The impact of different morphological and biochemical root traits on phosphorus acquisition and seed yield of *Brassica napus*

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ABSTRACT

Oilseed rape (*Brassica napus* L.) is an important crop in China. Although its yields are restricted by phosphorus (P) supply, the response of its root system to P supply has not been explored systematically. This study aimed to investigate the contribution of root morphological and biochemical traits to P acquisition from soils with deficient P (30 kg P_2O_5 ha^{-1}) and sufficient P (90 kg P_2O_5 ha^{-1}) supplies in the field at the leaf development, stem elongation, flowering, pod development and ripening stages. The total root length and root surface area in the surface soil (0−10 cm soil layer) were both reduced significantly by decreasing P supply. However, a larger root/shoot ratio and root length ratio were observed in plants with the deficient P supply at the flowering stage. Roots of plants with a deficient P supply also secreted more acid phosphatase and organic acid into the rhizosphere from stem elongation to pod development than plants with a sufficient P supply. Seed yield (SY), shoot dry weight (SDW) and total P content (TPC) were strongly correlated with root morphological traits at the leaf development and flowering stages, especially with the coarse root length (CRL) and root surface area in the surface soil. However, there were no correlations between SDW or TPC and root biochemical traits (rhizosphere pH, acid phosphatase activity and organic acid content). It is hypothesized that greater CRL in the surface soil (0−10 cm soil layer) at the leaf development and flowering stages, served as a scaffold for fine roots, enhancing soil exploration and P acquisition, and, thereby, increasing seed yield.

1. Introduction

Oilseed rape (*Brassica napus* L.) is one of the most important and profitable oil crops in the world (Hu et al., 2017; Friedt et al., 2018). More than 20 % of the world's planted area of oilseed rape is in China (FAO, 2007–2016), and 50–70 % of this is located in the Yangtze River

basin (approx. 7.52 Mha), which has weathered acid soils (mostly ulti-sols and oxisols) that are severely phosphorus (P)-deficient (Yan et al., 2006). Both aluminum (Al) and iron (Fe) oxides are enriched in these soils, which limits the phyto-availability of phosphate (Pi). This results in very low P-fertilizer use efficiency of *Brassica napus* crops in the growing season (Larsen, 1967; Parfitt, 1989; Holford, 1997) and, often, symptoms of P-deficiency, which include purpling of cotyledons, darkening of older leaves, and plants with fewer branches and reduced seed set (Ding et al., 2012; Shi et al., 2013a, b). Thus, strategies to improve P acquisition are essential to achieve commercially viable seed yields of *Brassica napus* in this region.

Previous studies have suggested that manipulating root system morphology can improve P acquisition and, thereby, increase crop yields and the efficiency by which P fertilizers are used in agricultural systems (Shen et al., 2011; Lynch and Brown, 2012; Lambers et al., 2006; White et al., 2013; Wang et al., 2018). Lynch and Brown (2001) observed that root systems with a shallow architecture have enhanced P foraging in the P-enriched topsoil, which is an advantage for plants growing in soils with low P availability. In addition, denser lateral root branching in the topsoil improves P acquisition in soils with low P availability, as observed, for example, in maize (Jia et al., 2018; Sun et al., 2018). Both Koscielny and Gulden (2012) and Thomas et al. (2016a) observe that the root length of *Brassica napus* plants measured at the seedling stage in the laboratory correlated positively with seed yield in field experiments. *Brassica oleracea* responds to reduced P supply by decreasing primary root length, increasing the diameter of roots, and producing more lateral roots and root hairs at the seedling stage in a 'pouch and wick' system (Hammond et al., 2009). However, *Brassica napus* produces fewer roots and has a shorter total root length (TRL) at the seedling stage when grown with a deficient P supply than when grown with a sufficient P supply in a rhizotron system filled with soil (Yuan et al., 2016). Moreover, at crop maturity the number of roots, TRL and root/shoot ratio of *Brassica napus* grown with a deficient P supply are far lower than when grown with a sufficient P supply (Yuan et al., 2016).

Rhizosphere chemical and biological processes, mainly driven by root exudates, also play an important role in the acquisition of soil P, especially when P is in limited supply (Neumann and Römheld, 1999; Dong et al., 2004; Tawaraya et al., 2013; White et al., 2013). Roots of *Brassica napus* can release malic and citric acids, which solubilize inorganic P salts, and acid phosphatases, which degrade organic P compounds, into the soil to increase the P supply to plants when P supply is low (Zhang et al., 1997; White et al., 2005; Akhtar et al., 2008; Wang et al., 2015; Lyu et al., 2016). However, little work has been undertaken to determine the relative contributions of root system morphology and root exudates on P acquisition and seed yield throughout the development of a *Brassica napus* crop grown in the field with either sufficient or deficient P supply.

In this study, four *Brassica napus* cultivars were used to characterize (1) the morphological and biochemical (exudation) traits of *Brassica napus* root systems grown with sufficient or deficient P supply and (2) the major root traits that contribute to greater shoot dry weight (SDW), total P content (TPC) and seed yield (SY) during the lifetime of *Brassica napus* grown in the field. These results will provide a better understanding of the root traits that might be targeted for breeding *Brassica napus* with greater P use efficiency and yield in both the Yangtze River basin and elsewhere on soils with limited Pi availability (Wang et al., 2018).

2. Materials and methods

2.1. Materials

Four *Brassica napus* cultivars with different root morphological traits (10C25, WY29, Haishen and Xinan28) were selected from an association panel collected from major breeding centers across China (Liu et al.,

2016) based on their root system architecture in a "pouch and wick" system (Wang et al., 2017). Cultivar WY29 had the longest total root length (TRL), primary root length (PRL) and lateral root length (LRL), and the largest lateral root number (LRN) of the four cultivars. Cultivar Xinan28 had the shortest TRL, PRL and LRL and the smallest LRN of the four cultivars (Supplemental Table 1).

2.2. Soil type and experimental design

Two years of field trails were conducted at the experimental site of Huazhong Agricultural University in Wuhan from October 2015 to May 2017 (114.3 °E, 30.5 °N). The soil was a yellow-brown soil (Alfisol), and its properties were as follows: pH 6.84 (1:5 soil solution ratio, AFNOR, 1994), organic matter 12.99 g kg^{-1}, NH$_4$OAc-extracted potassium (K) 141.14 mg kg^{-1}, total nitrogen (N) 0.70 g kg^{-1}, available N 51.30 mg kg^{-1}, and Olsen-P 9.79 mg kg^{-1}. All the plots received basal fertilizer, including 60 % of the total N applied (supplied as urea), and all the P (supplied as calcium superphosphate), K (supplied as potassium chloride) and boron (supplied as Na$_2$B$_4$O$_7$·10H$_2$O). The application rates were as follows: N 108 kg ha^{-1}, K 120 kg ha^{-1} and Borax15 kg ha^{-1}. There were two P treatments, namely (1) a sufficient P supply of 90 kg P$_2$O$_5$ ha^{-1} (farmers' fertilizer practice) (Zou et al., 2009; Lu, 2010) and (2) a deficient P supply of 30 kg P$_2$O$_5$ ha^{-1}. These fertilizers were thoroughly mixed and applied in bands near the crop rows. The remaining N was top dressed as urea in equal amounts at the four to five-leaf stage and at the stem elongation stage, respectively.

A split-plot design with four blocks was adopted in 2015−2016 and 2016−2017. The main plot treatments were P fertilizer dosages, and the sub-plot treatments were *Brassica napus* cultivars. The plot size was 2.4 m length × 2.0 m width, with 30 cm row spacing and 30 cm plant spacing. Seeds of *Brassica napus* were sown by bunch planting with 10–15 seeds per point on 10 October 2015 and 25 September 2016, respectively. Seedlings were thinned to one plant at each point at the five-leaf stage. The same planting and field management procedures were followed in both years.

