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Phosphorus depletion controls Cu and Zn biogeochemistry in canola and corn rhizosphere on a calcareous soil

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Abstract: Phosphorus (P) deficiency may trigger rhizodeposition, including protons and organic compounds, with possible effects on metal solubility and speciation. To explore the relevance of this process, we investigated biogeochemical changes in the rhizosphere of P-deficient canola (*Brassica napus* L.) and corn (*Zea mays* L.) cultivars grown in a pot experiment on calcareous soil. Depletion of total soluble (0.005 mol/L $Ca(NO_3)_2$-extractable) P in the rhizosphere varied with crop species and cultivar but was generally strong and negatively correlated with dissolved organic carbon (DOC) in canola (R^2 = 0.868) and corn (R^2 = 0.844) rhizospheres, indicating rhizodeposition in response to limited P availability. DOC was correlated with dissolved Cu, explaining 86% of its variation in the rhizosphere and bulk soil solution of canola and corn cultivars, respectively, suggesting Cu mobilisation *via* the formation of Cu-organic complexes. In line with lower Zn-organic complex stabilities, the effect of rhizodeposition was less pronounced for Zn mobilisation. We show that the P nutritional status of plants and the related variation of rhizodeposition among crops and cultivars represents a major control of metal solubility in soil, with possible effects on micronutrient supply and toxicity. Hence, targeted P availability control should be considered in the management of polluted and micronutrient-deficient soils.

Keywords: root activity; phytoremediation; metal contaminated soils; macro- and micronutrient availability; metal-organo ligand complexes

The availability of phosphorus (P) can be an important constraint to plant growth in calcareous soils. The P dynamics and mobility in calcareous environments are largely controlled by carbonates and iron oxide minerals (McBride 1994). Therefore, only a marginal proportion of bulk soil P is present as phosphate ions in the soil solution (0.1 to 10 µmol), which is rather low compared with the adequate P for optimal growth (Hinsinger 2001). However, plant root activities can induce changes in the rhizosphere soil properties and subsequently alter the P and micronutrient bioavailability (Jones and Darrah 1994, Hinsinger 2001, Hinsinger et al. 2009). Chemical processes that are induced by plant roots to change the mobility of P and micronutrients in the rhizosphere include mostly pH changes and exudation of a wide range of organic compounds (Jones and Darrah 1994, Hinsinger 2001, Nguyen 2003, Jones et al. 2009, Oburger et al. 2009). It has been well documented that the amount of rhizodeposition depends strongly

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on the plant's nutritional status and is stimulated by plant nutrient deficiency and exogenous stress conditions (Jones and Darrah 1994, Nguyen 2003). Many organic acids, which occur as anions under a wide range of soil conditions, are able to dissolve oxide minerals and calcium phosphates compounds and to liberate P for root uptake (Hoffland et al. 1989, Bolan et al. 1994, Jones and Darrah 1994).

While root exudates can increase the phytoavailability of P, iron (Fe) and zinc (Zn), this might also lead to the concomitant mobilisation or immobilisation of other micronutrients or toxic metals by the formation of metal-ligand complexes (Dessureault-Rompré et al. 2008, Hinsinger and Courchesne 2008, Puschenreiter et al. 2017). Dessureault-Rompré et al. (2008) showed that in low P conditions, root exudates of white lupin (*Lupinus albus* L.) resulted in the complexation and mobilisation of Fe and Zn and the solubilisation of copper (Cu) and lead (Pb) through mobilisation of soil organic matter. The formation of metal-organic complexes in the rhizosphere of P-deficient plants triggered the mobilisation of metallic micronutrients and pollutants with increasing their availability (Wenzel et al. 2011). Houben and Sonnet (2012) demonstrated that the Italian ryegrass plant (*Lolium multiforum* Lam.) mobilised Zn in the rhizosphere through complexation by organic exudates, associated with accelerated mineral ($ZnCO_3$) weathering.

As many polluted sites are characterised by nutrient deficiency (Tsao 2014), we hypothesise that such interactions of P nutrition and metal solubility require attention in the management and remediation of metal-polluted soils. Modifications of solubility and phytoavailability may support or counteract remedial actions such as phytoextraction or immobilisation techniques (Wenzel et al. 1999, Wenzel 2009). Apart from avoidance of unwanted effects, improved knowledge on metal complexation by rhizodeposition in response to limited P availability could provide angles for targeted management of P availability. Similarly, this could be of interest in micronutrient-deficient systems. Apart from soil management, the amount and quality of rhizodeposition is known to depend on plant properties and could therefore be controlled to some extent by the selection of crops and cultivars (Römheld and Marschner 1990).

