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Dynamics of root exuded carbon and its relationships with root traits of rapeseed and wheat

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Abstract: Quantifying carbon in root exudates and exploring their influencing factors are essential to understand soil organic carbon dynamics in cropland. A pot experiment was carried out to explore quantitative relations between root exuded carbon and root traits in wheat and rapeseed. The result showed that rapeseed had a similar pattern in root carbon exudation intensity (EI) as the wheat, but its EI per plant was obviously higher than that in wheat. Rapeseed plants had higher EI per root biomass than wheat plants in the early growth period but lower in the late growth period. EI per root biomass in both crops had significant exponential relationships with the specific root length (RL), surface area (RSA), volume (RV), root C/N ratio and root soluble sugar content. However, EI per plant of both crops had a markedly quadratic relationship with RL, RSA, RV and root biomass. During the whole growth period, the rapeseed had cumulative root carbon exudation of 14.09 g/plant, which was almost twice of that in the wheat plant. Root traits had close relations to root carbon exudation in both crops. Quantitative regression models between them could be referred to estimate root C exudation in rapeseed and wheat farmland.

Keywords: rhizosphere; *Brassica napus* L.; *Triticum aestivum* L.; root morphology; root biochemical traits

Rhizosphere deposition is the main source of unstable carbon in the soil. Around 5–21% of photosynthesis products were transported into the rhizosphere by root exudation (Bengtson et al. 2012), which are the key intermediary of interaction between the underground part of the plant and the microorganism, relating to the decomposition of soil organic matter (Shahzad et al. 2015). Quantifying root exudates is very important to study the carbon cycle of the soil-plant-microbe. However, once the root exudates enter the rhizosphere, it would be consumed by the microorganism immediately, so it's hard to quantify (Yin et al. 2013). Researchers have shown that there are many biological and abiotic factors regulating the rate of root carbon exudation and the composition of root exudates (Ostonen et al. 2007). Root morphological and chemical properties differ among plant species and varied in different development stages, which lead to changes in root carbon exudation rate (Paterson et al. 2006, Sun et al. 2017). However, few studies addressed the quantitative relations between root morphology and biochemical traits and root exudation. Different crops have their own distinctive root traits and growth characteristics; thus, root exudation varies a lot among them, which further regulate the soil carbon cycle in the farmland (Bais et al. 2006). Rapeseed rice and wheat-rice cropping are the major cropping systems in the middle reaches of the Yangtze River in China. These two cropping systems are different in soil organic carbon components and carbon emissions (Zhou et al. 2014). We think those divergences in the carbon cycle between them are somewhat induced by their root exudates. We reckon that root exuded carbon has close quantitative relationships with some root traits, and then its amount could be estimated dur-

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ing a crop life cycle. Therefore, this study aims to test two hypotheses: (1) Marked distinctions in the amount of root exuded carbon within the growing period exist between the rapeseed and wheat; (2) the root morphological traits of rapeseed and wheat are the driving factors of root exudation and their relationships could be quantitatively estimated.

MATERIAL AND METHODS

Pot experiment design and management. The pot experiment was conducted from 2018 to 2019 in Huazhong Agricultural University (30°28'N, 114°29'E) at Wuhan, China. The site is in a region with a humid mid-subtropical monsoon climate. The plastic pots with a diameter of 35 cm and a height of 50 cm were used. The local Haplic Alisol soil was collected from a nearby farm field. The basic soil properties as follows: soil organic carbon, 11.4 g/kg, total nitrogen, 1.0 g/kg; total phosphorus, 0.41 g/kg; total potassium, 7.14 g/kg; soil pH, 6.0. The soil was air-dried, crushed, and sieved (4 mm), then mixed with sand (soil:sand = 1:1). 18 kg of the substrate was placed in a plastic pot. 0.19 g N, 0.04 g P and 0.14 g K per kilogram of pot substrate mixture were applied. Total P fertiliser (phosphorus pentoxide), 40% of N fertiliser (urea) and 50% K fertiliser (potassium chloride) were used as basal fertiliser and mixed with the substrate before sowing. 30% of N fertiliser (urea) and 50% K fertiliser (potassium chloride) were applied at the rapeseed budding and wheat jointing stages. The rest, 30% of N fertiliser (urea), were top-dressed at rapeseed flowering and wheat heading stages. One of the local extended rapeseed (*Brassica napus* L.) cv. Huayouza 6 and wheat (*Triticum aestivum* L.) cv. Zhengmai 9023 were used. Rapeseed and wheat were sown on October 28 and November 2, 2018, respectively. After emergence, seedlings were thinned to 3 plants per pot. Crops grew outdoor under natural light. The pot was watered according to the rainfall to keep the substrate moist. Rapeseed and wheat matured on May 4 and 15, 2019, respectively. No damages from drought, water-logging, weeds and insects occurred during the crop growing period.