2.3. Sampling for shoot biomass and seed yield, and P analysis

Plants were sampled at five growth stages classified according to the BBCH development scale for oilseed rape (Lancashire et al., 1991) and winter oilseed rape (Böttcher et al., 2016) (Table 1): leaf development stage (BBCH 15−17), stem elongation stage (BBCH 30), flowering stage

Table 1
Sampling stages according to the BBCH development scale for *Brassica napus*.

Sampling		BBCH development scale (Plant stage)	Growth description
Stage 1	Year1: DAS 55 Year2: DAS 62	Leaf development (BBCH 15−17)	Plants have 5−7 leaves unfolded
Stage 2	Year1: DAS 107 Year2: DAS 105	Stem elongation (BBCH 30)	No internodes, stem begins to elongation
Stage 3	Year1: DAS 147 Year2: DAS 145	Flowering(BBCH 60−61)	Plants have less than 10 % of flowers on main raceme open, main raceme elongating
Stage 4	Year1: DAS 180 Year2: DAS 189	Pod development (BBCH 70−71)	Plants have less than 10 % of pods have reached final size
Stage 5	Year1: DAS 216 Year2: DAS 227	Ripening (BBCH 89)	Fully ripe: nearly all pods ripe

DAS: days after sowing.

(BBCH 60−61), pod development stage (BBCH 70−71) and ripening stage (BBCH 89). Each plot had a total of 48 plants with 8 rows and 6 plants in each row. One plant from each plot was sampled at each growth stage. Ten of the 43 plants remaining in each plot at maturity were selected randomly to determine seed yield (SY). All the plants sampled were divided into shoot and root. The shoot samples were exposed to 105 °C for 30 min. and then dried at 70 °C until a constant weight was obtained. The P concentration in dried plant material was determined using the vanado-molybdate method (Westerman, 1990). Total P acquisition (total P content, TPC) per plant was defined as the sum of the amounts of P in shoot and root material (Hammond et al., 2009; van de Wiel et al., 2016).

2.4. Root samples

Roots were sampled from excavated soil cubes. There were 27 soil cubes per plant, and the cube size was 10 cm × 10 cm × 10 cm (1000 cm^3). Cubes were dug one by one within a soil monolith of 30 cm × 30 cm × 30 cm. Each soil cube was kept in a plastic bag and labeled. The roots in each cube were carefully removed from the soil, and five roots at the seedling stage and three roots at the stem elongation, flowering, pod development and ripening stages were selected randomly for the collection of root exudates. The method for collection of rhizosphere exudates was modified from Pearse et al. (2006, 2007) and Lyu et al. (2016). Roots were shaken lightly to remove the bulk soil from the root system, and the soil adhering to the roots was defined as rhizosphere soil. The roots with rhizosphere soil attached were transferred to a 200 mL container containing 50 mL CaCl$_2$ (0.2 mM). Roots were gently immersed in the solution for 1−2 min to remove as much rhizosphere soil as possible and washed carefully to minimize root damage and leakage of solutes from damaged cells. Roots were then shaken lightly by hand and removed from the container. A subsample of the soil suspension (0.5 mL) was transferred to a 2 mL centrifuge tube to measure acid phosphatase activity (APA) (Alvey et al., 2001), which represents secreted acid phosphatase activity (Neumann and Römheld, 1999; Shu et al., 2005). The soil suspension was then left to stand for 5−10 mins. A second sub-sample (8 mL) of the supernatant from the soil suspension was transferred to a 10 mL vial, and then three drops of the microbial inhibitor Micropur (0.01 *g* L^{-1}; Sicheres Trinkwasser, Germany) and three drops of 85 % concentrated phosphoric acid were added to measure organic acids from the rhizosphere soil. The organic acids were quantified using a reversed phase high-performance liquid chromatography (HPLC) system as described previously (Wang et al., 2007; Wang et al., 2010; Li et al., 2016). Rhizosphere pH was measured on a third subsample (8 mL) of the original supernatant (Li et al., 2008, 2010). The remaining soil suspension (33.5 mL) was air dried and the dry weight of the rhizosphere soil was determined to calculate the secreted acid phosphatase activity and organic acid content (Li et al., 2008, 2010).

All roots collected from the soil monolith, including those used for measurements of acid phosphatase activity, organic acid release and rhizosphere pH were washed and stored at 4 °C for subsequent morphological analyses. For morphological analyses, roots were placed in a clear perspex tray with a film of distilled H$_2$O and scanned with a modified flatbed scanner (Epson V700, Nagano-ken, Japan). The root images were analyzed using WinRHIZO software (Regent Instruments Inc., Quebec, Canada). Brassica crops have a taproot and lateral roots of different orders. In this study, lateral roots with diameters of 0−2 mm were defined as fine roots (Jackson et al., 1997) and lateral roots with diameters of 2−5 mm were defined as coarse roots (Tufekcioglu et al., 1998; Bolte et al., 2004). The root length ratio (%) was defined as the ratio of root length in the surface soil layer (0−10 cm) to the total root length of the whole soil profile (0−30 cm).

2.5. Statistical analyses

Data were all analyzed using SPSS software (SPSS 19.0; IBM

Corporation, Armonk, NY, USA). A one-way ANOVA was used to compare SDW, RDW, TPC, SY, root morphological traits in 0−10 cm soil layer among four cultivars in each P treatment during the same growth stage. A two-way ANOVA was used to investigate the effect of P treatment on root morphological traits in 0−10 cm soil layer among four cultivars and root biochemical traits between two P supplies during the same growth stage. The effect of the growth stage and P treatment on root/shoot ratio, percentage of TRL in 0−10 cm soil layer and percentage of CRL in 0−10 cm soil layer of each cultivar were also investigated by a two-way ANOVA. A three-way ANOVA was used to investigate the effects of soil depth of sampling, P treatment and cultivar on root morphological traits. Means were compared using Tukey's test with *P* <0.05. In bar charts, significant differences were indicated by different letters above the bars. Pearson's correlation analysis was used to test the linear correlations between TPC, SDW and SY and their relationships with root traits observed in plants grown with P deficient and P sufficient supplies in the two experimental years, separately. Principal component analysis (PCA) was used to evaluate the relationships among SY, SDW, TPC, eight root morphological variables (TRL1, total root length in 0−10 cm; RSA1, root surface area in 0−10 cm; CRL1, coarse root length in 0−10 cm; FRL1, fine root length in 0−10 cm; TRL2, total root length in 10−30 cm; RSA2, root surface area in 10−30 cm; CRL2, coarse root length in 10−30 cm; FRL2, fine root length in 10−30 cm) and four root biochemical traits (APA, acid phosphatase activity; OAC, malic acid and citric acid; pH; rhizosphere pH) in plants grown with P deficient and P sufficient supplies in the two experimental years. The first two principal components were used to describe the relationships between the root morphological and biochemical traits.

3. Results

3.1. Differences in SDW, RDW and total phosphorus content among four Brassica napus cultivars grown with sufficient and deficient P supplies

The SDW and TPC of all cultivars increased gradually from the leaf development to the pod development stage in both P treatments (Figs. 1 and 2). In both years, all cultivars had the largest SDW and TPC at the ripening stage when grown with a sufficient P supply (Figs. 1 and 2a, b, e, f). Cultivar Haishen had a smaller SDW and TPC than other cultivars from the stem elongation to the ripening stage in both P treatments in both years. Cultivar Xinan28 had the largest SDW of all cultivars from the flowering to the ripening stage at both P supplies in both years.