The objective of this study was to investigate the influence of rhizodeposition triggered by P depletion in the rhizosphere of canola (*Brassica napus* L.) and corn (*Zea mays* L.) cultivars grown on a P-deficient, calcareous soil, on Cu and Zn solubility and speciation. We hypothesised that (1) the cultivars vary considerably in the amount of rhizodeposition; (2) rhizodeposition triggered by P depletion affects metal speciation and solubility; (3) Cu is more strongly complexed and solubilised than Zn; (4) the resulting magnitude of differences in metal solubility would be relevant for the management of polluted and micronutrient-deficient soils.

MATERIAL AND METHODS

Soil physicochemical properties. An agricultural calcareous topsoil (0–20 cm) which is classified as Haplic Calcisols (Loamic, Hypocalcic) in the WRB classification system was collected from a farmland area in the vicinity of a Zn-industrial town in the Iranian province of Zanjan (36°36'N, 48°26'E), air-dried and passed through a 2-mm sieve. Soil texture (Bouyoucos 1962), cation exchange capacity (CEC) (Blum et al. 1996), maximum water holding capacity (MWHC) or water saturation percentage (SP), pH in a saturated paste extract (USDA 1954), organic matter carbon (OC) (Walkley and Black 1934), calcium carbonate equivalent (Blum et al. 1996), potentially phytoavailable P based on the Olsen method (Kuo 1996), and K using 1 mol/L ammonium acetate (Helmke and Spark 1996) were determined (Table 1).

Soluble Cu and Zn were determined by single extraction using 0.005 mol/L $Ca(NO_3)_2$ (1:5 *w/v*; Muhammad et al. 2012). Potentially plant-available metals were measured using 0.005 mol/L DTPA (1:5 *w/v*; Norvell 1984). The near-total (*aqua regia* digest) Cu and Zn concentration in the experimental soil was determined according to Chen and Ma (2001) (Table 1).

Greenhouse experiment. A greenhouse experiment was conducted using three corn (*Zea mays* L.) cultivars (504, 301, and 704) and four canola (*Brassica napus* L.) cultivars (Zarfam, Okapy, Hyola401, and RGS003), which were obtained from the Seed and Plant Improvement Institute, Iran. The corn and canola cultivars were selected based on their differential potentials of biomass production and related P depletion (Rahjoo et al. 2018, Shiranirad et al. 2020).

Seeds were sterilised before cultivation by soaking them into 96% ethanol and 3% sodium hypochlorite for 1 min and 3 min, respectively, and then by four washes with sterile deionised water. Nitrogen, K, and P were applied to the experimental soil at a rate of 14, 11.5 and 9.12 mg/kg; respectively, in the forms

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Table 1. Physical and chemical properties of experimental soil. Values are mean ± standard error (*n* = 4)

Texture	pH	CEC	CCE	OC	MWH	K	P	Fe	Cu	Zn	Mn	Near-total Zn	Near-total Cu
		(cmol$_+$/kg)	(g/kg)			(mg/kg)							
Sandy loam	7.83 ± 0.03	13 ± 0.4	137 ± 2.6	5.8 ± 0.21	400 ± 12.8	243 ± 3.2	8.6 ± 0.3	2.3 ± 0.15	25.5 ± 1.3	28.1 ± 2.1	6.9 ± 0.2	174 ± 6.3	90.4 ± 2.0

Soil texture: Sandy loam containing 650 g/kg sand and 140 g/kg clay. CEC – cation exchange capacity; CCE – calcium carbonate equivalent; OC – organic carbon; MWH – maximum water holding capacity; K was determined using 1 mol/L ammonium acetate; P was measured using 0.5 mol/L $NaHCO_3$ at pH 8.5; Fe, Cu, Zn, and Mn were extracted by DTPA, pH (in saturation water extract)

of NH_4NO_3 and KH_2PO_4, as in a preliminary experiment, we observed severe P deficiency symptoms in both plant species. Fertilisation increased available P (Olsen) in unplanted bulk soil from 8.6 to 11.3 mg P/kg soil, which was still below the critical value of 13–15 mg P/kg reported for different crop plants in Iran (Khodshenas et al. 2019). Thus, we could conduct the experiment under mild P deficiency that allowed us at the same time to potentially achieve a larger range of soluble P concentration in samples from rhizosphere and bulk soil solution. Iron was added at a rate of 9 mg Fe/kg soil in the form of Sequestrene 138. Subsequent to fertilisation, the experimental soil was homogenised and incubated for 3 weeks at 40 °C with several wetting and drying. The plastic pots were prepared by adding 1 800 g air-dried soil for each treatment in four replications. In each pot, 6 corn or canola seeds were planted and reduced to 3 seedlings after 7 days of growth. Pots were kept in a greenhouse under controlled conditions at 20–23 °C and 23–26 °C for canola and corn, respectively, and in 380 and 400 μmol photons/m^2/s for a period of 14 h and 16 h for canola and corn, respectively. Soil water content was kept at 80–85% of maximum water holding capacity throughout the experiment. Four pots were kept without plants to represent the bulk soil. The pot experiment was conducted in a randomised complete design.

