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Effects of biochar, compost and straw input on root exudation of maize (*Zea mays* L.): From function to morphology

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ABSTRACT

Returning crop straw, such as biochar, into the field is increasingly recognized as a valid, environmentally-friendly agricultural strategy to improve soil quality, increase crop yields, and reduce combustion-induced air pollution. Root exudation has potential importance for plant performance, rhizosphere function and plant–soil interactions; however, the effects of crop straw input on maize root exudation remain unclear. After three seasons of a field experiment, a mesh bag experiment was conducted in situ in the fourth season to study the effects of three straw incorporation treatments (chopped maize straw, SD; compost produced by maize straw, SC; biochar produced by maize straw, BC) and control (no straw, CK) on root exudation and their underlying mechanisms in a maize cropping system. Discrimination in the metabolic profiling of the root exudates between the CK and all the straw-incorporated samples was clear, but the separation between the samples from the three different straw strategies was limited. Among the 32 assigned components of maize root exudates, the fluctuations in organic acids (up to 16-fold) were stronger than those in sugars and amino acids when straw was incorporated. The response of the organic acid exudates was linked to the transcript levels of the ZmMATE1 and ZmMATE2 genes, which are associated with root exudation (Spearman correlations, $P < 0.05$). Straw incorporation influenced the root phenotypic appearance and morphology, which are characterized by longer and thinner root, larger root surface area, and more root tips in BC treatment, whereas shorter and thicker root, and fewer root tips in SD treatment. The changes in the root morphology and metabolomes were responsible for the responses of the maize root exudates to straw incorporation. Regulation of genes (ZmCycD2; 2a, ZmEXPB8, ZmXTH1, and ZmARF1) that associated with root growth and development, and changes in the viability and anatomical structure of root tips were involved in the modifications of root morphology. This information will be useful for modulating rhizosphere processes and soil fertility, and for guiding and recommending residue management practices in crop production with relatively larger yields.

1. Introduction

Returning various straws or residues (including raw straw, straw-derived biochar, and compost) into the field has been widely recognized as a valid, environmentally-friendly agronomic strategy to improve soil quality and fertility, enhance crop yields, and reduce combustion-induced environmental pollution (Huang et al., 2018; Singh et al., 2018; Wang et al., 2019a). Maize (*Zea mays* L.) grows under a wide range of climatic conditions worldwide, and is an important crop with multiple applications in food, feed, fuel, and fiber (Sun et al., 2015). With the continuous increase in maize production, the disposal of large amounts of maize residues is substantially challenging (Tian et al., 2019). As a

monocotyledonous model plant, maize has a large root system and exhibits a complex root stock architecture that is highly responsive to soil environmental changes (Jansen et al., 2013; Palta and Yang, 2014).The root system is fundamental for plant growth, crop production and plant-environment interactions (Amendola et al., 2017; Nunes et al., 2019). Among the root functions, exudation plays a pivotal role in rhizosphere functions and plant–soil interactions, such as acquiring and mobilizing soil nutrients, repelling and attracting plant growth-promoting microorganisms, inhibiting the growth of competing plant species, and influencing information communication and energy transfer in the plant–soil system (Van Dam and Bouwmeester, 2016; Gargallo-Garriga et al., 2018; Herz et al., 2018). Plant roots will adjust

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the quantity and composition of exudates by sensing and adapting to the soil environmental disturbances. For example, plant roots frequently release the low-molecular-weight organic acids, such as acetate, malate, and oxalate in response to soil nutrient deficiency (Shang et al., 2019). The molecular transport mechanisms of plant export and secretion of compounds into the rhizosphere are complicated and contain multiple components. Multidrug and toxic compound extrusion transporters (MATEs) and Al-activated malate transporters (ALMTs) have been implicated in the efflux of various exudates across cell plasma membranes (PMs) into the rhizosphere (Ligaba et al., 2012; Adeleke et al., 2017; Coskun et al., 2017). A study on maize showed that the increase in organic acid exudates is linked to the over-expression of ZmMATE1 under biochar addition (Cheng et al., 2018). However, the effects of the incorporation of chopped straw, compost and biochar into soils on the quality and quantity of maize root exudates, and the exact mechanisms underlying this phenomenon remain unknown despite their potential importance. Untargeted metabolomics, a novel methodology derived from systematic biology can provide realistic insight into this process, and has been used to comprehensively investigate the metabolite profiles and composition of root exudates (Van Dam and Bouwmeester, 2016; Tsunoda and Van Dam, 2017; Guo et al., 2018). The sensitive and nondestructive omics technique of proton nuclear magnetic resonance (^{1}H NMR) spectroscopy is a powerful analytical platform used to explore rhizosphere processes, and has been applied to detect root exudates in barley, tomato, and maize (Fan et al., 1997; Escudero et al., 2014; Cheng et al., 2018).

Root exudation is positively correlated with plant biomass because the released root exudates originate from the photosynthetically fixed carbon of plants (Tückmantel et al., 2017). Exudation is not only driven by photoassimilate disponibility, and might be influenced by root morphology. Root system architecture and root branching were reported to qualitatively and quantitatively determine the composition of exudates (Haichar et al., 2014; Proctor and He, 2017; Rugova et al., 2017). For example, root exudation rates of maize increased with an increase in root branching (Groleau-Renaud et al., 1998). Accumulating evidence has showen that a much wider range of responses in root systems could be initiated by straw management via the direct and indirect interactions between crop roots and the incorporated straw materials. In our previous work, we found that root morphology, growth, and architecture were influenced to a remarkable extent by the addition of biochar produced from maize straw (Sun et al., 2017). Amendola et al. (2017) reported that fine root dynamics and morphology were affected by biochar application due to improvements in soil chemical and physical features. In terms of other patterns related to straw incorporation, Zhao et al. (2017) observed that crop residues can hamper root penetration in soils. Subsequently, the altered root morphology observed under straw management influenced root secretions. The plasticity of root morphology under straw management probably involves the regulation of cyclin proteins (which play fundamental roles in cell cycle control), expansin and xyloglucan endotransglucosylase/hydrolases (XTHs) (which are known to have a role in cell wall structure and cell enlargement), and auxin response factors (which contribute to the proliferation of branched and fine roots) (Geilfus et al., 2010; Na et al., 2011; Lara-Núñez et al., 2017). Additionally, root secretion largely relies on the inherent biology of plants, including root physiological status and metabolic changes (Rugova et al., 2017).

With the wide application of various straws, many previous studies have concentrated on the effects of different straw management strategies on soil properties and crop productivity. For example, the results from an extensive meta-analysis based on 156 published studies showed that crop residue return stimulated crop yield by 5.5 %, and N pool sizes in soil by 10.7 %, suggesting that this management functions to improve soil fertility (Wang et al., 2018). Recently, using a meta-analytical approach, the addition of biochar was found to have a large contribution to the stable soil organic carbon pool, and it also was found to have a positive impact on crop yield (Majumder et al., 2019). However,

the effects of straw management on root exudation and the relationships between the composition of root exudates and root morphological traits following straw management, that is, how and to what extent they are related, are largely unknown. A better understanding of these issues will greatly benefit the development of proper straw management strategies and allow the requirements for agricultural sustainability to be met. We hypothesized that maize root exudation would respond to straw management. Additionally, the modification of root morphology and the regulation of genes related to root exudation and growth played a substantial role in the response of exudation to straw management. To test this hypothesis, the effects of different straw inputting strategies, including chopped maize straw and its derived compost and biochar, on root exudation and root morphology were determined in a maize cropping system. The expression levels of genes associated with root exudation, root cell division and expansion, root branching, and viability and structure of root tips were investigated to determine the mechanism underlying the responses of root exudation and root morphology. The possible relationships of the exudates with the morphology and metabolism of the roots were described.