The RDW increased rapidly from the leaf development to the flowering stage in both P treatments, in both years and in all cultivars (Fig. 1c, d, g, h). All cultivars had largest RDW at the pod development stage in both P treatments in both years, except cultivar 10C25, which had its largest RDW at the ripening stage in both P treatments. Cultivar Haishen had a smaller RDW than other cultivars from the stem elongation stage to the ripening stage in both P treatments. The largest root/shoot ratio was observed at the flowering stage in all cultivars in both years and the root/shoot ratio was larger when plants were grown with a deficient P supply than when grown with a sufficient P supply at this growth stage (Fig. 3).

Seed yield had significant (*P* <0.05) positive correlations with SDW in the P deficient treatment at all growth stages, while significant (*P* <0.05) correlations between SY and SDW were only found at the pod development and at the ripening stages in the P sufficient treatment in 2016−2017 (Supplementary Fig. 1).

3.2. Differences in root morphological and biochemical traits among four Brassica napus cultivars grown with sufficient and deficient P supplies

The root morphological traits of total root length (TRL), fine root length (FRL), coarse root length (CRL) and root surface area (RSA) all increased rapidly from the leaf development stage to the flowering stage, and then decreased from the pod development stage to the
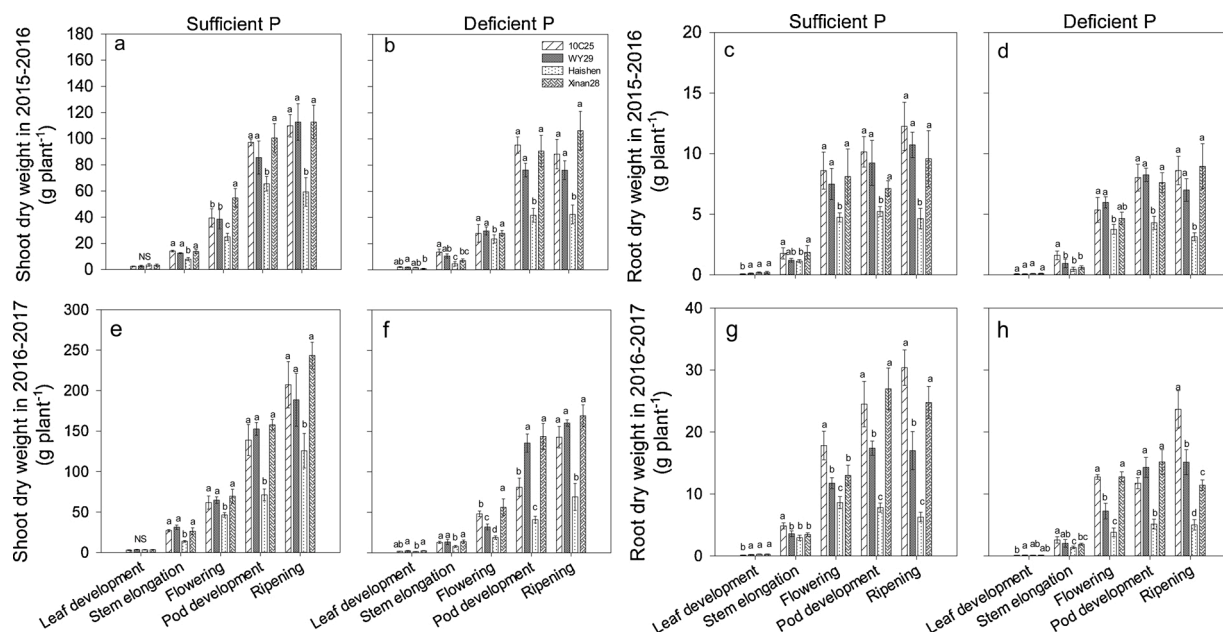
Fig. 1. (a, b, e, f) Shoot dry weight (SDW) and (c, d, g, h) root dry weight (RDW) of *Brassica napus* cultivars 10C25, WY29, Haishen and Xinan28 from the leaf development stage to the ripening stage when grown with (a, c, e, g) a sufficient P supply and (b, d, f, h) a deficient P supply in (a, b, c, d) 2015-2016 and (e, f, g, h) 2016-2017. The sufficient P supply was 90 kg P_2O_5 ha^{-1} and the deficient P supply was 30 kg P_2O_5 ha^{-1}. Each value is the mean (±SE) of four replicates. The different small letters above the column indicate significant difference among the cultivars in the same P treatment during the same growth stage at $P < 0.05$.
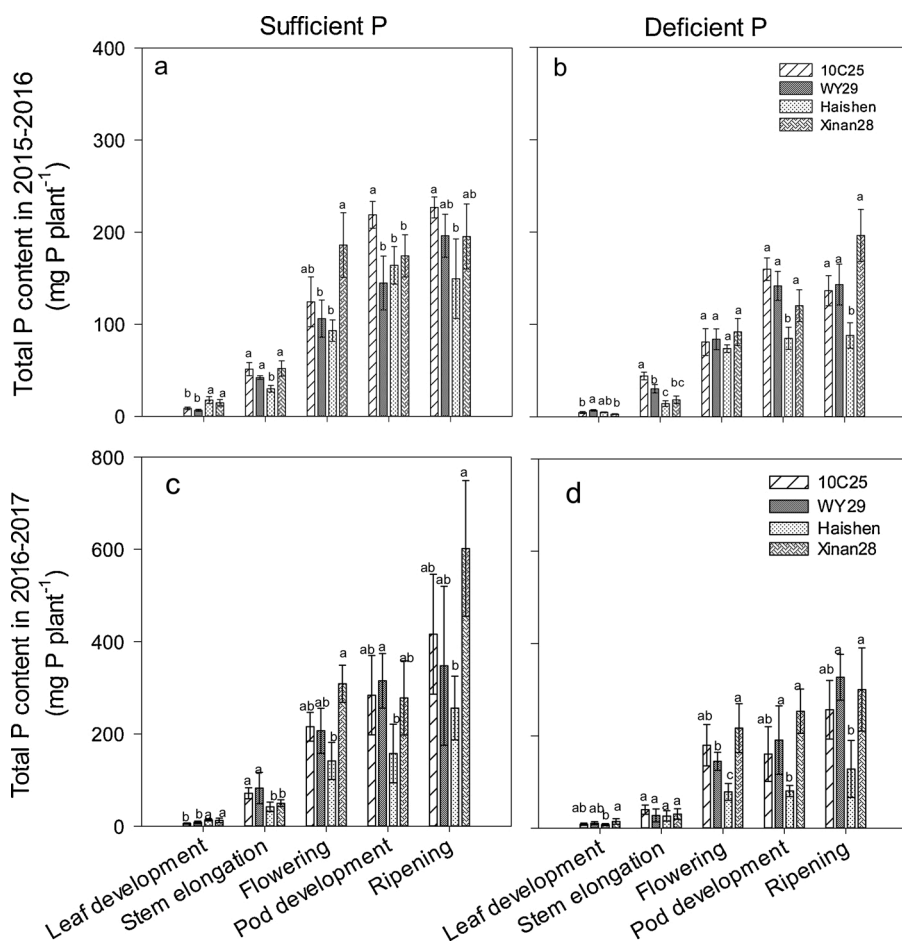
Fig. 2. Total P content of *Brassica napus* cultivars 10C25, WY29, Haishen and Xinan28 from the leaf development stage to the ripening stage when grown with (a, c) a sufficient P supply and (b, d) a deficient P supply in (a, b) 2015-2016 and (c, d) 2016-2017. The sufficient P supply was 90 kg P_2O_5 ha^{-1} and the deficient P supply was 30 kg P_2O_5 ha^{-1}. Each value is the mean (±SE) of four replicates. The different small letters above the column indicate significant difference among cultivars in the same P treatment during the same growth stage at $P < 0.05$.
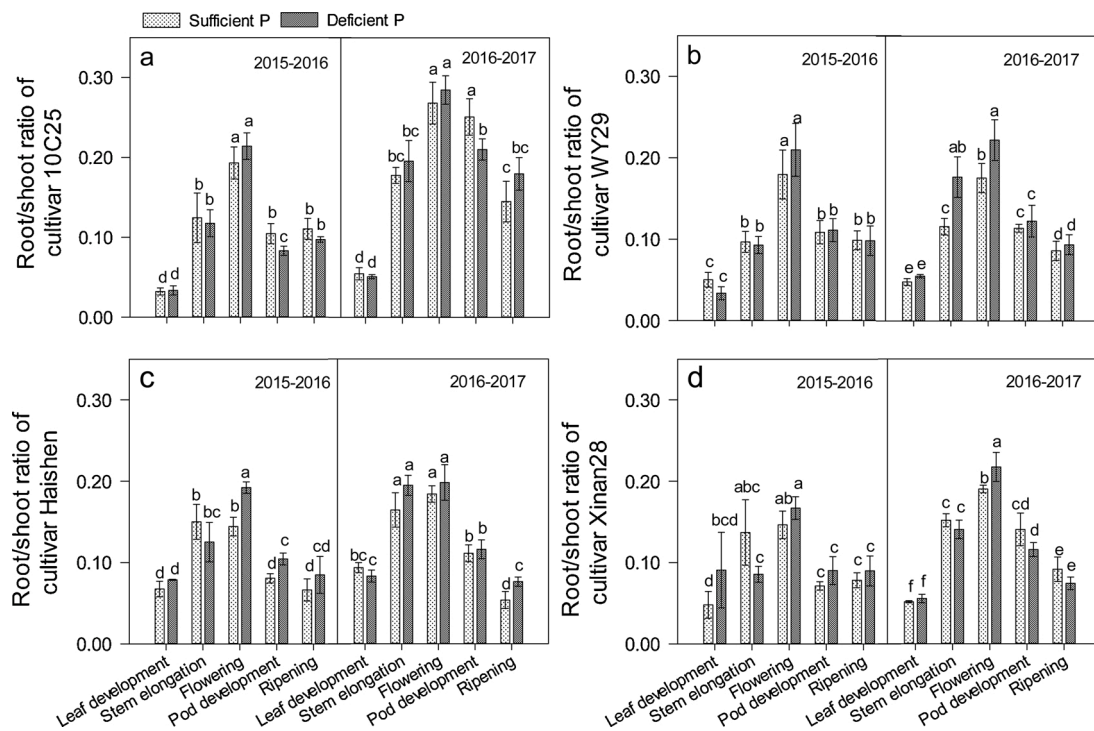
Fig. 3. Root/shoot ratios, expressed on a dry weight basis, at five growth stages for four *Brassica napus* cultivars grown with either a sufficient or a deficient P supply in 2015-2016 and 2016-2017. The sufficient P supply was 90 kg P_2O_5 ha^{-1} and the deficient P supply was 30 kg P_2O_5 ha^{-1}. Each value is the mean ($\pm$SE) of four replicates. The different small letters above the column indicate significant difference between two P treatments among five growth stages each year at $P < 0.05$.