Soil and plant analysis. Plant shoots were harvested after 45 days, washed with tap water and then with deionised water, dried at 70 °C for 48 h in the oven; fresh and dry matter yields were determined gravimetrically. To collect rhizosphere soil, roots were removed and shaken gently, thus separating the soil adhered to plant roots, which was considered as the rhizosphere soil. The latter was gently sieved to separate small roots; the smaller remaining roots were separated by tweezers. All roots collected from the soil were rinsed with tap water, then washed using 0.05 mol/L $CaCl_2$ in an ultrasonic bath for 10 min to minimise root-attached soil particles and remove Cu^{2+}, Zn^{2+} and phosphate from apoplast. Subsequently, the roots were thoroughly rinsed 3 times with deionised water, then dried at 70 °C, and weighed.

Macro and micronutrients concentrations in roots and shoots were determined after wet digestion at 225 °C using a mix of HNO_3 and $HClO_4$ (4:1 *v/v*) (Muhammad et al. 2012) by AAS using a Shimadzu AA-670 with graphite furnace. Nutrient concentrations in different soil extracts were determined by ICP-MS (Elan DRCe 9000, Waltham, USA).

Table 2. Phosphorus (P) concentration and content in canola and corn cultivars' shoot and root

Cultivar	P concentration (mg/kg)	Dry weight (g)	P content (mg/pot)
Shoot			
Canola Okapy	2 040 ± 20^{b}	5.70 ± 0.49^{b}	11.6 ± 0.96^{b}
Zarfam	2 160 ± 50^{ab}	5.07 ± 0.22^{b}	10.9 ± 0.40^{b}
Hyola401	2 200 ± 50^{a}	7.74 ± 0.44^{a}	17.0 ± 1.03^{a}
RGS003	2 220 ± 40^{a}	7.68 ± 0.38^{a}	17.0 ± 0.61^{a}
Corn 504	2 420 ± 90^{a}	12.2 ± 0.44^{a}	29.5 ± 1.79^{a}
301	2 370 ± 30^{a}	13.4 ± 0.51^{a}	31.9 ± 1.75^{a}
704	2 130 ± 70^{b}	13.0 ± 0.77^{a}	27.7 ± 1.22^{a}
Root			
Canola Okapy	1 820 ± 40^{a}	2.82 ± 0.09^{b}	5.20 ± 0.25^{b}
Zarfam	1 920 ± 40^{a}	1.78 ± 0.07^{d}	3.43 ± 0.16^{c}
Hyola401	1 990 ± 70^{a}	2.49 ± 0.10^{c}	4.93 ± 0.18^{b}
RGS003	1 860 ± 60^{a}	4.10 ± 0.04^{a}	7.6 ± 0.28^{a}
Corn 504	2 090 ± 80^{a}	4.24 ± 0.19^{c}	8.9 ± 0.62^{b}
301	1 930 ± 50^{b}	7.70 ± 0.14^{b}	14.9 ± 0.47^{a}
704	1 820 ± 50^{c}	8.44 ± 0.32^{a}	15.38 ± 0.68^{a}

Values are mean ± standard error (*n* = 4). For each type of plant, within columns, values followed by the same letter are not significantly different (Duncan test, $P < 0.05$)

Soluble Cu and Zn in bulk and rhizosphere soil solution were determined using 0.005 mol/L $Ca(NO_3)_2$ (1:5 *w/v*) (Muhammad et al. 2012). Additionally, pH, DOC (Brandstetter et al. 1996), main anion and cation concentrations in the rhizosphere and bulk soil were determined in the same extract. All chemicals used in the experiments were standard laboratory grade.

Thermodynamic modelling and statistics. Speciation of total 0.005 mol/L $Ca(NO_3)_2$-extractable Cu, Zn and P as a surrogate of the soil solution was conducted using Visual MINTEQ (Gustafsson 2014) thermodynamic model in the rhizosphere and bulk soil solution to predict the activity of free Cu^{2+}, Zn^{2+}, and phosphate ($H_2PO_4^-$ and HPO_4^{2-}), metal complexed by dissolved organic matter (DOM), and the proportion of free Cu^{2+} and Zn^{2+} to total dissolved Cu and Zn. We used the constant for single DOM binding affinity (Gaussian DOM), which results in relatively lower mole fractions of complexed Cu and Zn compared to the NICA-Donnan model that also considers fulvic acids. Therefore, the presented mole fractions should not be interpreted quantitatively but are used to identify relative differences between bulk and rhizosphere soils. We also performed runs with the NICA-Donnan model, showing that the differences between bulk and rhizosphere soil are equally depicted by both models.