2. Materials and methods

2.1. Site description and setup of mesh bag field experiment

A long-term field experiment initiated on the spring of 2015 was conducted in a randomized block design with four replicates at the National Field Observation and Research Station of Shenyang Agroecosystems on the Liaohe Plain, Northeast China (N41°31′, E123°24′). The experimental plots had four different treatments, namely, no straw input (CK), straw directly incorporated in situ (SD, approximately 6000 kg ha^{-1} year^{-1} chopped maize straw), compost incorporation (SC, approximately 2000 kg ha^{-1} year^{-1} maize straw-produced compost), and biochar addition (BC, approximately 2000 kg ha^{-1} year^{-1} of maize straw-derived biochar). The chopped maize straw, compost and biochar were incorporated into the topsoil (0–20 cm) using a rotary cultivator. Each plot was formed by 9 rows (0.55 m width and 30 m length) with 1 m length buffer zone and sowed with maize cultivar "Fuyou 9″ at a plant population density of 5.5 plants m^{-2}. The conventional NPK fertilizer (urea, diammonium phosphate and potassium chloride) with 90, 75, and 75 kg ha^{-1} N, P$_2$O$_5$, K$_2$O, respectively, were applied yearly before planting, and side-dressing with 90 kg ha^{-1} N was applied during the jointing stage. The current study, a part of this long-term experiment, was established on the spring of 2018, and the mesh bag approach was performed according to Cui et al. (2016) with modifications. The sampling points were marked and numbered in each rows on the basis of plant population density. The topsoil was collected on each sampling point using a sampler with 18 cm internal diameter. The dug holes were retained to install the 75-μm nylon mesh bag filled with in situ collected soil. The soil cores formed in the mesh bags were approximately 13 cm in diameter and 17 cm in height. Maize seeds were sowed into the mesh bags, and other cultivation practices were the same as those in previous field experiments. The basic physicochemical characteristics of soil and various straws were listed in Table S1 and Table S2, respectively. After one month growth corresponding to the three-leaf stage, the mesh bags containing maize plant were lifted from the field soil. Then, the plants were gently separated from the soil and carefully collected through washing using sieves and running water. Afterward, the plant and root samples with six replicates of each treatment were divided into different parallel groups to measure root exudation, root morphology, expression level of genes, and structure of root tips, respectively.

2.2. Collection of root exudates and ^{1}H NMR analysis

After careful washing, the maize plant was immediately transferred to a tube containing 50 mL of ultrapure water covered with aluminum

foil, and cultured for 6 h to collect the root exudates. After filtering, the root exudate solutions were frozen at −70 °C and lyophilized for subsequent analysis. ^{1}H NMR analysis of metabolite profiling was performed using a Bruker Avance 600 spectrometer (Bruker Biospin, Germany), as described by Sun et al. (2017). The ^{1}H NMR spectra were processed on TopSpin 2.1 software (Bruker Biospin, Germany), and the spectral intensities of integrated regions with uniform width (0.04 ppm) were calculated on MestRe-C 4.9.9.9 software (Mestrelab Research S.L., Santiago, USA). The metabolites were assigned on the basis of previous studies and publicly available databases (Fan et al., 1997; Cheng et al., 2018). The response ratio of exudates between the control and different treatments was calculated based on the semiquantitative data from ^{1}H NMR. The identified compounds were showed in Fig. S1.

2.3. Root morphological characteristics

The plant samples for morphology measurement were separated into above and belowground parts. To obtain photos, the root part was dispersed in water to manipulate and locate the best position using an Epson perfection V850 photo scanner (Japan). The images with 400-DPI resolution were processed and analyzed using WinRHIZO (WinRHIZO Pro2017, Regent Instruments Inc., Quebec, Canada) to measure the total root length (RL), total root surface area (RSA), total root volume (RV), average root diameter (RD), and total root tip number (RTN). To determine the shoot and root dry weight (RW), they were dried in an oven at 80 °C until reaching a constant weight after root scanning. Six plants per treatment were measured to assess the root traits.

2.4. Cell cycle analysis

The samples were prepared according to Galbraith et al. (1983) and Soltys et al. (2014), with slight modifications to measure the cell cycle in the root tips. Freshly cut root tips (0.5 cm long; 5 tips per treatment) were chopped using a sharp razor blade and placed in a Petri dish with 3 mL nucleus-isolation buffer (3 mM Tris, 0.5 mM Na_2EDTA, 0.1 mM Spermin, 16 mM KCl, 4 mM NaCl, 0.1 %, v/v, Triton X-100, 0.11 %, v/v, β-Mercaptoethanol; pH 7.5). The suspension was passed through a 45-μm mesh nylon filter and then was centrifuged. After collection, the sample was incubated with 1 mL propidium iodide (PI) working fluid for 30 min at 4 °C in the dark. The sample was immediately run on a LSR-Fortessa flow cytometer (BD Company, USA) with a total of 20 000 cells collected and analyzed in the PI-based cell cycle phase. The distributions of cells in the gap phase 1 (G1), synthesis phase (S), and gap phase 2 (G2) were analyzed on ModFit LT 4.1 software (Verity Software House, Topsham, ME, USA).

2.5. Root tip viability and anatomical structure

The freshly excised root tips (0.5 cm) were stained with a mixture of fluorescein diacetate 12.5 μg mL^{−1} and PI (5 μg mL^{−1}) for 10 min at 4 °C according to Ishikawa and Wagatsuma (1998) to evaluate their cell viability. After staining, the cells were washed with distilled water for three times and sealed with antifluorescence quencher (Beyotime Institute of Biotechnology, Beijing, China). The root tips were observed and captured using a confocal fluorescence microscope (Leica, SP8., Wetzlar, Germany). Red and green fluorescence indicate the dead and viable cells in the root tips, respectively. To perform anatomical structure observation, the root tips excised from the secondary lateral roots of each fresh seedling were immediately fixed with 4 % (v/v) glutaraldehyde in 100 mM PBS buffer, overnight at 4 °C. Afterword, the samples were cryo-protected through infiltration with 10 % sucrose solution for 30 min and were embedded and longitudinally sectioned at 10 μm thickness using a cryostat microtome (Leica CM1850, Germany).

2.6. RNA extraction and gene expression analysis

The roots for gene expression analysis were sampled, immediately frozen, and stored at −70 °C until subsequent analysis by qRT-PCR according to Cheng et al. (2018). The total RNA of the root was extracted using a TRIpure Reagent Plant RNA Mini Kit (BioTeke, Beijing, China) following the supplier's instructions. First-strand cDNA was synthesized using a high-capacity cDNA reverse transcription kit (StepOnePlus™ Real-Time PCR System, ThermoFisher Scientific, USA) on the basis of the manufacturer's instructions, and a StepOnePlus™ Real-Time PCR system (ThermoFisher Scientific, USA) was used to perform qRT-PCR. PCR amplification conditions were described as follows: 10 min at 95 °C (pre-denaturation, 1 cycle), 30 s at 60 °C, 30 s at 72 °C (PCR, 40 cycles); 15 s at 95 °C, 1 min at 60 °C, 1 s at 95 °C (melting curve, 1 cycle). The relative expression levels of genes compared with the *ZmUbiquitin* gene was calculated using the $\Delta\Delta C_T$ method. The primers used to quantify gene expression are summarized in Table S3.