ripening stage in all soil layers in both P treatments ($P < 0.001$) and in both years ($P < 0.05$), apart from the CRL increased from the leaf developmene stage to pod development and then decreased at the ripening stage in 2015-2016 (Tables 2 and 3). Root morphological traits

in the 0−10 cm soil layer, including TRL1 and FRL1, were significant ($P < 0.05$) smaller from the leaf development stage to the flowering stage in both years, and RSA1 and CRL1 were significant ($P < 0.05$) smaller at the leaf development, stem elongation, flowering and ripening stages in

Table 2
The effects of P supply on total root length (TRL), root surface area (RSA), fine root length (FRL) and coarse root length (CRL) in different soil layers at five growth stages of *Brassica napus* in 2015-2016.

Year	Growth stages	Soil depth	P treatments	TRL (mm plant^{-1})	RSA (mm^2 plant^{-1})	FRL (mm plant^{-1})	CRL (mm plant^{-1})
2015−2016	Leaf development stage	0−10 cm	Sufficient P	168.2a[a]	49.9a	157.4a	7.5a
			Deficient P	128.3b	33.6b	122.2b	3.8b
		10−20 cm	Sufficient P	15.3c	2.5c	14.5c	
			Deficient P	8.5c	1.5c	8.5c	
	Stem elongation stage	0−10 cm	Sufficient P	1597.3a	366.2a	1546.7a	39.5a
			Deficient P	1103.0b	260.2b	1076.0b	27.5b
		10−20 cm	Sufficient P	427.4c	81.1c	424.5c	2.7c
			Deficient P	214.1d	40.8d	213.2d	0.8d
		20−30 cm	Sufficient P	144.7e	29.0d	141.7e	1.4cd
			Deficient P	74.9f	14.5e	77.8f	0.3d
	Flowering stage	0−10 cm	Sufficient P	2371.0a	644.1a	2259.1a	105.0a
			Deficient P	1764.7b	493.4b	1654.0b	82.6b
		10−20 cm	Sufficient P	495.3c	102.6c	484.7b	9.8c
			Deficient P	385.7c	76.7c	379.1b	6.9c
		20−30 cm	Sufficient P	46.5d	9.0ad	40.6d	0.3d
			Deficient P	103.8d	20.8d	101.7c	1.9d
	Pod development stage	0−10 cm	Sufficient P	1893.1a	600.9a	1675.3a	120.2a
			Deficient P	1716.2a	545.0a	1580.4a	100.1b
		10−20 cm	Sufficient P	673.7b	150.6b	685.1b	16.4c
			Deficient P	621.5b	143.6b	599.5b	17.4c
		20−30 cm	Sufficient P	239.3c	48.5c	236.8c	2.0d
			Deficient P	215.1c	45.3c	210.8c	4.1d
	Ripening stage	0−10 cm	Sufficient P	955.4a	447.0a	812.7a	103.7a
			Deficient P	871.0a	373.9b	758.2b	83.5b
		10−20 cm	Sufficient P	238.9b	70.2c	225.5c	10.8c
			Deficient P	238.5b	64.6cd	227.3c	10.8c
		20−30 cm	Sufficient P	172.7c	42.0de	167.2d	4.1d
			Deficient P	120.1c	28.4e	117.5d	3.2d

[a]Within a column for a given each growth stage, means of four *Brassica napus* cultivars followed by different letters are significantly different between two P treatment among different soil depth during the same growth stage at $P < 0.05$.

Table 3
The effects of P supply on total root length (TRL), root surface area (RSA), fine root length (FRL) and coarse root length (CRL) in different soil layer at five growth stages of *Brassica napus* in 2016-2017.