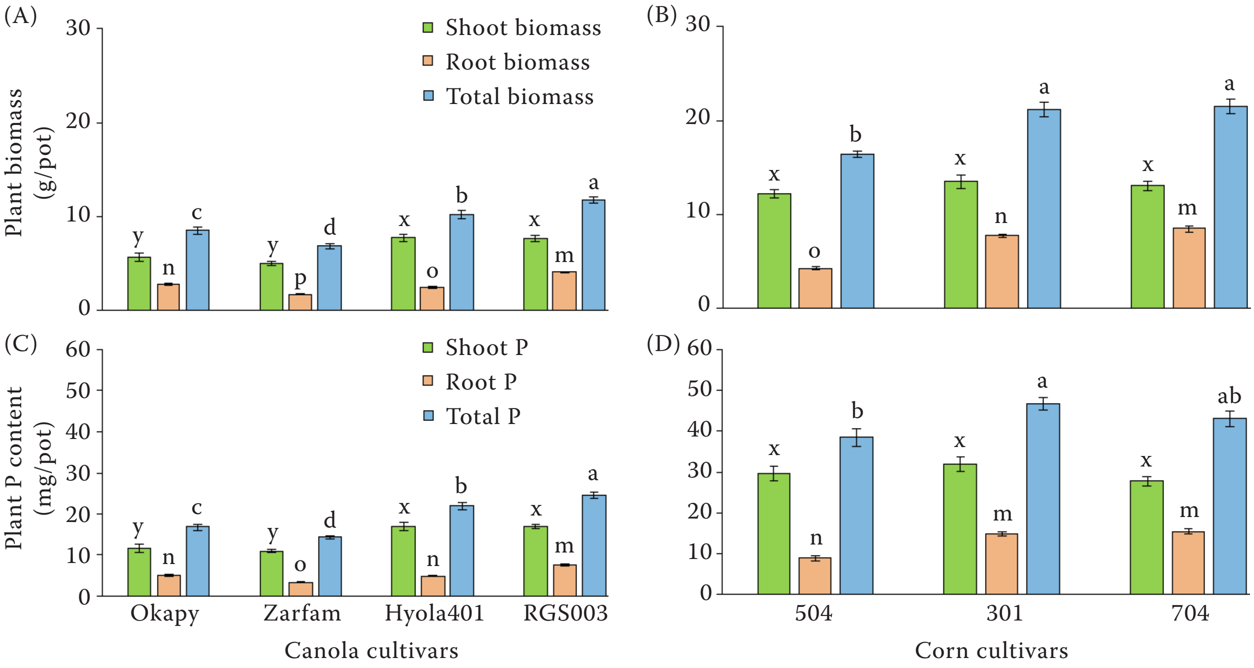

Figure 1. Biomass and phosphorus (P) uptake in different canola and corn cultivars. Shoot, root, and total biomass in different cultivars of (A) canola and (B) corn. P uptake in different cultivars of (C) canola and (D) corn. Values are mean ± standard error (*n* = 4), and bars sharing the same letter are not significantly different according to the Duncan test ($P < 0.05$)

Table 3. Total cation, anion, dissolved organic carbon (DOC) concentrations and pH in the rhizosphere and bulk soil solution extracted by 0.005 mol/L $Ca(NO_3)_2$. Values are mean ± standard error (*n* = 4)

Cultivar	Na	K	Ca	Mg	P	DOC	*I*	Cu	Zn	pH
	(mg/L)						(mol/L)	(µg/L)		
Canola Okapy	9.72 ± 0.37**	3.56 ± 0.18**	220 ± 0.29**	10.8 ± 0.38	0.041 ± 0.004**	9.22 ± 0.27**	0.017 ± 0.00**	245 ± 7.1**	8.75 ± 3.0**	7.61 ± 0.01
Zarfam	9.69 ± 0.38**	3.66 ± 0.06**	218 ± 0.73**	11.5 ± 0.46	0.050 ± 0.004**	8.00 ± 0.19**	0.017 ± 0.00**	242 ± 6.1**	9.5 ± 2.1**	7.65 ± 0.02
Hyola401	9.31 ± 0.19**	3.78 ± 0.14**	205 ± 0.24**	10.2 ± 0.37*	0.001 ± 0.000**	10.6 ± 0.36**	0.016 ± 0.00**	353 ± 12.1**	8.05 ± 1.8**	7.64 ± 0.02
RGS003	10.7 ± 0.5**	3.29 ± 0.21**	213 ± 0.60**	10.2 ± 0.23**	0.014 ± 0.003**	11.6 ± 0.48**	0.016 ± 0.00**	326 ± 12.1**	7.9 ± 2.9*	7.64 ± 0.01
Corn 504	4.83 ± 0.19	3.31 ± 0.15**	264 ± 2.32*	12.6 ± 0.58	0.083 ± 0.005**	5.80 ± 0.28	0.019 ± 0.00*	144 ± 8.1**	9.1 ± 2.0**	7.65 ± 0.02
301	5.19 ± 0.14	3.42 ± 0.19**	248 ± 2.8	11.6 ± 0.30	0.045 ± 0.003**	7.01 ± 0.44**	0.018 ± 0.00	182 ± 6.9**	10.9 ± 2.4**	7.56 ± 0.03
704	5.00 ± 0.25	3.57 ± 0.05**	243 ± 1.93*	12.2 ± 0.37	0.054 ± 0.003**	6.90 ± 0.19**	0.018 ± 0.00*	187 ± 6 .9**	8.25 ± 1.6**	7.54 ± 0.04
Bulk	4.15 ± 0.46	9.32 ± 0.46	253 ± 2.53	11.9 ± 0.40	0.125 ± 0.006	4.98 ± 0.31	0.019 ± 0.00	94.4 ± 20.1	6.10 ± 2.0	7.55 ± 0.03