2.7. Statistical analysis

To reveal the difference in metabolic profiling of root exudates between treatments, principal component analysis (PCA) was performed ON SIMCA-P+ (version 13.0; Umetrics, Sweden) software package. Spearman's correlation coefficient was calculated to explore the associations between the root exudates and the expression levels of genes related to exudation using SPSS version 18.0 for Windows (SPSS Inc., Chicago, IL, USA). Heatmap analysis was performed on R package (version 3.1.1) to visualize the associations. Canonical correspondence analysis (CCA) was performed to determine the relationship between the root exudates of maize seedlings and their morphological traits using CANOCO 4.5 (Biometris, Wageningen, Netherlands). To assess how root exudates and root metabolomic data matrices are associated or correlated, we performed Procrustes analyses on package of R (version 3.1.1) according to Lisboa et al. (2014) and Perea et al. (2014). Other data were analyzed on SPSS 18.0 to examine the differences between the treatments. Statistically significant differences ($P < 0.05$) were identified through ANOVA and Student–Newman–Keuls method.

3. Results

3.1. Metabolic profiling and components of root exudates

The representative ^{1}H NMR profiles showed that the signals of amino acids, sugars, and low-molecular-weight organic acids dominated the polar extracts (Fig. 1a), whereas fatty acids, lipids, and phenols were enriched in the nonpolar extracts (Fig. 1b). The NMR spectra revealed that the maize root exudates from the three treatments had more abundant carbohydrates and amino acid molecules than that from CK, which had more abundant fatty acids. The score plots of PCA showed a clear separation in polar metabolite profiling between the CK and straw management, but the sample points representing the three straw treatments were overlapped and clustered together, suggesting that straw management exerted an obvious influence on the quantity and composition of maize root exudates, and the three strategies showed a limited difference among each other (Fig. 1c). The influence of the three strategies on the nonpolar metabolite profiling of exudates was limited (Fig. 1d).

Thirty-two root exudates were assigned, and the calculated fold changes were used to quantify their fluctuations under straw incorporation compared with the no straw control. Generally, the influence of straw input on organic acids was stronger than on sugars, amino acids, and nonpolar components in maize exudates (Fig. 2). Eleven amino acids had significantly higher level in SD treatment than in CK ($P < 0.05$) (Fig. 2a). However, only the levels of two amino acids, namely, Phe and Tyr, were significantly higher in SC and BC treatments than in CK, and the level of Ile was significantly decreased in BC
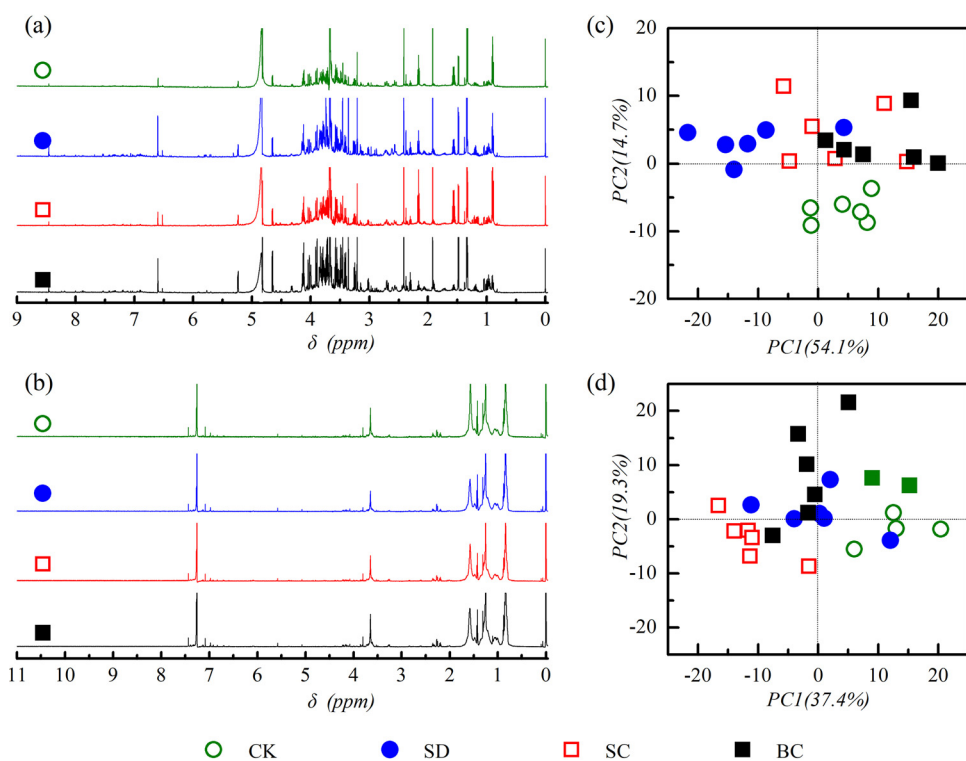
Fig. 1. Representative ^{1}H NMR spectra of polar (a) and nonpolar (b) extracts, and score scatter plots of principal component analysis for the metabolic profiling (c, d) of the maize root exudates from different straw treatments. The shapes and colors represent the different treatments. CK: no straw; SD: chopped maize straw incorporation; SC: compost incorporation; BC: biochar incorporation.
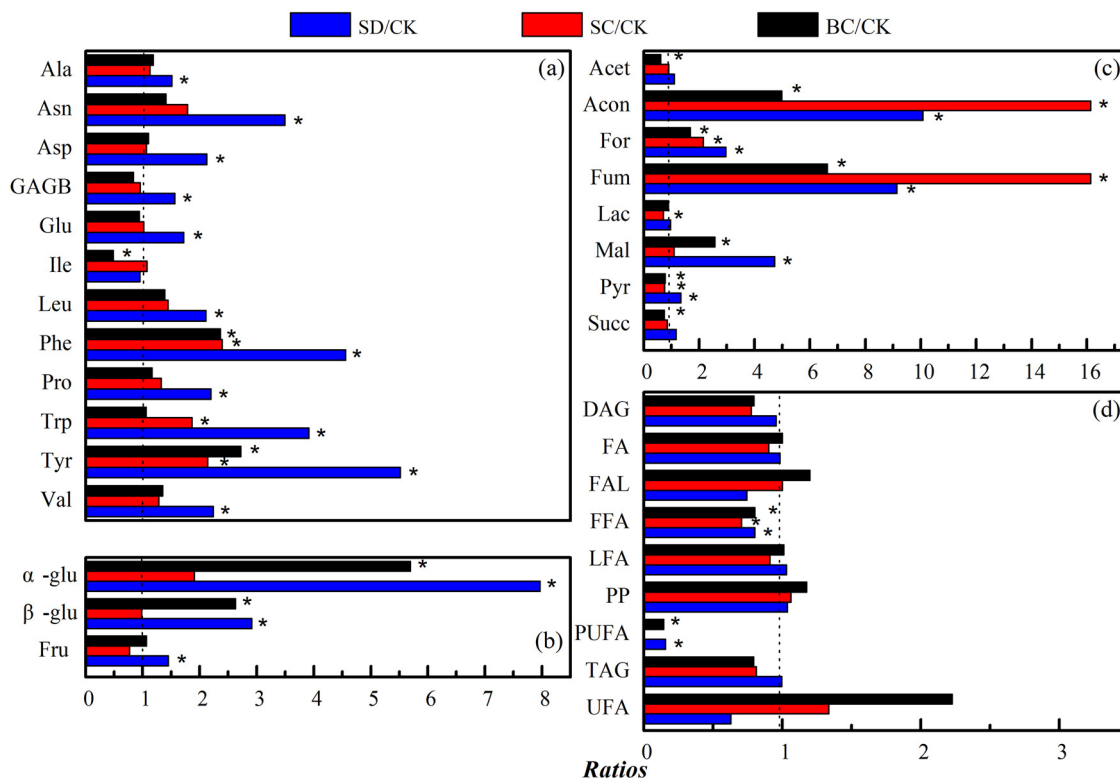