Year	Growth stages	Soil depth	P treatments	TRL (mm plant^{-1})	RSA (mm^2 plant^{-1})	FRL (mm plant^{-1})	CRL (mm plant^{-1})
2016−2017	Leaf development stage	0−10 cm	Sufficient P	517.7a[a]	67.1a	509.8a	5.7a
			Deficient P	398.6b	52.4b	392.6a	3.7b
		10−20 cm	Sufficient P	104.9c	9.5c	104.9c	
			Deficient P	81.0c	7.1c	81.0c	
	Stem elongation stage	0−10 cm	Sufficient P	1015.7a	452.2a	864.1a	119.8a
			Deficient P	822.8b	390.2a	686.3b	112.8a
		10−20 cm	Sufficient P	403.4c	161.4b	352.9c	45.2b
			Deficient P	310.6c	149.7b	249.9d	51.8b
		20−30 cm	Sufficient P	205.5d	95.5c	170.2d	29.7be
			Deficient P	85.5e	39.9d	69.7e	14.6e
	Flowering stage	0−10 cm	Sufficient P	1694.0a	3712.9a	1135.0a	289.0a
			Deficient P	1419.3b	2845.5b	1078.5b	239.5b
		10−20 cm	Sufficient P	482.8c	182.4c	432.0c	40.2c
			Deficient P	379.4c	137.6c	341.7c	31.6c
		20−30 cm	Sufficient P	221.9d	65.8d	211.6d	9.8d
			Deficient P	169.6d	50.1d	162.4d	6.6d
	Pod development stage	0−10 cm	Sufficient P	848.9a	557.3a	593.8a	66.6a
			Deficient P	757.1a	404.6b	558.8a	48.0b
		10−20 cm	Sufficient P	429.9b	180.9c	374.0b	43.5b
			Deficient P	368.4b	126.4d	340.1b	22.9c
		20−30 cm	Sufficient P	234.9c	78.6e	211.4c	14.4d
			Deficient P	259.0c	84.6e	243.2c	13.4d
	Ripening stage	0−10 cm	Sufficient P	702.5a	578.2a	556.0a	101.1a
			Deficient P	655.4a	427.4b	521.1a	86.8a
		10−20 cm	Sufficient P	352.3b	209.8c	266.0b	63.1b
			Deficient P	332.9b	165.4d	274.6b	42.6b
		20−30 cm	Sufficient P	172.4c	77.6e	146.2c	20.6c
			Deficient P	167.1c	62.0e	151.1c	13.5c

[a]Within a column for a given each growth stage, means of four *Brassica napus* cultivars followed by different letters are significantly different between two P treatment among different soil depth during the same growth stage at $P < 0.05$.

2015−2016 and significant ($P < 0.05$) smaller at the leaf development, flowering and pod development stages in 2016−2017 when plants were grown with a deficient P supply than when grown with a sufficient P supply (Tables 2 and 3). On average, more than 50 % of the TRL and more than 60 % of the CRL were found in the surface soil (0−10 cm) in both sufficient and deficient P treatments across the whole growth period in both years (Supplementary Figs. 2 and 3). Averaged over both years, TRL1 was 23.4 % less with a deficient than with a sufficient P supply at the leaf development stage, 25.0 % less at the stem elongation stage, 20.7 % less at the flowering stage, 10.1 % less at the pod development stage, and 7.8 % less at the ripening stage. CRL1 was 42.2 % less at the leaf development stage, 18.1 % less at the stem elongation stage, 19.2 % less at the flowering stage, 22.3 % less at the pod development stage, and 16.8 % less at the ripening stage (Tables 2 and 3).

More acid phosphatase activity and organic acid (malic acid, citric acid and fumaric acid) were found in the rhizosphere soil from the stem elongation stage to the pod development stage in plants grown with a deficient P supply than in plants grown with a sufficient P supply. In both years, a lower rhizosphere pH was also observed in the rhizosphere soil when plants were grown with a deficient P supply than when plants were grown with a sufficient P supply (Supplementary Table 2).

3.3. Correlations between SY, SDW, TPC and root morphological and biochemical traits of four Brassica napus cultivars growth with sufficient and deficient P supplies

When plants received a sufficient P supply, TPC had significant ($P < 0.02$ to 0.008) positive correlations with CRL1 and RSA1 in the 0−10 cm soil layer at the leaf development stage, and significant ($P < 0.05$ to 0.001) positive correlations were also found between SY and CRL1 and RSA1, and between TPC and CRL1 and RSA1 at the flowering stage in both years (Fig. 4a, c, e, g).

When plants received a deficient P supply, SY had significant ($P < 0.05$ to 0.001) positive correlations with CRL, RSA, CRL1 and TRL1 at the flowering stage in both years (Fig. 4b, d; Supplementary Fig. 4a, b).

The TPC had significant ($P < 0.043$ to 0.001) positive correlations with TRL and RSA (TRL1 and RSA1) at the flowering stage in both years (Fig. 4b, d; Supplementary Fig. 4a, b). At the leaf development stage, SDW had a significant ($P = 0.013$) positive correlation with RSA1 in 2015−2016, and with CRL1 in 2016−2017 (Fig. 4b, d).

When plants received a sufficient P supply, significant ($P < 0.05$ to 0.001) positive correlations were observed between SY and APA at the leaf development stage, between SY and OAC at the flowering stage in 2015−2016 and at the ripening stage in 2016−2017. When plants received a deficient P supply, SY had significant ($P < 0.05$) correlations with APA at the flowering stage in 2015−2016 and at the leaf development stage in 2016−2017 (Fig. 4e, f, g, h). There were no correlations ($P > 0.05$) between TPC or SDW and root biochemical traits (APA, OAC or Rhizos-pH) in either year at either P supply (Fig. 4e, f, g, h).

3.4. Root morphological and biochemical traits of Brassica napus cultivars at the leaf development and flowering stages

The results of PCA, with SY, SDW, TPC and eight root morphological traits (RSA1, CRL1, FRL1, TRL1 and RSA2, CRL2, FRL2, TRL2) and four root biochemical traits (CA, MA, APA, pH) of plants grown with a sufficient P supply showed that the first axis explained 33.3 % and 28.2 % in 2015−2016, and 32.3 % and 37.5 % in 2016−2017 of the variation among the twelve root traits at the leaf development and flowering stages, respectively (Fig. 5a, c, e, g). The first axis was mostly associated with root morphological traits in the 0−10 cm soil layer (TRL1, RSA1, CRL1 and FRL1) in plants with a sufficient P supply at the flowering stage in both years (Fig. 5c, g). When plants were grown with a deficient P supply, the first axis of the PCA explained 30.6 % and 33.6 % in 2015−2016, and 38.8 % and 32.3 % in 2016−2017 of the variation among the twelve root traits at the leaf development and flowering stages, respectively, and was also mostly associated with root morphological traits in 0−10 cm soil layer (TRL1, RSA1, FRL1 and CRL1) (Fig. 5b, d, f, h).