*$P < 0.05$; **$P < 0.01$ probability level compared to bulk soil. *I* – ionic strength was predicted by the Visual MINTEQ thermodynamic model. Cation and anion concentration imbalances were less than 9% and 12% in different canola and corn rhizosphere soil solutions, respectively

Data analyses were performed with ANOVA ($P < 0.05$) in SAS software 9.2 (Institute Inc., Cary, USA), followed by *LSD* (least significant difference) and Duncan-test. Simple linear regression analysis was also conducted in SAS. The differences in metal concentrations between bulk and rhizosphere soil were evaluated using a paired *t*-test ($P < 0.05$). Principal component analysis (PCA) was used to explore the interrelationship among 12 variables (such as total soluble P, Cu and Zn; DOC concentration, free metal and metal-ligand activities in the rhizosphere; plant biomass; plant P concentration) and 7 plant cultivars. Using the software (R ver. 3.6.1, Vienna, Austria), the principal components were calculated and two-dimensional biplots generated.

RESULTS

As shown in Table 2 and Figure 1A, B, total biomass in corn was on average ~2.1 times greater than that of in canola. Shoot and root biomass in corn were on average ~1.97 and ~2.48 times larger than in canola cultivars, respectively. We also found significant differences (Duncan test, $P < 5\%$) in total biomass produced among cultivars of canola and corn by factors of ~1.72 and ~1.31, respectively.

Shoot and root P concentrations ranged between ~1.8–2.3 g/kg in both plant species and all cultivars (Table 2). There were significant differences in plant P content between plant species and cultivars (Duncan test, $P < 5\%$) (Table 2; Figure 1C, D). As shown in Figure 1C, D, total P content in corn was on average 2.2 times larger than that of in canola and varied among the cultivars of canola and corn species by factors of ~1.71 and ~1.22, respectively. While RGS003 had the highest plant P content (~24.6 mg/pot) among the canola cultivars, Zarfam had the lowest plant P content (~14.4 mg/pot). Among corn cultivars, corn 301 and corn 704 had the highest plant P content (~43.1–46.7 mg/pot).

$Ca(NO_3)_2$-extractable P (total soluble P concentration) in the rhizosphere of the cultivars after growth ranged between ~0.001–0.083 mg/L (Table 3). Compared to the unplanted bulk soil (0.125 ± 0.006 mg/L), this indicates considerable P depletion by plant roots, with variation by a factor of 83 among the cultivars.

We found no significant pH difference between bulk soil and the rhizosphere of the corn and canola cultivars (Table 3). The concentration of DOC significantly (paired *t*-test, $P < 0.05$) increased by

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a factor of ~2–3 in the rhizosphere of canola cultivars relative to bulk soils, while only two of the corn cultivars showed significant but smaller increases of DOC in their rhizosphere (Table 3).

As shown in Table 1, the near-total Cu and Zn in the experimental soil was 90.4 and 174 mg/kg, respectively, indicating slight to moderate pollution (MEF 2007). The DTPA-extractable Zn and Cu concentrations were 28.1 and 25.5 mg/kg bulk soil, respectively (Table 1). Total soluble Cu ($Ca(NO_3)_2$-extractable) significantly increased on average in the rhizosphere soil solution compared to unplanted bulk soil by factors of ~3.08 and ~1.81 in canola and corn, respectively (paired *t*-test $P < 0.05$). Total soluble Cu in the rhizosphere varied among the canola and corn cultivars by factors of ~1.46 and ~1.30, respectively. Total soluble Zn significantly increased in canola and corn rhizosphere compared to bulk soil solution on average by factors of ~1.41 and ~1.55, respectively (paired *t*-test $P < 0.05$.), and varied among the cultivars of canola and corn by factors of ~1.2 and ~1.32, respectively (Table 3).

To explore relations between the variables, in particular between P, DOC and metals in the rhizosphere, we conducted a PCA. Some of the main results are shown in the biplot in Figure 2. Principal component 1 explains 63.1% of the variation and clearly separates the two crops, i.e., corn and canola. According to the eigenvector loadings, PC 1 is closely associated with total soluble P (TSP), DOC, total soluble Cu (TSCu), free Cu activity (FCu), Cu and Zn complexed by DOM (CuDOM, ZnDOM), and to a lesser extent to free and total soluble Zn (FZn, TSZn). Component 2 explains 13.6% of the variation and tends to separate the cultivars of each crop according to their root biomass production and root P concentration. Given that principal components could explain more than 75% of variances, the positive or negative relations between variables can reliably be depicted (Figure 2).