Plants sampling, collection and determination of root exudates. A total of 9 sampling events for rapeseed and a total of 12 sampling events for wheat took place at different growth stages. Five pots were taken each time. When sampling, the plastic pots were carefully cut, then gently removed the whole plant from the soil, and rinsed the roots repeatedly with deionised water. Then the water on the plants was carefully wiped off with absorbent paper. The washed whole plants were put in the beaker and immersed the whole roots in ultra-pure water (sterilised) indoors under light conditions for 2 h to collect root exudates. Then, the extract in the beaker was filtered through a 0.45 μm filter membrane to remove particles and most of the microbial biomass present in the solution. The carbon content in the filtered extract was measured using a TOC/TN analyser (ShimadzuTOC-Vcsh,Tokyo, Japan).

Determination of root morphological indexes and dry weight. After the extraction, root length, root surface area, and root volume were measured by WinRHIZO^{TM} (Positioning System for LA2400 scanner, Seiko Epson Corp., Nagano, Japan). Then, the sampled plants were disassembled into roots, stems, leaves, reproductive organs and then were dried at 80 °C. The specific root length (SRL), specific root surface area (SRSA) or specific root volume (SRV) at a sampling event was calculated by dividing the respective values of the whole root with the root dry matter. Root carbon and nitrogen concentration were measured with an elemental analyser, and root soluble sugar concentration in fresh roots was determined by anthrone colorimetry.

Calculations and statistical analyses. The carbon exudation intensity per plant (EI_{plant}, mg/plant/h) at each sampling event was determined according to the concentration of carbon in the extracted filtrate (mg/L), the volume of extracted filtrate (L) and the hours of extraction (h). Then, the carbon exudation intensity per root weight (EI_{root}, mg/g root/h) was calculated as the divisor of EI_{plant} by the root dry weight per plant. The cumulative carbon exudation between two sampling events was calculated according to EI_{plant} and the number of days between two adjacent sampling events.

Analysis of variance was performed in SPSS 16.0 (Chicago, USA). The regression functions for the dynamic of carbon exudation (CE), EI_{plant} and EI_{root} between plant traits were figured out by the curve estimation in SPSS 16.0. All the figures were plotted in Microsoft Excel 2010 (Microsoft Corp., Washington, USA) and Origin 2019 (OriginLab, Massachusetts, USA).

RESULTS

Dynamics of root carbon exudation intensity and dry matter of rapeseed and wheat. The dynamics of carbon exudation intensity per plant and carbon exudation per root biomass presented a similar trend in the rapeseed and wheat plants (Figure 1). The trend of EI_{plant} increased with growing and then decreased in the later growth period. However, an apparent increase in EI_{plant} occurred when plants were close to maturity. The maximum of EI_{plant} appeared in the early flowering stage of rapeseed to 5.31 mg C/plant/h and in the panicle differentiation stage of wheat to 4.26 mg C/plant/h, respectively. Overall, rapeseed plants had significantly higher EI_{plant} than wheat plants at most sampling events during the entire grow period (Figure 1A). Two peaks in EI_{root} emerged in the growing period of both plants, with the highest value of 2.88 mg C/g root/h in the budding stage of rapeseed and of 3.39 mg C/g root/h in the jointing stage of wheat (Figure 1B). However, the EI_{root} of rapeseed was remarkably higher than those in wheat in the early sampling events while showed an opposite trend in the later growth period. The rapeseed plant gained higher dry matter (DM) accumulation in the whole plant and root than wheat (Figure 1). However, the gap in root DM between both crops was greater than that in whole plant DM.