Fig. 2. Fold changes in amino acids (a), sugars (b), organic acids, (c) and nonpolar metabolites (d) under different straw treatments. The fold change in each exudate is obtained from the mean relative quantitative value of the exudate in the treatment divided by its mean relative quantitative value in the control. CK: no straw; SD: chopped maize straw incorporation; SC: compost incorporation; BC: biochar incorporation. Star indicates significant difference at $P < 0.05$. Acet; acetate; Acon, aconitic acid; Ala, alanine; Asn, Asparagine; Asp, aspartate; DAG, diacylglyceride; FA, fatty acid; FAl, fatty alcohols; FFA, free fatty acids; For, formate; Fru, frucose; Fum, fumarate; GABA, γ-amino-butyrate; Glu, glutamate; Ile, isoleucine; Lac, lactate; Leu, leucine; LFA, linoleyl fatty acid; Mal, malonate; Phe, phenylalanine; PP, polyphenols region; Pro, proline; PUFA, polyunsaturated fatty acids; Pyr, pyruvate; Succ, succinate; TAG, triacylglyceride; Trp, tryptophan; Tyr, tyrosine; UFA, unsaturated fatty acid; Val, valine; α-Gluc, α-glucose; β-Gluc, β-glucose.

treatment ($P < 0.05$). These results indicated that the influence of SD treatment on amino acids was stronger than that of BC and SC managements. The Gluc content in the exudates significantly increased in SD and BC treatments ($P < 0.05$), with the highest fold (7.97-fold) in SD (Fig. 2b). Five out of eight organic acids in SD and three organic acids in SC significantly increased ($P < 0.05$) (Fig. 2c). In particular, Acon and Fum increased by 10.08-fold and 9.14-fold in SD treatment and 16.13-fold and 16.12-fold in SC treatment, 4.98-fold and 6.63-fold in BC treatment, respectively, compared with the control group. The levels of Acet and Succ in BC treatment significantly decreased ($P < 0.05$), whereas they remained unchanged in SD and SC treatments. Compared with polar metabolites, nonpolar metabolites did not show significant changes under straw returning compared with nostraw control, except for FFA and PUFA, which significantly decreased in all treatments ($P < 0.05$) (Fig. 2d).

3.2. Expression levels of genes encoding root exudation

The selected genes, including ZmMATE1, ZmMATE2, ZmALMT2, and ZmMHA2, were based on previous studies linked to the function of controlling root exudation. The SD treatment resulted in a significant increase in ZmMATE1 expression (> 2-fold), which was accompanied by a significant decrease in the expression of ZmMATE2 (< 0.5-fold) compared with CK (Fig. 3a, b), implying that these genes may have specific roles in response to straw incorporation. After BC treatment, the transcript abundance of ZmMATE1 and ZmMHA2 was altered compared with the control (Fig. 3a, c). The expression of ZmALMT2 was not modified under the three straw treatments (Fig. 3d). We performed Spearman's correlation analysis to investigate the relationships between the organic acids with the expression levels of the genes encoding root exudation. A positive correlation between Acon, For, and Mal with ZmMATE1, whereas a negative correlation between Acon, For, and Fum with ZmMATE2 was observed (Fig. 3e). However, most of the assigned organic acids did not show significant correlation with ZmALMT2 and ZmMHA2.
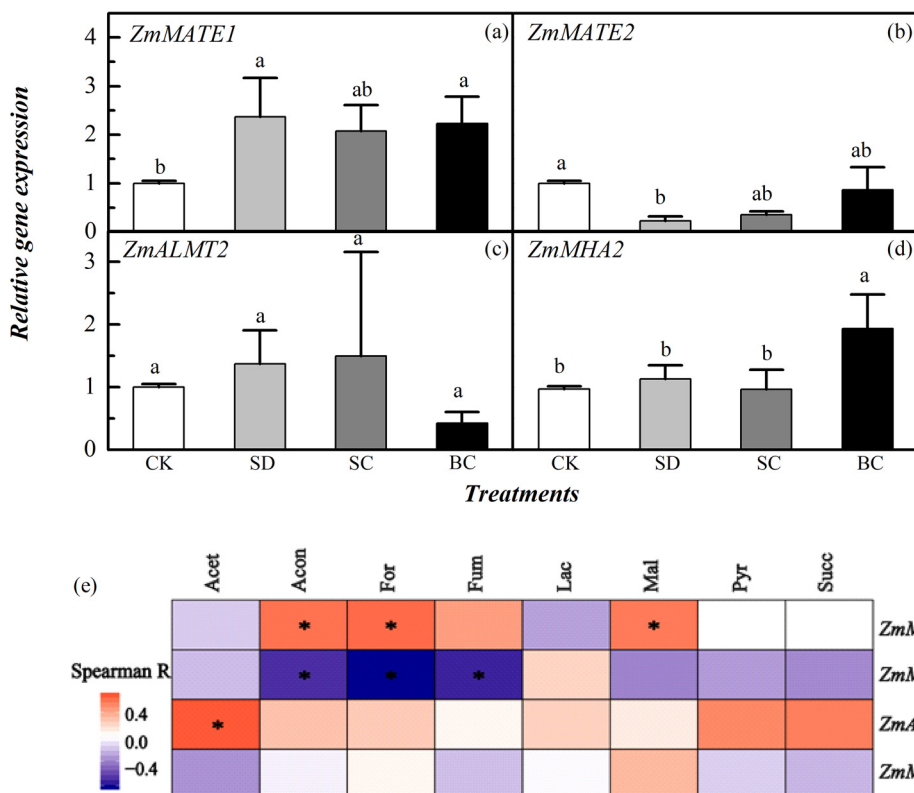
3.3. Root morphology, root tip viability, and anatomical structure

As shown in Fig. 4a, the root phenotypic appearance, which was characterized by root number, length, and diameter, was influenced by different straw treatments. The shoot weight was the largest in SC treatment, whereas the root weight remained stable compared with CK, indicating the partitioning of resources to root biomass in response to compost incorporation (Fig. 4b).The effects of straw management on the total root length was different, where the RL was the largest in BCand lowest in SD (Fig. 4c). Compared with CK, biochar incorporation significantly improved root elongation ($P < 0.05$). On the contrary, root diameter was the largest in SD and smallest in BC, indicating that the roots became prone to thickening under chopped straw input. The total root volume significantly varied between SD and SC treatments with similar trends to those of the root surface area (Fig. 4c). Root tip number was the highest in BC, followed by BC, CK, SD, and SC. As shown in Fig. 4d, the red fluorescence revealed that the dead cells in the root tip of control plants were more abundant than that in straw-treated plants. The border cells, which were detached from the outer peripheral part of the root cap, were produced by all straw-treated roots, and their number significantly increased in SD-treated roots compared with other treatments (Fig. 4e). The root cap of SD-treated plants was longer than that of the others.