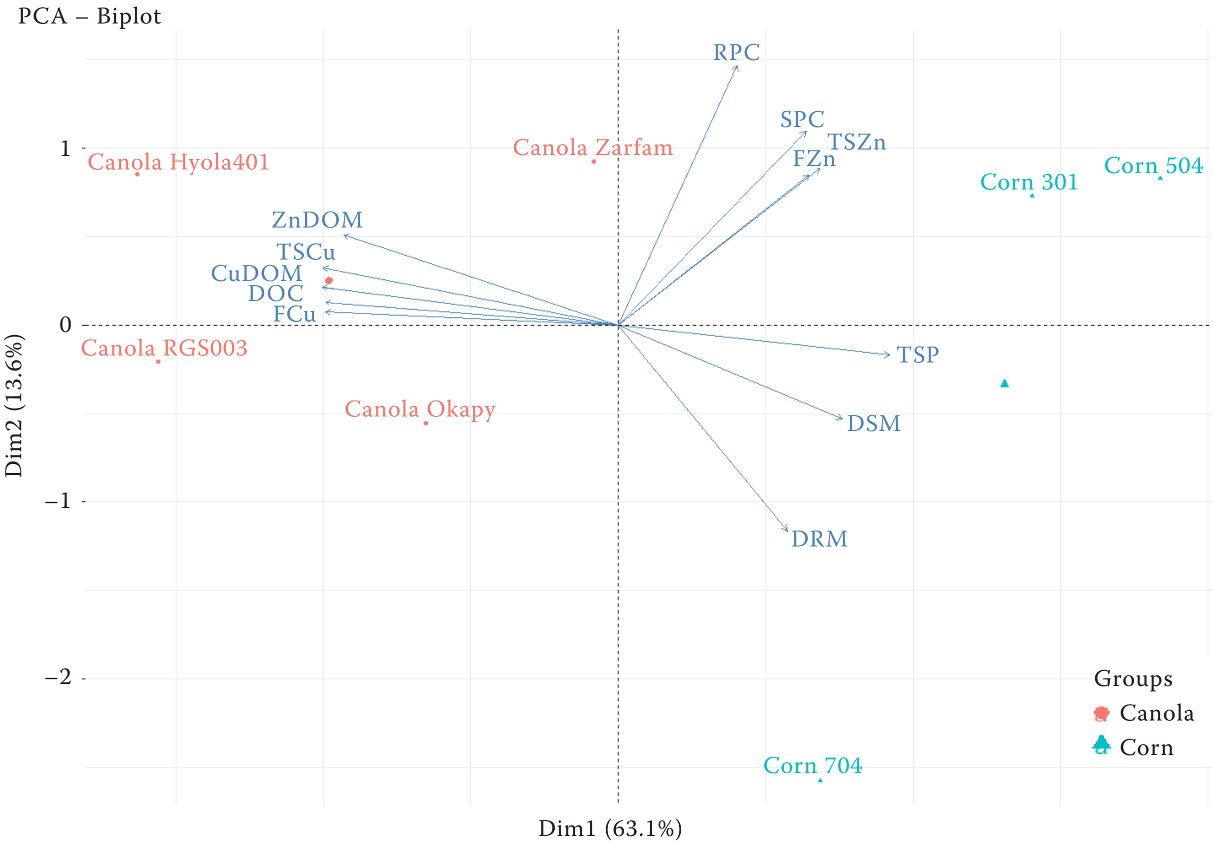

Figure 2. Biplot of the first two principal components of canola and corn cultivars' rhizosphere soil (7 observations) according to their variables: SPC – shoot phosphorus (P) concentration; RPC – root P concentration; TSP – total soluble P; TSCu and TSZn – total soluble Cu and Zn; FCu and FZn – activity of free Cu and Zn; CuDOM and ZnDOM – activity of Cu and Zn complexed by dissolved organic matter (DOM) in the rhizosphere soil solution; DRM – root dry mass; DSM – shoot dry mass

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The PCA indicates the strong inverse correlation between TSP and DOC while DOC is positively related to CuDOM, ZnDOM, FCu and TSCu. Component 2 is associated with high positive loadings for root P concentration and is negatively related to the root dry mass. Overall, the PCA indicates a strong connection between total soluble P in the rhizosphere and DOC on the one hand and DOC and metal complexation by DOM on the other hand.

DISCUSSION

Based on the results of the PCA, we first discuss the relation between soluble P as an indicator of depletion and related changes in the rhizosphere, and secondly, explore the effects of DOC on metal solubility and speciation.