Cumulative carbon exudation in rapeseed and wheat. The trends in cumulative carbon exudation in rapeseed and wheat initially increased slowly and then accelerated, finally decelerated during the later growth period, which were in accordance with the logistic model ($R^2 = 0.999$) (Figure 2). The estimation of CE over the whole growth period of rapeseed and wheat was 14.09 g/plant and 7.48 g/plant according to the logistic model, respectively. The rapeseed had significantly higher CE than the wheat in each respective growth period. The proportion of root exudate carbon to the whole plant biomass in the corresponding period (PEB) changed with the plant growing (Table 1). PEB in rapeseed was greater at the seedling stage while lower at the mature stage. The highest PEB in wheat occurred at the jointing stage, while the smallest value showed at the mature stage. PEB across the whole period of rapeseed was significantly greater than that of wheat.

Relationships between root traits and carbon exudation intensity per plant and root biomass. The relationship between carbon exudation intensity per plant and root length (RL), root surface area (RSA), root volume (RV), and root biomass (RB) of rapeseed and wheat conformed to the quadratic regression

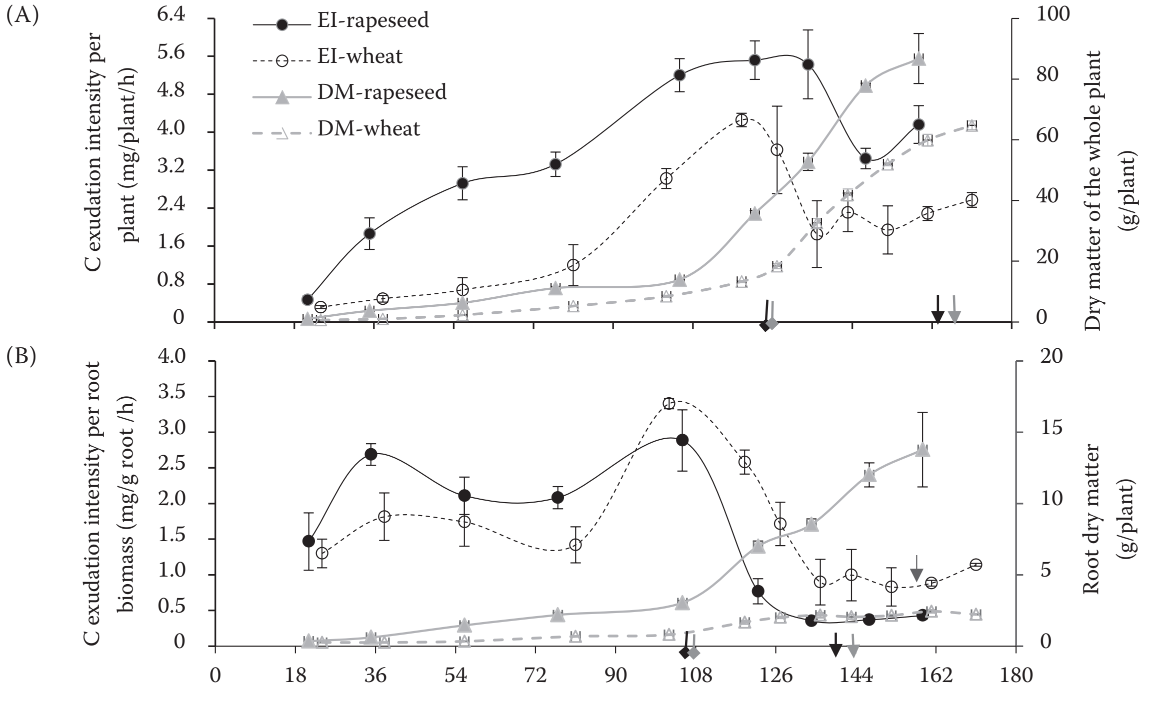

Figure 1. Dynamics of carbon exudation intensity (EI) per (A) plant and (B) root biomass, and changes in dry matter (DM) of the whole plant (A) and (B) root of rapeseed and wheat. ♦ and ♦ – rapeseed budding and wheat jointing, respectively; ↓ and ↓ – rapeseed flowering and wheat heading, respectively