3.4. Cell cycle and expression of genes related to root growth

The cells at G2 phase remarkably increased, whereas the cells at S and G1 phases decreased when chopped straw was inputted compared with nostraw control (Fig. 5a), indicating that many cells could pass from S phase to G2 phase under this condition. The cells at G1 phase decreased under the other two straw management conditions, suggesting that the expression of specific cell cycle genes could be regulated. To explore the underlying mechanism of cell cycle regulated by straw treatment, the expression levels of one cell cycle gene, ZmCycD2;2a, combined with ZmEXPB8, ZmXTH1, and ZmARF1, which
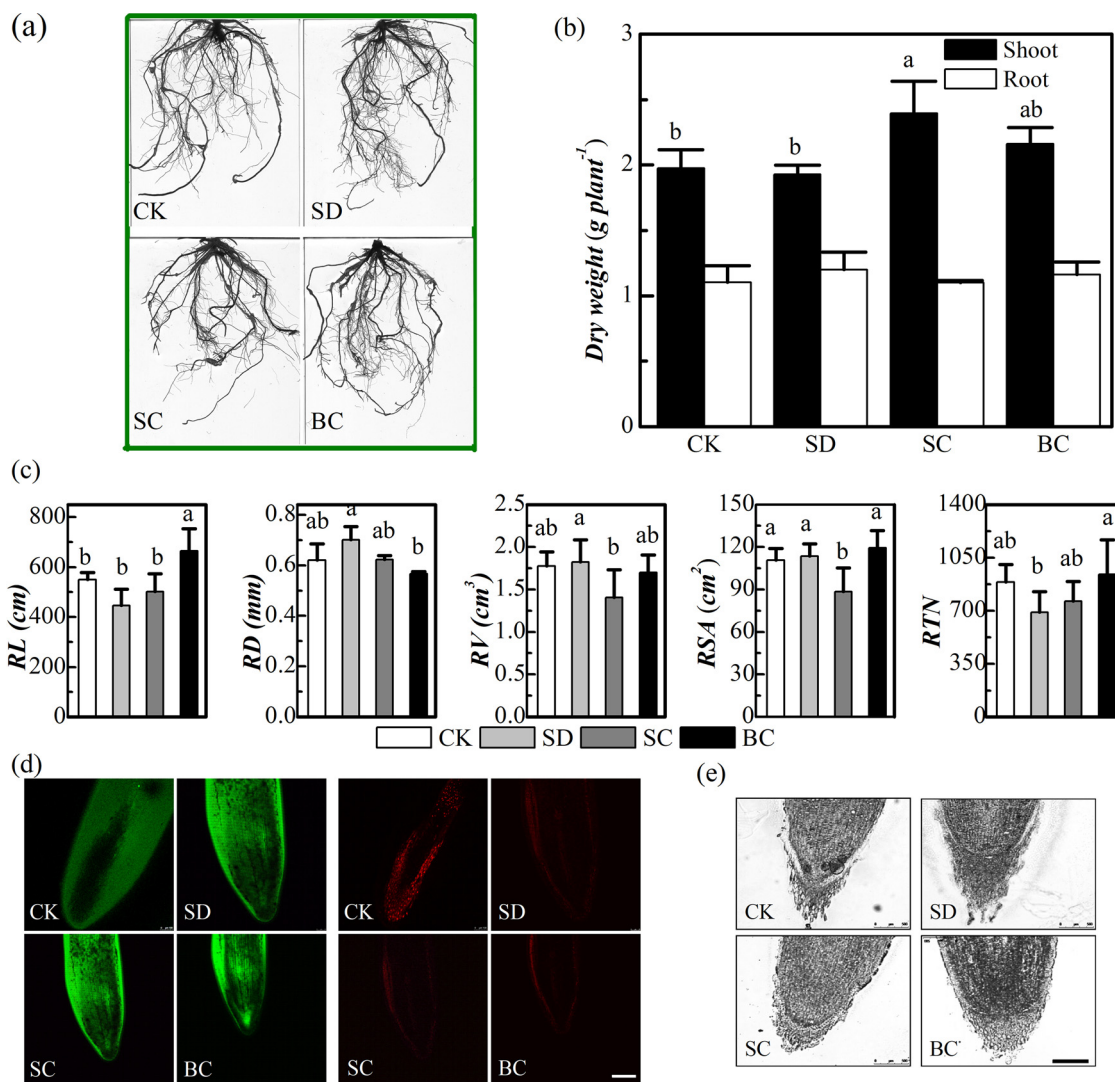

Fig. 3. Expression levels of genes encoding root exudation (a-d) and Spearman correlations between the levels of gene expression and exudated organic acids (e) of maize roots under different straw treatments. CK: no straw; SD: chopped maize straw incorporation; SC: compost incorporation; BC: biochar incorporation. The means (± SE) with different letters in the columns are statistically significant at $P < 0.05$ in (a), (b), (c), and (d). The stars in (e) indicate correlations with $P < 0.05$.
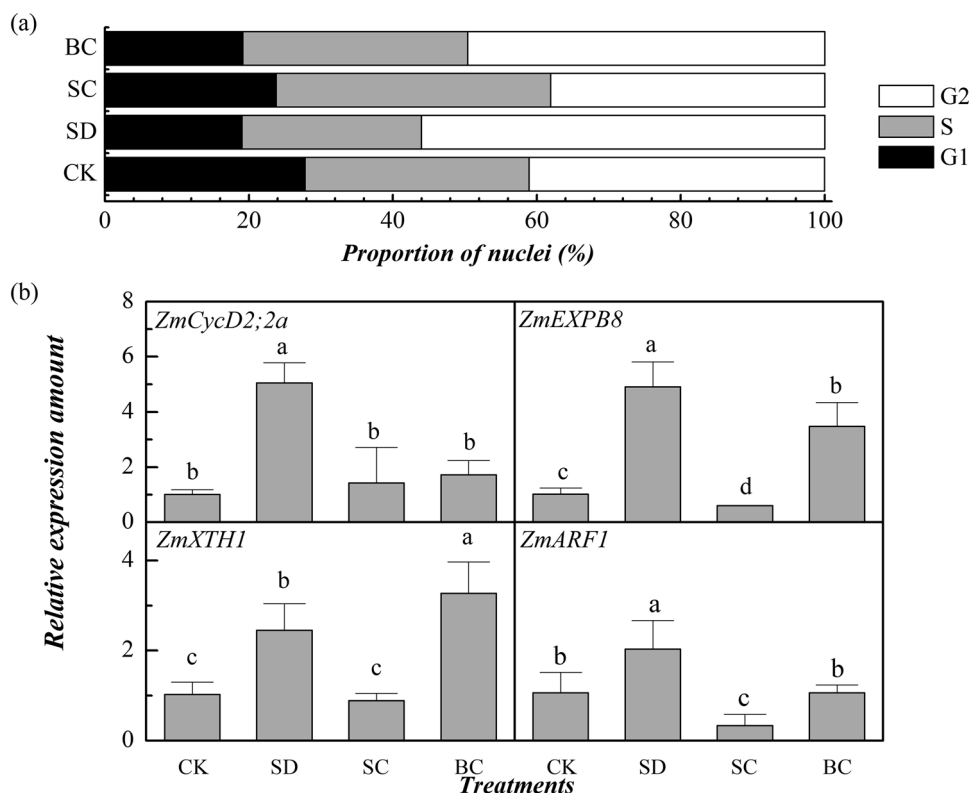
Fig. 5. Cell cycle (a) and the expression levels of genes controlling cell division, cell wall expansion, and root branching (b) of maize roots under different straw treatments. The mean (± SE) with different letters in the columns are statistically significant at $P < 0.05$ in.(b); $n = 3$.