Phosphorus depletion and related changes in the rhizosphere. In the unplanted bulk soil, total dissolved P extracted with 0.005 mol/L $Ca(NO_3)_2$-solution was 0.125 ± 0.006 mg/L (Table 3; ~4 µmol/L), indicating low soluble P in the calcareous experimental soil (Hinsinger 2001, Marschner 2012).

As shown in Table 2, shoot and root P concentrations (~1.8–2.3 g/kg) in all plant cultivars were below the range of critical values (3–5 g/kg) for optimal plant growth (Marschner 2012). In addition, we observed slight P deficiency symptoms in some plants at harvest time.

While we found strong depletion in soluble P in the rhizosphere of all plant cultivars as compared to the bulk soil, the variation among the crops and cultivars was still large, as indicated by the factor of 83 between the smallest and largest soluble P concentration (Table 3). Soluble P remaining in the rhizosphere soil solution at the termination of the experiment was inversely related to P uptake for both corn and canola cultivars (Figure 3A). At the same rate of removal, P concentrations in the canola rhizosphere were decreased to lower levels than that of corn.

To cope with exogenous stresses, including shortage of P, plants have evolved various mechanisms such as exudation of protons/hydroxyl ions and organic compounds that influence the chemical conditions (pH, concentration of organic ligands) at the root-soil interface and can mobilise of soil P from unavailable sources in the solid phase (Jones and Darrah 1994, Hinsinger 2001).

While we found no relevant differences of pH between bulk and rhizosphere soil (Table 3), the concentration of DOC in the rhizosphere of all cultivars except corn 504 was significantly larger than that of in bulk soil (paired *t*-test $P < 0.05$) (Table 3), indicating rhizodeposition in response to P depletion (Jones et al. 2009).

As it was depicted by PCA (Figure 2), we found strong inverse linear relations between total soluble P and DOC for all crops and cultivars, as well as for each of the two individual crops (Figure 3B).

These observations indicate that DOC concentration in the rhizosphere and bulk soil solution was generally

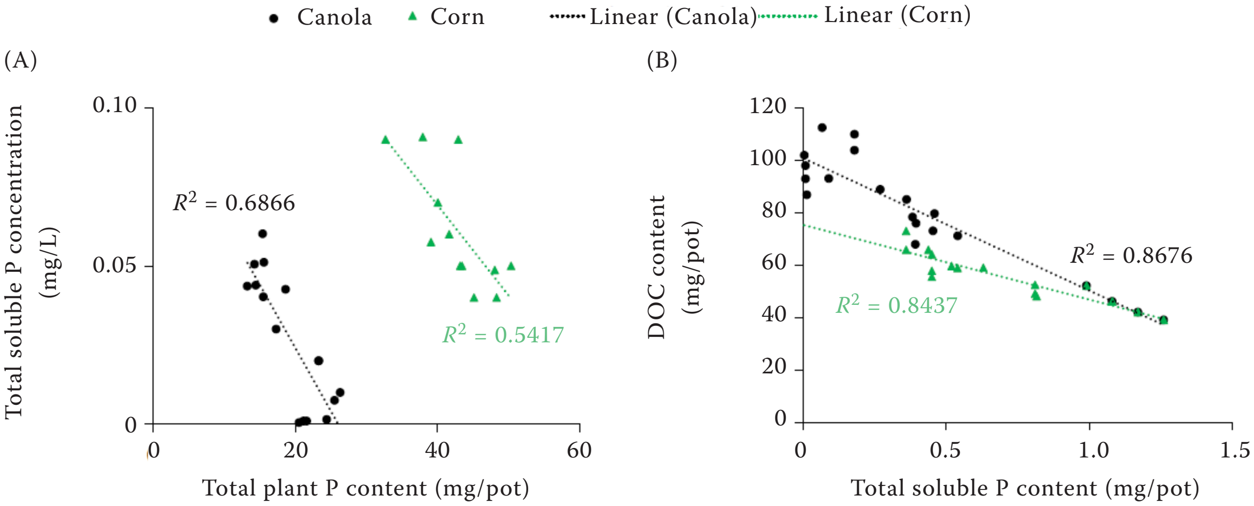

Figure 3. The relationship between total plant phosphorus (P) content (canola and corn cultivars) and total soluble P concentration in rhizosphere soil solution (A), and the relationship between total soluble P and dissolved organic carbon (DOC) concentration (B)

strong and inversely correlated with total soluble P in canola (R^2 = 0.868) and corn (R^2 = 0.844) cultivars, indicating the exudation of organic compounds into the rhizosphere (i.e., rhizodeposition) in response to P depletion. We are aware that the enhanced rhizosphere DOC in response to P depletion might have partly been due to the mobilisation and destabilisation of soil organic matter (SOM) through ligand exchange and co-dissolution of oxides (Dessureault-Rompré et al. 2008). However, even if we do not know the exact source of DOM, we observe root-induced changes enhancing DOM in the rhizosphere and related metal (Cu) mobilisation and speciation.