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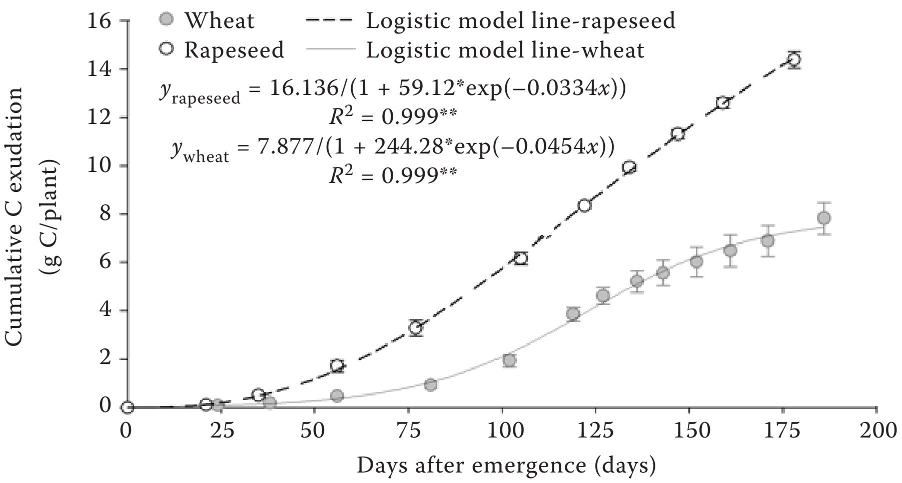

Figure 2. Logistic model of cumulative carbon exudation of rapeseed and wheat

model (Figure 3). With the growth of the root system, EI_{plant} had been climbing up until reaching the peak and then decreased. According to those regression models, EI_{plant} could be estimated by the measurement of the root morphological traits. The rapeseed showed higher EI_{plant} than the wheat when at the same value of a certian root morphological traits (Figure 3). Regression analysis also showed that carbon exudation intensity per root biomass increased exponentially with the increase in SRL, SRSA, SRV and root N concentration (RN) (Figure 4A–D). In comparison, rapeseed plants had higher EI_{root} than wheat plants under the same SRL, SRSA and SRV. Furthermore, the rising rate of EI_{root} along with the SRL, SRSA, SRV in rapeseed were greater than those in wheat (Figure 4A–C). Nevertheless, the RN of rapeseed and wheat had comparable effects on EI_{root} dynamics (Figure 4D). Root C/N ratio and soluble sugar content in root had a significant influence on EI_{root}, which fitted the negatively exponential growth models (Figure 4E, F). EI_{root} of rapeseed declined faster than those in wheat with the increasing of root C/N ratio soluble sugar content (Figure 4E, F).

DISCUSSION

Previous reports showed that the amount of root-released compounds were estimated at 0.6% to 27% of plant dry weight (Krassilnikov 1958). In the present study, cumulative carbon exudation of rapeseed shared about 14.75% of the plant dry weight, while CE of wheat was about 10.85% of the plant dry weight (Table 1). Root exudation rate varies during the growth period of the plant (Oburger et al. 2014), which supports our results. The root exudation of rice was found the least at the seedling stage; it was gradually rising up to the peak in the flowering stage and then decreasing in the mature stage (Aulakh et al. 2001). Similarly, the peak of EI_{plant} came out at the early flowering stage of rapeseed and panicle differentiation stage of wheat (Figure 1). This phenomenon might correlate to the larger root system and higher root vigor at these stages of the two crops. The relative abundance of young roots with very small diameters, which usually grow in prophase and more vigorous, controls the rate of exudation (Groleau-Renaud et al. 1998). Then, the roots grew slowly in the late growth period and declined in carbon exudation intensity (Figure 1).