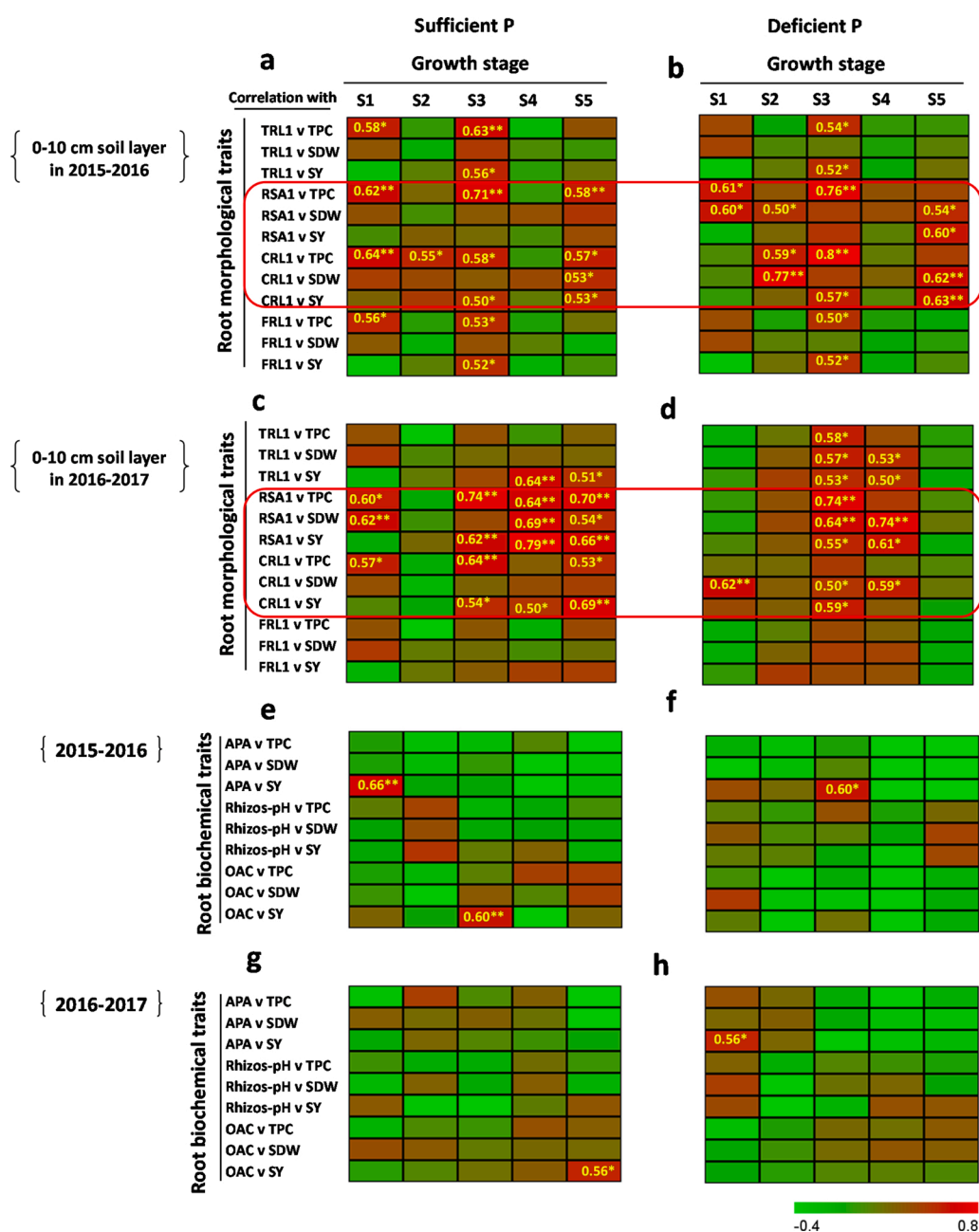In the surface soil (0−10 cm soil layer), the FRL1 had significant (P

Fig. 4. Pearson's correlations between total P content (TPC), shoot dry weight (SDW) as well as seed yield (SY) and (a, b, c, d) root morphological traits (TRL1, RSA1, CRL1, FRL1) in the 0-10 cm soil layer and (e, f, g, h) root biochemical traits for (a, b, e, f) 2015-2016 and (c, d, g, h) 2016-2017 in plants grown with (a, c, e, g) a sufficient P and (b, d, f, h) a deficient P supply at different growth stages (S1 to S5). S1, leaf development stage, S2, stem elongation stage, S3, flowering stage, S4, pod development stage, S5, ripening stage. The sufficient P supply was 90 kg P_2O_5 ha^{-1} and the deficient P supply was 30 kg P_2O_5 ha^{-1}. *$P \leq 0.05$; **$P \leq 0.01$.

=0.001) correlation with CRL1 at the leaf development stage when plants were grown with a sufficient P supply in 2015−2016, and significant ($P <0.028$ to 0.001) correlations with CRL1 at the flowering stage when plants were grown with either P supply in both years (Fig. 6a, b). The TPC had significant ($P <0.043$ to 0.023) correlations with fine root surface area and coarse root surface area at the leaf development stage when plants were grown with a sufficient P supply in both years (Fig. 6c, e), and significant ($P <0.049$ to 0.004) correlations with fine root surface area and coarse root surface area at the flowering stage when plants were grown with either P supply in both years (Fig. 6d, f).

4. Discussion

Four *Brassica napus* cultivars (10C25, WY29, Haishen, Xinan28) with contrasting root morphological and biochemical traits were used in this study. The TRL and LRL of cultivar Xinan28 were shorter than those of the other three cultivars when grown without P in a 'pouch and wick' system for 14 days (Supplementary Table 1). A previous study by Thomas et al. (2016b) reported that seed size could explain variation in total root length, primary root length and lateral root length of seedlings of *Brassica napus* grown with a deficient P supply. However, in the field trials reported here, it is unlikely that seed size was a major influence on root traits, even at the leaf development stage (Figs. 1 and 2). Compared to other cultivars, Haishen had the smallest TRL1, RSA1, CRL1 and FRL1 (Supplementary Table 3), and the smallest RDW, SDW and TPC from the flowering stage to the ripening stage when grown with either P supply in the field (Figs. 1 and 2), but had the largest 1000-seed weight (Supplementary Table 1). Similarly, there were no significant differences in these traits among cultivars 10C25, WY29 and Xinan28 whether grown with a deficient or a sufficient P supply (Figs. 1 and 2), yet their 1000-seed weight varied from 3.93 to 5.38 g (Supplementary Table 1).

Both root morphological and biochemical traits are important for resource acquisition and, therefore plant growth and crop yield. Root

2015-2016
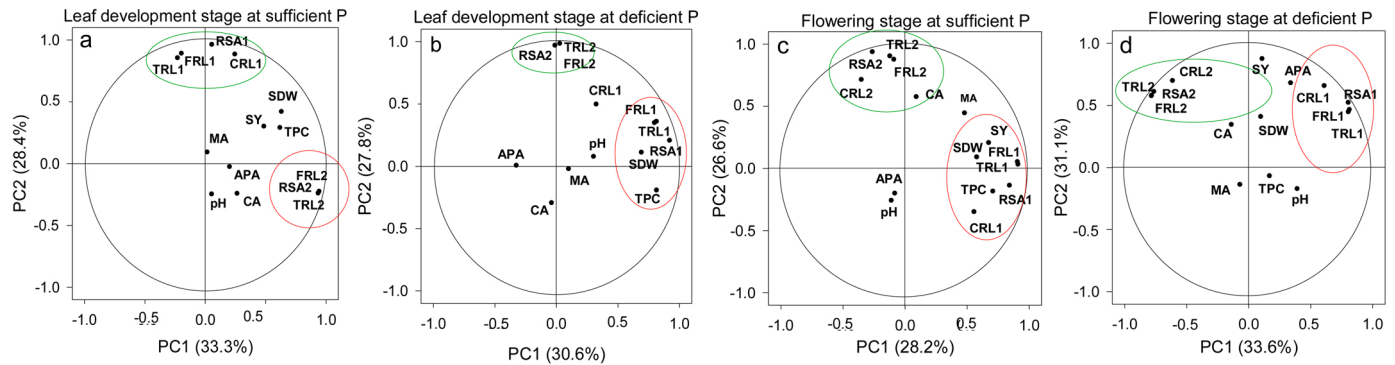
2016-2017
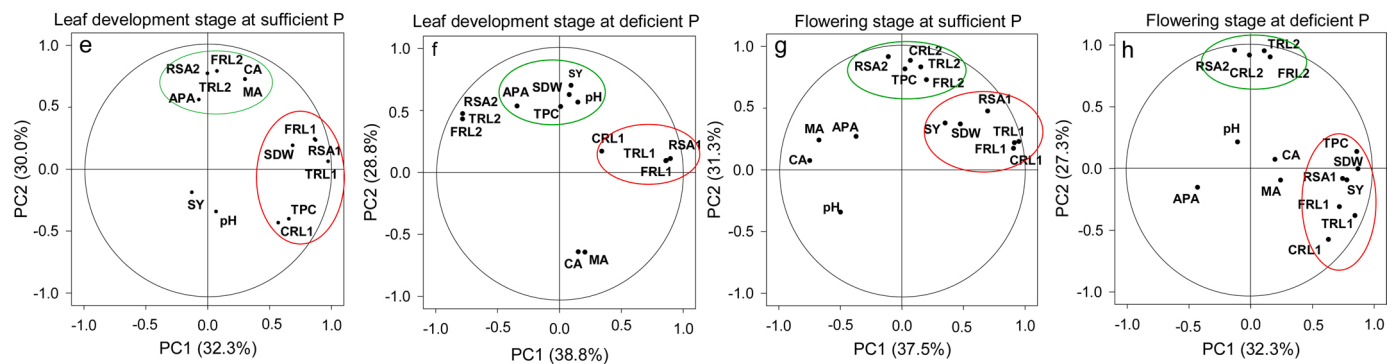
Fig. 5. Principal component analysis (PCA) of SY, SDW, TPC, eight root morphological traits (recorded at 0–10 cm and 10–30 cm) and four root biochemical traits determined on four *Brassica napus* cultivars grown with (a, c, e, g) a sufficient P supply or (b, d, f, h) a deficient P supply at (a, b, e, f) the leaf development and (c, d, g, h) the flowering stages in (a, b, c, d) 2015-2016 and (e, f, g, h) 2016-2017. The sufficient P supply was 90 kg P_2O_5 ha^{-1} and the deficient P supply was 30 kg P_2O_5 ha^{-1}. *$P \leq 0.05$; **$P \leq 0.01$.