related to soil properties (Bukovsky-Reyes et al., 2019). Straw management strategies could influence soil factors and ultimately impact the activity and quality of root exudates. For example, biochar input could increase soil aeration and macroporosity, and decrease penetration resistance or soil hardness (Zhang et al., 2013; Majumder et al., 2019). The metabolic costs of soil exploration for plant roots will be increased, and root exudation, especially water-soluble compounds, will be stimulated when the soil penetration resistance increases (Groleau-Renaud et al., 1998; Bengough et al., 2011; Colombi et al., 2018). The decreasing oxygen content in soil is believed to increase exudation into the rhizosphere through the shifting of carbohydrate fermentation from aerobic respiration (Tückmantel et al., 2017). In this work, the component level of exudates immensely varied under different straw treatments. Maize roots exude more amino acids and sugars in SD treatment, whereas they exude more organic acids in SC treatment (Figs. 1 and 2). The decrease in bulk density and increase in oxygen supply potentially caused by straw management did not explain the fluctuation of root exudation. However, apart from the influence of soil penetration resistance and oxygen content, exudate fluxes are mainly driven by soil temperature. The active exudation processes, which depend on metabolic energy, and the passive part of exudation, including membrane stability and diffusion speed, are temperature-dependent and are affected by the rhizosphere temperature (Uselman et al., 2000; Arai-Sanoh et al., 2010; Tückmantel et al., 2017). Thus, the observed increase in root exudates could be due to the increasing temperature caused by straw management (Zhang et al., 2013; Wang et al., 2019b). Furthermore, the abundance of microorganisms can affect rhizodeposition and enhance root exudation in straw-incorporated soil with higher microbial activity and abundance than those in soil without straw (Zhao et al., 2017; Wang et al., 2019a,Wang et al., 2019b). This work aimed to evaluate the effects of soil modifications induced by straw management on the variation of root exudation rather than on soil properties.

The variations in exudate amount and differences in its composition

could play a crucial role in the regulation of nutrient availability, soil ecology, plant adaptation, and crop productivity (Adeleke et al., 2017; Proctor and He, 2017; Guyonnet et al., 2018; Chang, 2019). The plasticity of root exudation can enhance the nutrient acquisition by individual plants and minimize the competition between plants under limited or heterogeneous nutrient sources (Giles et al., 2017). The organic acids andsugars, which are secreted in large quantities, are the dominant components of exudates. Therefore, we focus on the two classes of compounds in terms of their possible ecological importance in agriculture tillage system with straw management. In a rhizosphere ecology system, plant-released sugars and sugar alcohols can serve as the main sources of energy and signal molecules for rhizosphere processes (Escudero et al., 2014; Cheng et al., 2018). Glucose is a common sugar in soil and root exudates (Baetz and Martinoia, 2014; Swenson et al., 2015). Here we demonstrated that straw or biochar management resulted in the high glucose levels of in root exudates (Fig. 2b), suggesting that the root morphological and physiological traits of maize may respond to straw input. This finding was supported by the work of Zhou et al. (2019), who revealed that the enhanced sugar exudation improves the root morphological and physiological plasticity of soybeans. Hayden et al. (2019) observed that glucose is scarce in the soil where it might be rapidly metabolized by rhizosphere microorganisms. Therefore, the non-increase of glucose in maize root exudates under SC addition (Fig. 2b) may be partly ascribed to the rapid microbial turnover caused by the incorporated compost with abundant microbial community. In this work, organic acids exudates, such as Acon and Fum, increased more in SD and SC treatments than that in BC treatment (Fig. 2c). This result may be partly because the decomposition of straw could release organic acids to the soil microenvironment (Wang et al., 2019a,Wang et al., 2019b). The link between the increased organic acid exudation and better plant adaptation has been well established because organic acids, which is a major component of root exudates, can alter the bioavailability of nutrients and the toxicity of trace elements, maintain cytosolic charge balance in root cells, and influence soil
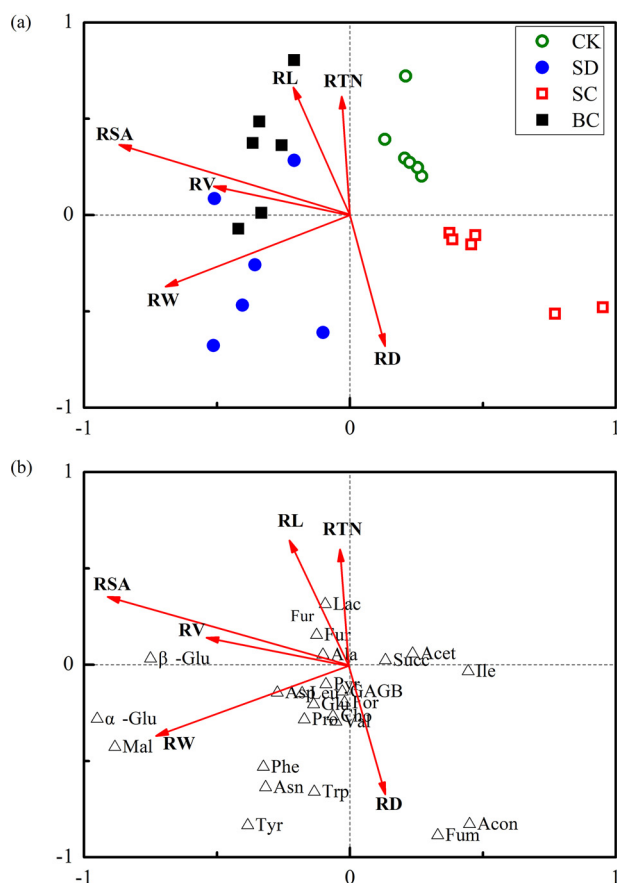
Fig. 6. Canonical correspondence analysis of exudate composition (polar-extracted) and root morphological traits. The arrows indicate the direction and magnitude of the root morphological traits associated with the root exudate composition. Abbreviations: RL, total root length; RD, average root diameter; RV, total root volume; RSA, total root surface area; RTN, total root tip number; RW, root dry weight; CK, no straw; SD, chopped maize straw incorporation; SC, compost incorporation; BC, biochar incorporation.

bacterial communities (Giles et al., 2017; Zhou et al., 2019; Chang, 2019). Only several parameters of plant nutrition were measured in this work (Table S4). However, plant nutrient status should be considered in the future to interpret the influence of straw input on root exudates in more depth.