Table 1. The proportion of cumulative carbon exudation to dry matter per plant (%) in different growth stages of rapeseed and wheat

Growing period	Rapeseed	Wheat
Emergence-December 15	33.77^{a}	14.95^{b}
December 15-rapeseed budding/wheat jointing	27.91^{a}	17.21^{b}
Rapeseed budding-flowering/wheat jointing-heading	11.46^{a}	8.80^{a}
Rapeseed flowering-maturity/wheat heading-maturity	10.51^{a}	10.97^{a}
Whole growth period	14.76^{a}	10.86^{b}

Different lowercase letters in the same row indicate the significant difference between rape and wheat

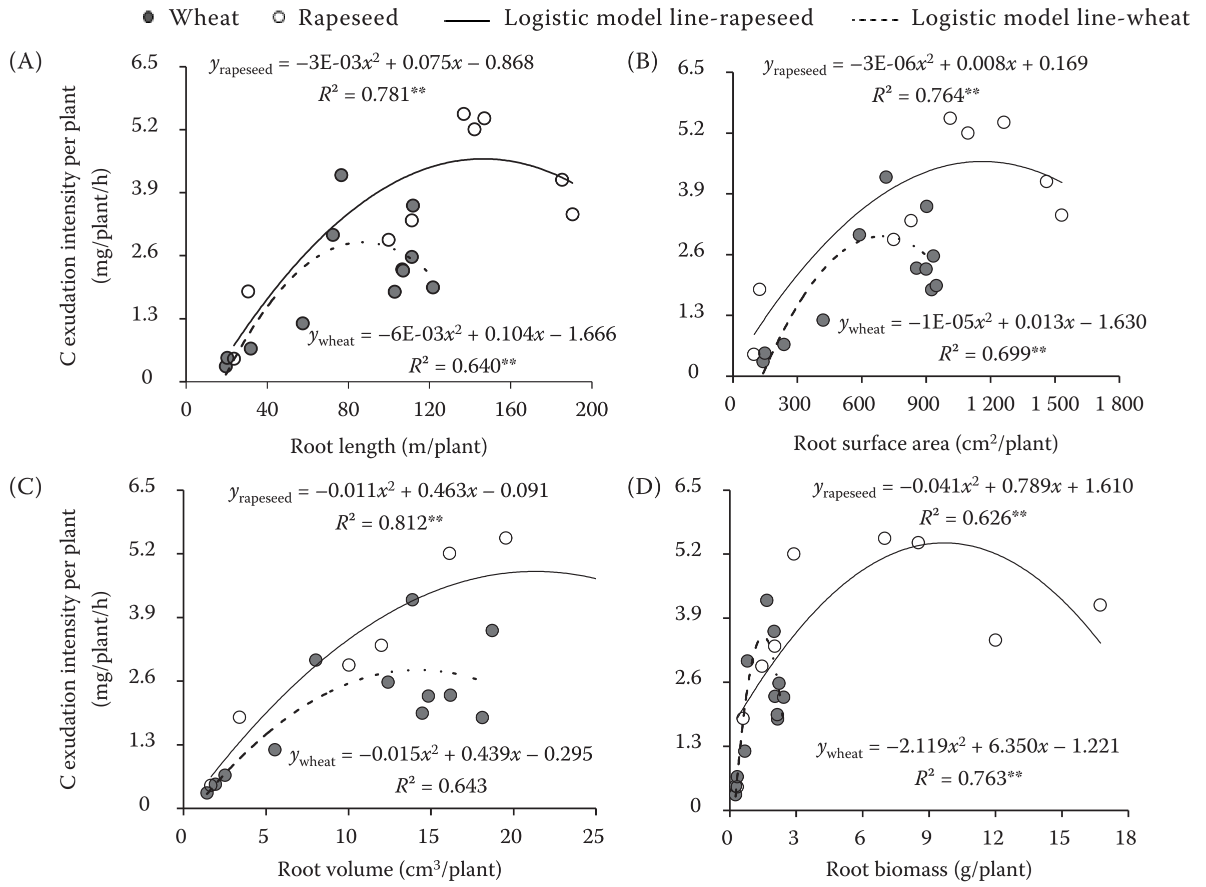

Figure 3. Relationships of (A) root length; (B) root surface area; (C) root volume and (D) root biomass on carbon exudation intensity per plant in rapeseed and wheat