traits show great plasticity in the adaptive responses of *Brassica napus* plants to P deficiency (Ligaba et al., 2004; Zhang et al., 2009, 2010; Yang et al., 2010; Hu et al., 2010; Lyu et al., 2016). The results presented here suggest that root morphological traits play a more important role in the adaptation of *Brassica napus* to low soil P supply than root biochemical traits (Figs. 4 and 5). There were strong positive correlations between SY, SDW and TPC and CRL1, FRL1, TRL1 and RSA1 in the topsoil (Fig. 4; Supplementary Fig. 4). The PCA results also indicated that root morphological traits in the topsoil at the leaf development and flowering stages were most strongly associated with SDW and SY (Fig. 5). Although the rhizosphere of plants grown with a deficient P supply had a lower pH, more acid phosphatase activity and greater organic acid content than plants grown with a sufficient P supply at the flowering stage in both years (Supplementary Table 2), there were no correlations between SDW or TPC and root biochemical traits (APA, OAC or pH) at any growth stage at either P supply (Fig. 4e, f, g, h). This might be because the APA and OAC in the rhizosphere are influenced by both microorganisms and soil properties (Rovira, 1959; Husain and McKeen, 1963; Jones et al., 2003). To our knowledge, this is the first study to compare the relative contributions of root morphological and biochemical adaptations to P supply on TPC, SDW and SY of *Brassica napus* in the field over the whole growth cycle.

Plants grown with a deficient P supply had larger total root length ratio and coarse root length ratio in the surface soil (0−10 cm soil layer) and a larger root/shoot ratio at the flowering stage than plants grown with a sufficient P supply (Fig. 3; Supplementary Figs. 2 and 3). This is consistent with the observations of Yuan et al. (2016) for *Brassica napus* grown in soil in a rhizobox. It is anticipated that a greater root length and surface area in the surface soil will benefit P foraging by *Brassica napus* in the field (Liu et al., 2011; Lyu et al., 2016; Yuan et al., 2016).

When P is deficient, many *Brassica* species respond by thickening

primary roots, and investing biomass in both lateral roots and root hairs to explore a greater volume of soil and thereby access more P (White et al., 2005; Hammond et al., 2009). In the present study, increased distribution of coarse roots in the surface soil (0−10 cm soil layer) appeared to enable *Brassica napus* to increase P acquisition at the leaf development and flowering stages (Fig. 6). Cornish et al. (1984) found that P acquisition was positively correlated with root extension and that greater root length could improve root contact with the soil and increase P uptake. Thus, more coarse roots provide a larger root surface area, which enables greater P uptake from the soil (Fig. 5; Cornish et al., 1984; Haling et al., 2013).

Normally, fine roots have a greater effect on P acquisition than coarse roots (Föhse et al., 1991; Gahoonia and Nielsen, 2004), since they comprise most of the root length and surface area responsible for water and nutrient uptake (Zobel et al., 2007; Liu et al., 2010; Ulas et al., 2012). Furthermore, fine roots enable plants to explore a larger volume of soil with a smaller metabolic investment in root tissue (Eissenstat, 1992; Miller et al., 1998). In this study, TPC significantly correlated with FRL1 at the leaf development and flowering stages when plants were grown with a sufficient P supply, and at the flowering stage when plants were grown with a deficient P supply in 2015−2016 (Fig. 4). Although there were no significant correlations between TPC and FRL at any growth stage or P supply in 2016−2017 (Fig. 4), significant correlations were observed between TPC and fine root surface area at both P supplies in both years (Fig. 6). Moreover, in the surface soil, there were significant correlations of FRL with CRL at the leaf development stage when plants were grown with a sufficient P supply in 2015−2016, and at the flowering stage when plants were grown with either P supply in both years (Fig. 6). Plant TPC showed a greater correlation with the length of coarse roots in the surface soil (CRL1) than with fine roots in the surface soil (FRL1) in the present study (Fig. 4). This positive effect of coarse
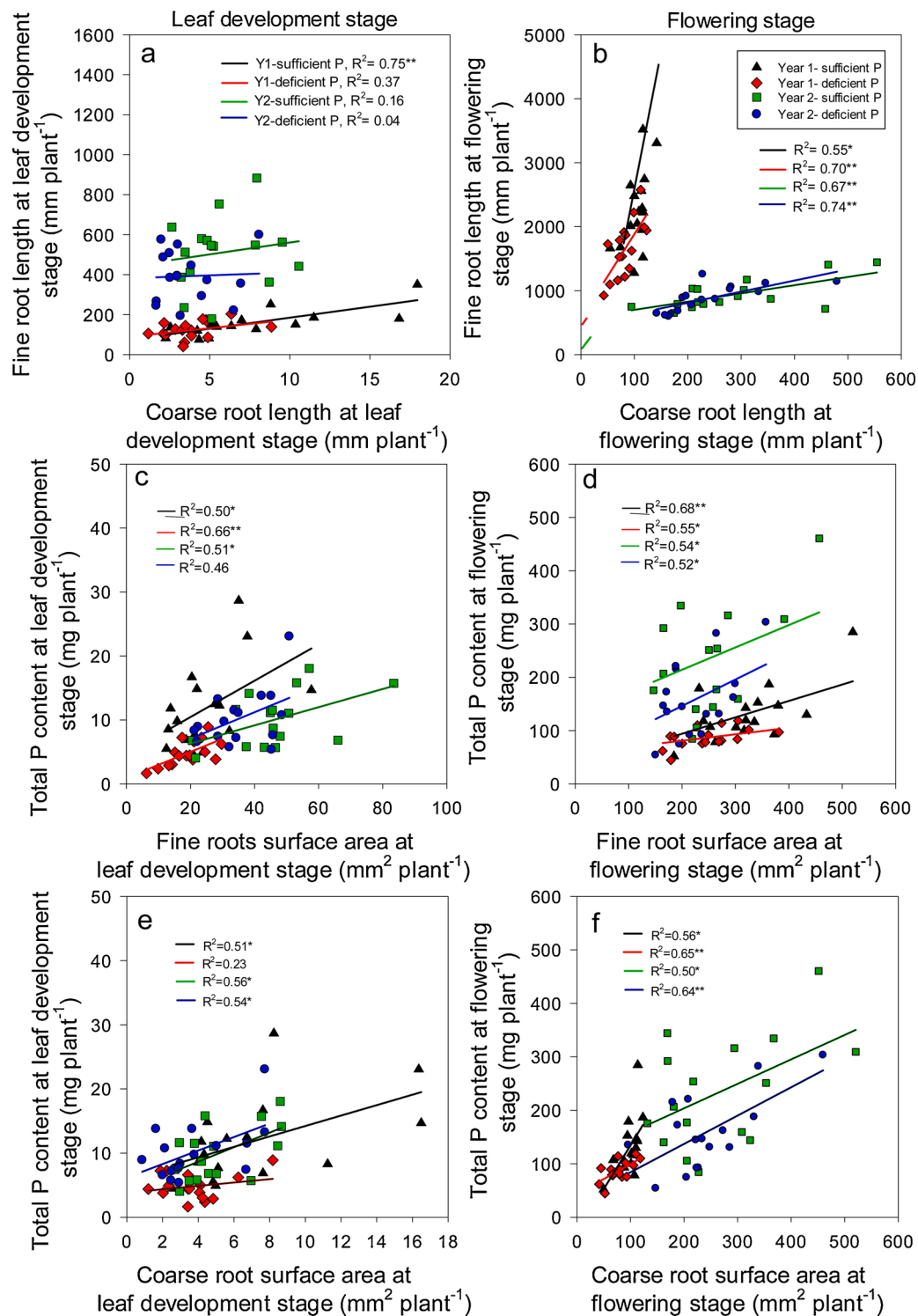
Fig. 6. Pearson's correlation between (a, b) fine root length (FRL) and coarse root length (CRL), total P content (TPC) and (c, d) fine root surface area, (e, f) coarse root surface area in the topsoil (0-10 cm soil layer) of plants grown with a sufficient P and a deficient P supplies at the leaf development and flowering stages, respectively. The sufficient P supply was 90 kg P_2O_5 ha^{-1} and the deficient P supply was 30 kg P_2O_5 ha^{-1}. *$P \leq 0.05$; **$P \leq 0.01$.

