbial exudation.

The data acquired in this study contrast the often-stated view that soil EPS of both microbial and plant origin, directly originated or transformed from mucilage are predominantly composed of carbohydrates (Bacic et al. 1986; Nazari 2021; Nazari et al. 2022, 2023). The idea is that EPS-carbohydrates perform a more protective role than EPS-proteins (Li et al. 2022). The structural proteins contained in the EPS-protein fraction, i.e., so-called scaffoldins, are highly responsible for EPS elasticity and for increasing the persistence of EPS-embedded extracellular enzymes (Costerton et al. 1981; Redmile-Gordon et al. 2015). Additionally, the presence of supporting glycoproteins and scaffoldin-like structures in EPS can enhance the hydrolytic activity of enzyme-complexes (Redmile-Gordon et al. 2015; You et al. 2012). The current contrasting results during crop maturation may result from the decrease in organic matter quality and the greater production of recalcitrant compounds by plants after subjected to drought (Pugnaire et al. 2019). This process might be closely related to the increase in proteinaceous EPS after a whole plant growth season under drought.

The contents of EPS-proteins and EPS-carbohydrates are in the range of those obtained by previous studies. Kakumanu et al. (2019) observed that EPS-carbohydrates varied between 50 and 100 μg g^{-1} soil without a clear effect of intermediate drought, but soil-specific increases at strong drought regimes. Hale et al. (2021) found EPS-carbohydrates in the range of 180 to 470 μg g^{-1} soil, which increased with SOC content and with deficit irrigation at 50% and 75% of full irrigation. In contrast, Sher et al. (2020) found only 10 and 14 μg EPS-carbohydrates g^{-1} soil in a marginal Arenic Plaggic Anthrosol, which were not affected by drought. In the study of Redmile-Gordon and Chen (2017), EPS-protein was at approximately 100 μg g^{-1} soil and EPS-carbohydrates at approximately 200 μg g^{1} soil in a sandy grassland soil (Cambic Arenosol), which resulted in a nearly identical EPS-carbohydrate/EPS-protein ratio as the current dataset.

EPS must be produced during microbial growth in the hotspots of substrate availability. However, dormant soil microorganisms also require protection by EPS as suggested by relatively small fluctuations during the season. This indicates that the CER method described by Redmile-Gordon et al. (2014) is a useful, reproducible, and reliable approach for the extraction of soil EPS (Bérard et al. 2020). As the contribution of hotspots to bulk soil is probably low (Joergensen and Wichern 2018; Kuzyakov and Blagodatskaya 2015), i.e., the analysis of drought effects of bulk soil dilutes on crops and their rhizodeposition. Further analysis of rhizosphere soil is necessary to better understand this mechanism.

Effects of cropping system on MBC, MBN, and EPS

MBC and MBN remained roughly constant throughout the plant stages of winter wheat, without any effects of drought. Several factors need to be analysed in conjunction to the differences in crop management, and which might have contributed to such results. Despite the specific and contrasting fertilization and weed management regimes, the DOK long-term field trial has been long implementing regenerative practices such as shallow tillage, cover cropping, and incorporation of grass-clover into the crop rotation in all cropping systems, treatments that, additionally to the soil type, with high amounts of silt, and the largely dormant microbial population of the bulk soil, likely buffered the actual differences in water and substrate availability. As a result, the soil microbial biomass only responded to the long-term differences in crop management.

The data showed differences between cropping systems, already reported before, highest MBC and MBN in the biodynamic system BIODYN with farmyard manure application and no pesticide application, and lowest in the conventional system CONMIN with solely mineral fertilization and use of pesticides and herbicides (Joergensen et al. 2010; Krause et al. 2022; Mäder et al. 2002).

Cropping system-dependent drought effects on EPS-carbohydrates and EPS-protein were not detected (Table 2), suggesting that under drought stress, the influence of cropping system was low. Kost et al. (2024) has, similarly, reported no effect of cropping system on the level of resilience of the microbial community to drought, which might be closely related to our findings, as EPS exudation increases microbe resilience to harsh environments (Roberson and Firestone 1992). Finally, we have to reject our third hypothesis, as the application of organic amendments and/or the reduction of pesticide use in both BIODYN and CONFYM did not result in higher amounts of microbial biomass and therefore more EPS under drought. Plant stage-specific effects on both indices did not show a clear direction (Fig. 1). Maximum EPS-protein contents coincided with minimum MB-C/N ratios at flowering, which indicates high N availability to microbes during this plant stage, contrary to the observation of higher EPS-protein contents under N deficit (Redmile-Gordon et al. 2015).

The ratios of EPS-carbohydrates / MBC and EPS-protein / MBN were lowest in BIODYN and highest in CONMIN,

which suggests that the biodynamic system buffered drought stress, so that less plant mucilage and rhizodeposition was diverted to microbial EPS and more to MBC and MBN. EPS-carbohydrates / MBC and EPS-protein / MBN ratios are therefore, important tools for assessing the reliability of EPS measurements. Whereas MBC and MBN mainly discern cropping systems, EPS-carbohydrates and EPS-proteins were mainly sensitive to drought and to a lower extent to crop growth stage. Their combination with MBC and MBN seems to form useful indices for the actual soil fertility status, as driven by crop-microorganism interactions, and may gain similar importance as the MBC / SOC ratio (Anderson and Domsch 1989, 2010) and the metabolic quotient qCO_2 (Anderson and Domsch 1990, 2010) in the future.

Conclusions

Drought simulation presented a greater effect on bulk soil EPS accumulation at early stages of wheat development compared to later growth stages indicating a high contribution of mucilage exuded by roots. However, EPS-carbohydrates and -proteins were affected differently over plant stages, reflected in a higher ratio of EPS-carbohydrates / EPS-proteins at stem elongation than at ripening. The ratios of both EPS fractions to microbial biomass were highest in the CONMIN system and lowest in the BIODYN system, where the higher level of SOC might have improved the buffering capacity against drought stress. The reason might be that less rhizodeposits were diverted in the long-term to EPS than to microbial biomass. However, this explanation needs more experimental evidence by analysing the transfer of rhizodeposits into EPS and microbial biomass in rhizosphere soil, using stable isotopes. This should be done particularly during early stages of plant development, i.e., considerably before stem elongation.

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Declarations

Conflict of interest The authors declare no conflicts of interest.

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