The root system of rapeseed was reported to be lignified in the later growth stage, but wheat has no such changes (Yin et al. 2017). This phenomenon might lead to the results of wheat plants kept higher EI_{root} than rapeseed in the latter growth period (Figure 1B). In addition, this study found that EI_{plant} slightly increased when plants were close to maturity, which is not in line with previous studies (Swinnen et al. 1995). The possible reason is that the root cells died, and their contents flowed out due to senescence approaching maturity and resulted in the abnormal increase of EI. However, further studies are needed to verify the findings of the abnormal increase of EI.

A previous study found that the morphology of the root varied greatly at different developmental stages and environmental conditions and resulted in the change of root exudation rate and quantity (Xiong et al. 2020). Regression analysis showed a positive exponential function between EI_{root} and SRL, SRSA or SRV in this study (Figure 4A–C). Our findings almost agree with the recent report by Meier et al. (2020), who found an exponential relation between root exudation and specific root length in trees. Root nutrient content also has a certain effect on root carbon exudation. A positive correlation between root exudates and root nitrogen concentration was found (Xiong et al. 2020), which nearly consists with our findings of a positive exponential relationships in EI_{root} and RN in wheat and rapeseed plants (Figure 4D). Possibly, the higher concentration of nitrogen in the root leads to a higher root respiration rate (Guo et al. 2004), provides more energy to support root growth and enhances root exudates (Makita et al. 2009). Root exudation was reported a positive correlation with the concentration of soluble sugar in the fine roots of aspen seedlings (Karst et al. 2017). However, the present study found a negative correlation between EI_{root} and root soluble sugar content of the two crops (Figure 5F). Root C/N ratio was also found a negative relationship to the EI_{root} in both crops (Figure 4E). In the current study, the rapeseed plant had obviously higher RN and lower root C/N than the wheat plant in the early growth stage regarding to its higher EI_{root} in the respective