roots in the topsoil on P acquisition might be achieved: (1) by providing a scaffold in the topsoil for the production of fine roots to forage for P (Fig. 7; Atkinson and Wilson, 1980; Rubio et al., 2003; Kosola et al., 2007) or (2) by facilitating the root system of *Brassica napus* to penetrate the heavy clay soil effectively (Lin et al., 2013; Jin et al., 2017).

Many studies indicate that a topsoil foraging phenotype improves P acquisition from the soil (Bonser et al., 1996; Ge et al., 2000; Liao et al., 2001; Lynch and Brown, 2001; Lynch, 2011; White et al., 2013). In

China, most of the *Brassica napus* crop is grown in yellow-brown soils, which have very sticky texture and a very poor soil pore system (Xi, 1998). Coarse roots can resist buckling and might facilitate the penetration of densely compacted soils by the root system (Pietola and Smucker, 1998; Price et al., 2000; Clark et al., 2002; Chimungu et al., 2015). Fine roots arising from the coarse roots might then follow soil pores created by coarse roots or subterranean biota for greater resource acquisition and improved plant growth (Jin et al., 2017). Thus, the
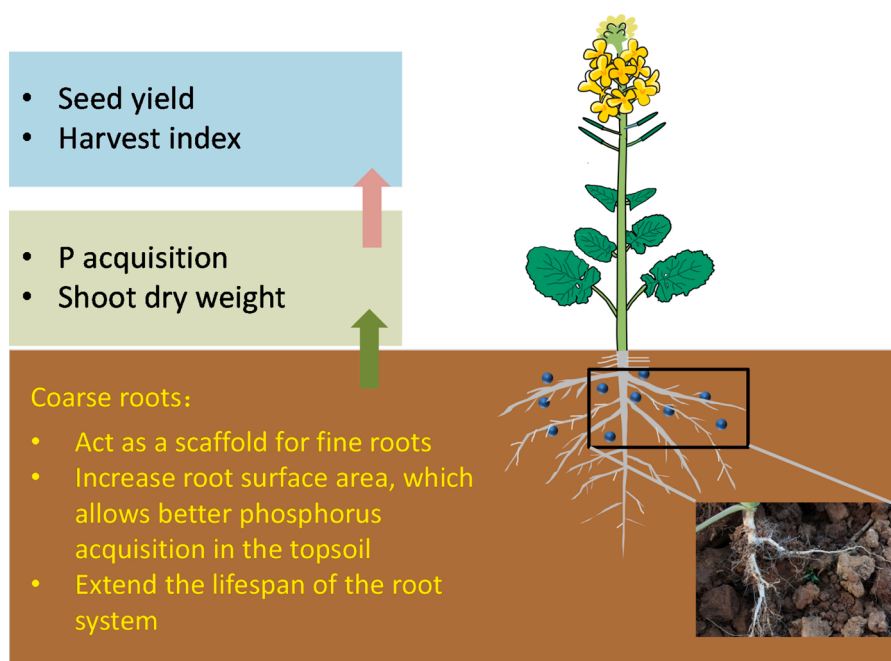
Fig. 7. A proposed diagram of how coarse roots of *Brassica napus* contribute to P acquisition.

establishment of coarse roots at the leaf development and flowering stages of *Brassica napus* would benefit penetration of the soil, and subsequent root branching and root exploration to improve P acquisition (MacDuff et al., 1986; Yuan et al., 2016). It was also observed that the SY of *Brassica napus* was significantly correlated with CRL1 at the flowering stage (Fig. 4). Compared with fine roots, coarse roots might also increase the lifetime of the root system and extend the duration of resource acquisition (Gill and Jackson, 2000; Gill et al., 2002; Adams et al., 2013; Hunter et al., 2014). This would allow the root system of *Brassica napus* to acquire not only P but also other nutrients to maintain the branches and seed pods formed in the later stages of crop development (Berry et al., 2010; Ding et al., 2012; Shi et al., 2013a, b). This is very important for yield formation in *Brassica napus* (Luo et al., 2015). Maintaining an appropriate functional equilibrium between the root, supplying mineral nutrients, and the shoot, supplying photosynthate, throughout the growth cycle of a crop is likely to maximize both crop yields and quality attributes (Fu, 2000; Hermans et al., 2006). Therefore, increasing the proportion of coarse roots in the topsoil might provide multiple benefits to the *Brassica napus* crop (Fig. 7).

5. Conclusions

Plant biomass and root morphology at the leaf development and flowering stages are very important for the seed yield of *Brassica napus*. At these two stages, the length and surface area of coarse roots in the topsoil were correlated with P acquisition, shoot biomass and eventual seed yield of plants grown in the field with either a sufficient or a deficient P supply. Root morphological traits played a more important role than root biochemical traits in the adaptation of *Brassica napus* to a deficient soil P supply. It is hypothesized that increasing the length of coarse roots in the topsoil allowed greater topsoil foraging by enabling the root system to penetrate the heavy clay soil and by providing a scaffold for the development of fine roots.

CRediT authorship contribution statement

Xianjie Duan: Investigation, Methodology, Formal analysis, Writing - original draft. **Kemo Jin:** Conceptualization, Methodology, Supervision, Writing - review & editing. **Guangda Ding:** Writing - review & editing. **Chuang Wang:** Writing - review & editing. **Hongmei Cai:** Writing - review & editing. **Sheliang Wang:** Writing - review & editing. **Philip J. White:** Writing - review & editing. **Fangsen Xu:** Writing - review & editing. **Lei Shi:** Supervision, Funding acquisition, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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.fcr.2020.107960.

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