The exact mechanisms in which maize roots shape their metabolic profiling of exudates under straw managements remain unknown, whereas the differences in the expression levels of genes related to root exudation provide a likely molecular evidence. The specific efflux carriers and channels, which mediate the efflux of organic aicds, might finely tune the exudation flux by regulating gene expression or post-translational modification (Canarini et al., 2019). The link between organic acid exudation and transporter families of MATE or ALMT has been well established (Ligaba et al., 2012; Zhu et al., 2016; Coskun et al., 2017). In the present study, the expression of ZmMATE1 was upregulated compared with controls and positively related to the increase in Acon, For, and Mal. By contrast, ZmMATE2 was down-regulated and negatively related to the increase in Acon, For, and Fum (Fig. 3). These findings implied that the two genes may have different and specific roles in response to straw incorporation. The expression of ZmMATE1 was mostly localized to root tissues, whereas that of ZmMATE2 was not specifically expressed in any particular tissue (Maron et al., 2010). Furthermore, MATE proteins not only mediate citrate and flavonoid transport, and are involved in the detoxification of endogenous secondary metabolites and exogenous agents (Zhu et al., 2016). Differing from the MATE family in terms of substrate specificity

and transport type, the ALMT-type proteins mediate a highly selective and bidirectional transport of inorganic and organic anions (Ligaba et al., 2012). The presented gene expression data in maize roots suggested that ZmALMT2 may play roles in plant mineral nutrition in response to straw management rather than in organic exudation. Two PM H^+-ATPase genes, namely, MHA1 and MHA2, were reported in maize (Santi et al., 2003). In this work, the ZmMHA2 gene expression is significantly high in BC treatment (Fig. 3). The lack of correlation between the ZmMHA2 expression and organic acids made it difficult to explain its role in driving the secondary active transport across the root surface in response to straw management. However, PM H^+-ATPase was considered to play various physiological roles in plants (Sorgonà et al., 2011). Thus, the link between the expression of MAH2 gene and organic acid exudation did not appear as expected. Although the roles of different MATEs, ALMTs and MHAs in maize roots have not been firmly established, the regulations of these genes provide molecular evidence of the fluctuation in organic acid exudates under straw management.

4.2. Effects of straw input on root morphology

The development of root system with a complex branching architecture must be plastic, dynamic, and highly responsive to the soil environment for providing better fitness and larger yield (Ron et al., 2013). Detailed analysis of root morphology, combined with a quantification of exudation, is a suitable approach to better understand the rhizosphere processes of maize plants. Some previous studies have indicated that biochar incorporation has a positive effect on root growth through the following mechanisms: i) by improving soil physical structure, ii) by acting as a direct nutrient source indirectly enhancing nutrient availability, and iii) by creating a habitat for beneficial soil microorganisms (Prendergast-Miller et al., 2014; Amendola et al., 2017; Sun et al., 2017). In this work, we found that the number of roots under biochar incorporation increased and became longer and thinner than those in the control, whereas the root dry matter remained constant (Fig. 4a–c). T The root elongation rate significantly increased and accompanied by a decrease in diameter when the roots experienced a decreased physical constraint in the soil (Bengough et al., 2011; He et al., 2017; Colombi et al., 2018). Thus, the decrease in soil bulk density with straw amendment might play important roles in determining maize root growth (Groleau-Renaud et al., 1998; Colombi et al., 2018). The RL remained constant with the decrease in bulk density caused by chopped straw input. Contrary to the findings in BC treatment, we found that the reduction in RL corresponded to an increase in RD in SD treatment (Fig. 4c). The strength contact between the root and soil can enhance water and nutrient uptake, thus the positive effect of soil compaction and mechanical impedance on root growth should be considered (He et al., 2017; Colombi et al., 2018). Deep root growth and an increased number of roots could increase the soil volume available for root exploration, thus increasing nutrient and water translocation and absorption, thereby improving plant growth and crop performance (Nunes et al., 2019). Thick roots with large diameters can push soil particles that blocked the root tips and are favorable in penetrating hard soils (Bengough et al., 2011). Thus, deep or thick roots are considered to be adaptive in response to environmental variations, and the interpretation of the influence of straw input on root morphology should be based in terms of yield.

The root tip is plastic, flexible, and can change its size and shape when it experiences resistance, and the root cap, which is shown to be major site of exudation for mucilagineous fractions, may fall off (Haichar et al., 2014). The observation of the viability and anatomical structure of root tips may reflect the functionality and physiological state of roots better than the morphological traits to explain the growth regulation mechanism induced by straw management. A substantial decrease in the number of dead cells in the root tip was observed in straw-treated plants (Fig. 4d). Moreover, longer root cap and more border cells were found in SD-treated roots than those in the control
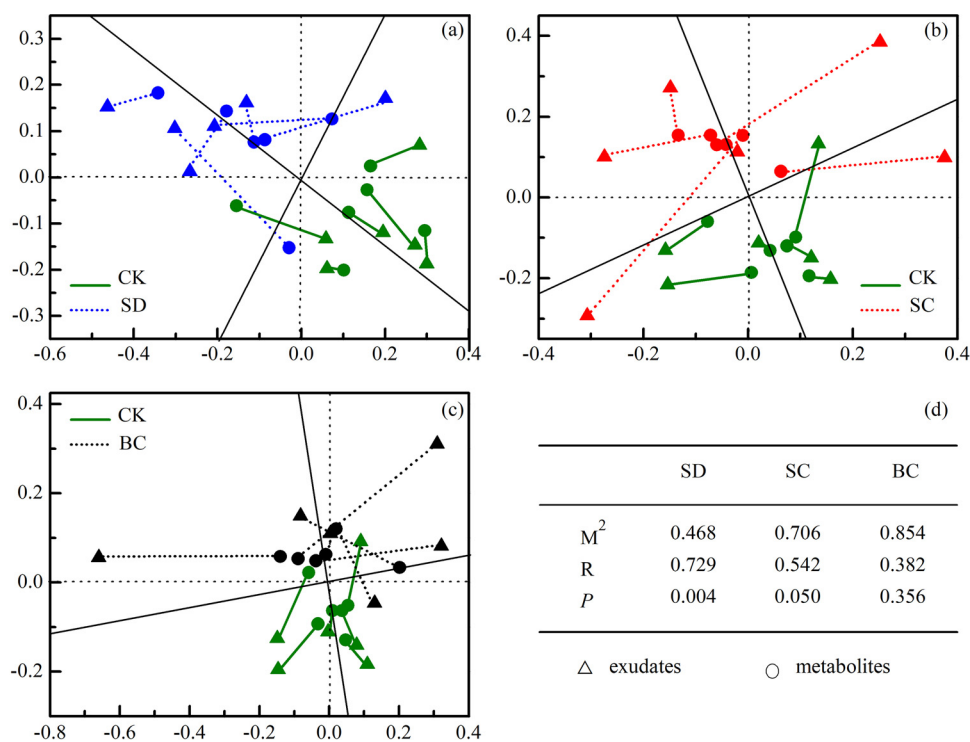
Fig. 7. Procrustes analysis of the root exudates and metabolites of maize under different straw treatments. The triangle represents the root exudates samples, and the circle denotes the root metabolite samples. The distance between them is the Procrustean residual (R) and is indicated by the connecting line. The short lines represent similar object positioning on the plots. M^2 is the Procrustes sum of squares.

	SD	SC	BC
M^2	0.468	0.706	0.854
R	0.729	0.542	0.382
P	0.004	0.050	0.356

△ exudates   ○ metabolites

group (Fig. 4e), denoting the changes in root cap function after chopped straw incorporation. The border cells, which were produced by the root cap, were related to the sensitivity of roots to the rhizosphere environment (Na et al., 2011; Canarini et al., 2019). Root caps maintain a margin of independence from other root zones, and the determination of the correlations between root growth and the number/viability of border cells might be more complicated than expected (Soltys et al., 2014). Root growth and anatomy were influenced by the joint changes in soil penetration resistance, soil temperature, moisture, and pH or the interaction induced by straw input. The effects of each factor were difficult to separate. Therefore, the influence of straw input on root morphology should be carefully interpreted.