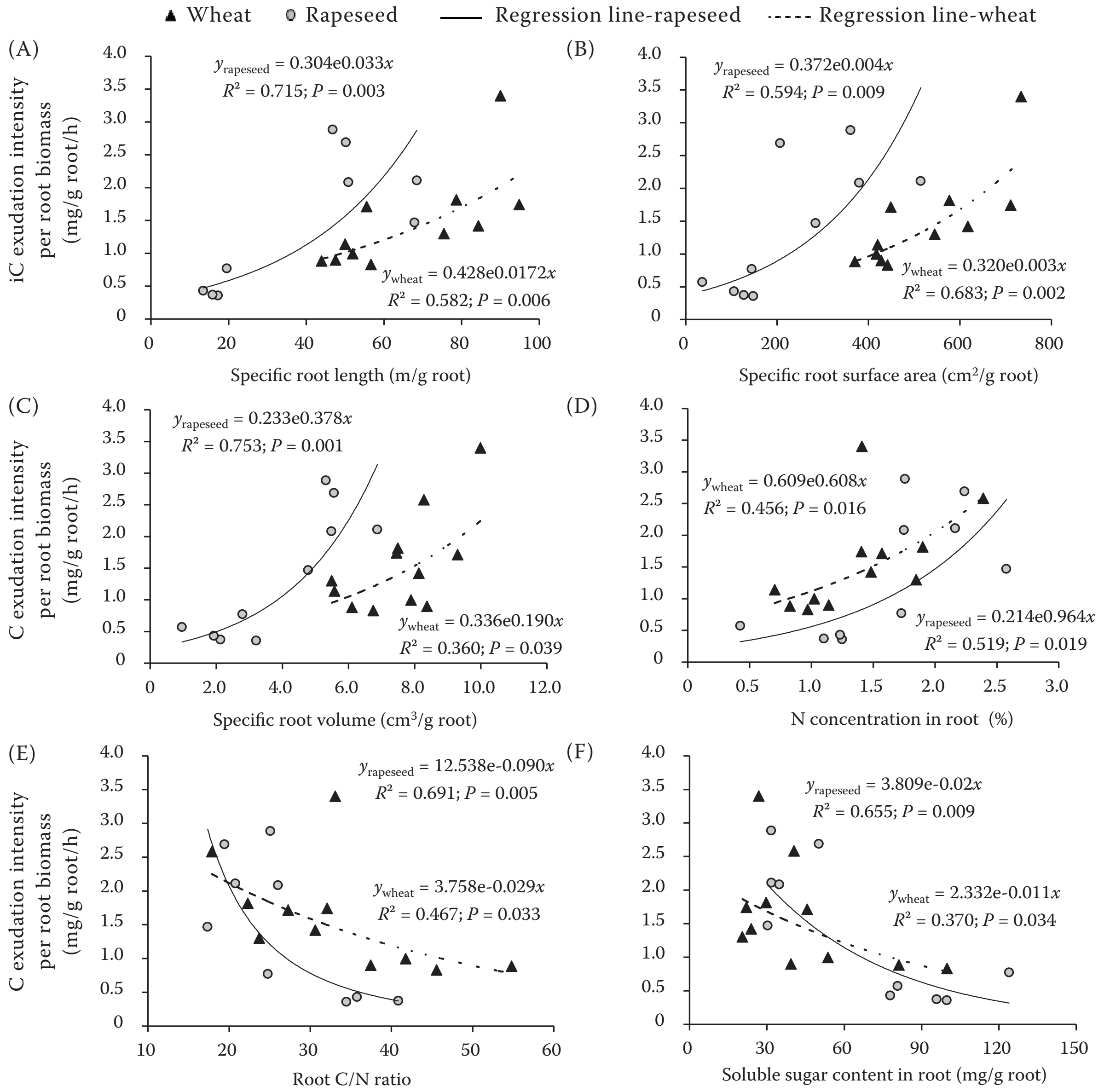

Figure 4. Relationships of (A) specific root length; (B) specific root surface area; (C) specific root volume; (D) root nitrogen concentration; (E) root C/N ratio and (F) root soluble sugar content on carbon exudation intensity per root biomass in rapeseed and wheat

growth period (Figure 1B). Moreover, the rapeseed had significantly higher carbon exudation intensity and cumulative carbon exudation per plant in the whole growth period than the wheat plant (Figures 1 and 2). It is possibly due to greater root length, surface area and volume in rapeseed than in wheat (Figure 3). Similar findings on the quadratic functions between EI_{plant} and root morphological traits have not been found in the previous reports and need further verification.

Collecting root exudates is an important issue that has perplexed most researchers in root exudates research. Hydroponics, soil culture or substrate culture are widely using to collect root exudates (Marschner et al. 1987). Collecting root exudates using the method of soil-hydroponic-hybrid is simple to operate and can be applied to plants grown in the field, reflecting the true growth of plant roots (Oburger and Jones 2018). However, physical damage to the roots due to the complicated cleaning procedures left debris of roots (Oburger and Jones 2018). Moreover, small fine roots could not be completely taken apart from the substrate. These two aspects possibly influenced the root integrity for root exudation collection in the next procedure of hydroponics for 2 h. Thus, in this study, in order to reduce the damages from sampling, the plastic pots were carefully sawn apart in our study and conducted to keep the whole root system for rinsing and extraction. Although growth conditions and plant metabolism under soil-hydroponic-hybrid experiments differ from plants grown in the field, our findings provide more understandings of root carbon exudation dynamics and their connections to plants traits in rapeseed and wheat. We think the quantitative regression models between EI and root morphological and biochemical traits could be applied to estimate root carbon exudation in rapeseed and wheat farmland.

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