The process of root growth and development combines cell division, expansion, and differentiation where the cell cycle regulation is of pivotal importance (Na et al., 2011). A family of proteins named cyclins (Cyc), plays crucial roles in cell cycle control and transitions of three main control points, which constitute an important driving force to facilitate cell cycle progression (Buendía-Monreal et al., 2011; Lara-Núñez et al., 2017). In this work, only ZmCycD2;2a, which is involved in the regulation of G1 and G1/S transitions, was analyzed as the representative of this group. Together with the reduction of the proportion of cells in the G1 phase of the cell cycle induced by straw input, an upregulation of ZmCycD2;2a was observed compared with the non-straw control (Fig. 5), suggesting that straw management stimulated cell proliferation in maize roots. Cell wall proteins play a vital role in the regulation of cell wall extensibility and cell expansion control. Among the investigated cell wall proteins, expansins are the first identified and have unique ability to promote acid-induced cell wall loosening and regulate cell enlargement during various developmental processes, including root growth (Geilfus et al., 2010; Zhang et al., 2014). In maize, β-expansins (EXP B) are more abundantly expressed compared with α-expansins (EXP A) (Wu et al., 2001). Along with expansin, enzymes like XTHs, which can cleave and frequently rejoin xyloglucan molecules in primary cell walls, have been included in the modification of cell wall structure (Genovesi et al., 2008; Geilfus et al., 2010). The upregulation of the ZmExpB8 and ZmXTH1 transcripts in SD- or BC-incorporated roots may contribute to cell wall loosening, degradation, alterations in the composition, and structure of primary

cell walls and account to the improvement of maize root growth when chopped straw or biochar inputted (Fig. 5b). By contrast, the down-regulation or maintenance of these genes in SC-treated roots was related to the inhibition of cell expansion and elongation. A study on *Arabidopsis* showed that touch, as an abiotic factor, can regulate the expression levels of XTH genes, thereby indicating that the change in soil mechanical impedance induced by chopped straw or biochar input might play a role in the regulation of ZmXTH1 gene in SD and BC treatments (Genovesi et al., 2008). Auxin signaling plays a crucial role in root patterning, lateral root formation and root meristem establishment. Auxin response factors (ARFs) can regulate the expression levels of auxin responsive genes by binding specifically to the auxin response elements in promoters (Jansen et al., 2013; Zhang et al., 2015). In this work, the regulation of ZmARF1 transcripts in maize roots was not related to the RTN under different straw input conditions (Figs. 4 and 5). The proliferation regulation of branched and fine roots was achieved through multiple mechanisms involving multiple molecular players, which were not investigated in this study.

4.3. Links between root exudation, root morphology, and root metabolism under straw input

The morphology of the entire root, which is related to plant genetics and physiology, can influence the flux of carbon from each root to the rhizosphere (Haichar et al., 2014; Steinauer et al., 2016). As reported in previous studies, for grasses, forbs, and forest plants, root mass is the most important trait that influence the exudation carbon flux from the roots to the soil (Tückmantel et al., 2017; Herz et al., 2018). Consistent with these results, good concordance was observed between root biomass and exudate composition in SD treatment. In particular, the close links of root biomass with the exudate contents of Gluc and Mal were indentified (Fig. 6). Similarly, Canarini et al. (2016) observed positive correlations between saccharide exudates and sunflower roots. However, one aspect in root research is frequently ignored, that is, root biomass is rarely distinguished in different functionally and morphologically distinct orders, thereby hindering the ability to determine the roles of root morphology in exudation (Tsunoda and Van Dam, 2017). A study on *Fagus sylvatica* L. has revealed that the RSA plays a key role in

the variations of root exudates composition (Tückmantel et al., 2017). In addition to RW, RSA was the significant factor influencing the Gluc content in maize root exudates based on CCA (Fig. 6b). The seasonal carbon efflux rates could be well predicted by the number of fine roots or root branching intensity (Proctor and He, 2017; Tückmantel et al., 2017). Furthermore, the concentration changes in exuded metabolites could be sensed by the root tips and the signals could be translated to modify root growth and architecture (Canarini et al., 2019; Sun et al., 2019). This condition could be attributed to the fact that RTN determines the number of root caps, elongation zones, and lateral branching, which have been identified as the major sites of exudation for mucilagineous fractions and soluble compounds, respectively (Haichar et al., 2014). Root exudates are frequently categorized in terms of their water solubility into insoluble fractions, which are composed of the high-molecular-weight mucilage compounds, and soluble fractions, which are composed of organic acids, amino acids, carbohydrates, and various secondary metabolites (Martin et al., 2014). Different compounds were released from different parts of the root system, suggesting that the spatial variability of root exudation existed at different root regions (Lesuffleur and Cliquet, 2010; Li et al., 2018). In this work, we found that the root caps were immensely affected by straw management (Fig. 4e). Although the term root exudate was used in this work, only the soluble fractions with low molecular weight were detected. This condition partially explained the results that the RTN was not expected to significantly influence the exudate composition in maize roots (Fig. 6b).

Root morphology might affect exudate composition via physical patterns, such as impacting transport carriers and channels, providing sites of exudation, and improving the amount of fine-haired roots, however, the root exudation is also highly dependent on the metabolic features of plant roots (Haichar et al., 2014). The concentration gradient of exuded metabolites between the root cell cytoplasm and soil solution might be large at many orders of magnitude (Martin et al., 2014; Proctor and He, 2017). The release of exudates from the roots would be inevitably influenced by the synthesis and concentration of metabolites in the roots (Canarini et al., 2019). Consistently, PA of metabolite profiles in roots and exudates revealed good concordance between root exudates and metabolites under chopped straw management ($M^2 = 0.468$, $P < 0.05$, Fig. 7a). Root exudation was not a purely unregulated passive process and involved active exudation mechanism, which may affect the magnitude and composition of exudates by expressing and regulating efflux carriers (Martin et al., 2014; Tsunoda and Van Dam, 2017; Canarini et al., 2019). Root exudates and root metabolites did not significantly match when biochar was incorporated ($M^2 = 0.854$, $P > 0.05$), conforming that the exudate amount released from the root did not necessarily equate to its internal concentration. The microbial consumption in the rhizosphere, active retrieval of key compounds, and porosity of root cell walls can be compound specific, thereby leading to poor concordance between the root exudates and metabolites.

5. Conclusion

In this study, the profiles and compositions of exudates varied under different straw treatments, and their fluctuations were partially explained by the expression levels of genes associated with root exudation. Maize subjected to biochar incorporation tended to develop longer and thinner roots, more root tips, and a larger root surface area compared with those of maize in SD treatment, indicating that the chopped straw input induced a change from the elongation to the thickening of maize roots. The expression levels of the selected genes related to root growth presented a contrasting behavior among the straw treatments, suggesting that the root system sensed the straw managements, differentially regulated the genes at the transcriptional level, and modified the root architecture. Our hypothesis about the relationship between root exudation and morphology was also largely supported. The root

morphology played a substantial role in the composition of root exudates under different straw management strategies. These results can be used to guide and recommend residue management practices in agroecosystems.

Declaration of competing interest

None.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.agee.2020.106952.

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