Interestingly, corn cultivars showed larger P removal and uptake ability than those of canola, in spite of lower amounts of rhizodeposition and remaining larger concentrations of soluble P in the rhizosphere (Figure 3A, B). The differential nature and quality of organic compounds and ligands in corn and canola rhizosphere soil might explain why DOM in corn rhizosphere could be much more efficient than that of canola to mobilise and resupply P from the soil-solid phase through ligand exchange (Hinsinger 2001). Another explanation of larger P concentration in corn rhizosphere might be greater P efflux by corn (Elliot et al. 1984). Phosphorus efflux is not only dependent on plant P nutritional status but also severely affected by soil solution P concentration (Marschner 2012). Elliott et al. (1984) demonstrated that the P efflux: influx ratio was 0.68 at 0.2 µmol/L P concentration in the external solution; however, this ratio followed a sharp reduction to 0.08 when P concentration increased to 2 µmol/L.

Changes of Cu and Zn solubility and speciation in the rhizosphere. There is ample evidence in the literature demonstrating that root exudates in P (and some micronutrients) deficiency can enhance the weathering of solid phases and the dissolution of associated metals and metalloids (Hinsinger and Courchesne 2008, Wenzel et al. 2011). The main soil solution factors controlling metal solubility are generally pH and DOC (Sauvé et al. 2000), which may both be influenced by plant roots in low P conditions. As the PCA indicated a strong positive relation between DOC, metal complexation and Cu solubility, we further explored our data using metal speciation modelling and correlation analysis to explain the enhanced total soluble Cu and Zn concentrations in the canola and corn rhizospheres.

As shown in Figure 4A, we found a strong positive correlation between DOC concentration and $Ca(NO_3)_2$-extractable Cu in bulk and rhizosphere soil solution of different canola and corn cultivars. Variations in DOC concentrations explain 86% of changes in total dissolved Cu in bulk and rhizosphere solution of canola and corn cultivars suggesting the formation of Cu-organic complexes and related Cu solubilisation. Such relations between DOC and total Cu concentration in soil solutions have been reported both in the presence and absence of plants (Zhao et al. 2007, Bravin et al. 2012).

These observations show that rhizodeposition and possibly mobilisation of SOM triggered by root activities in response to P depletion is the key driver of dynamic Cu solubility and speciation. Solution speciation on the data of the 0.005 mol/L $Ca(NO_3)_2$ extraction using the

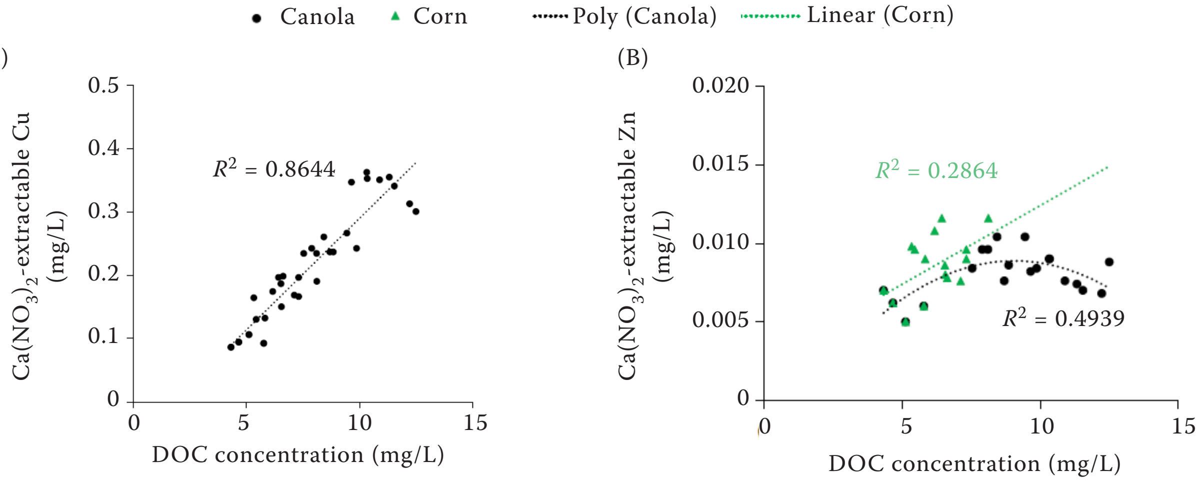

Figure 4. Relation between dissolved organic carbon (DOC) concentration and total soluble Cu (A) and Zn (B) in bulk and rhizosphere soil solution of canola and corn cultivars

Gaussian DOM model (single DOM binding affinity) in Visual MINTEQ indeed showed that ~16–33% of total Cu was complexed by DOM in bulk soil and rhizosphere of the different plant cultivars. Due to the inherent limitations of the Gaussian DOM model, the DOM-complexed proportion of Cu was likely underestimated. This interpretation is supported by reports of considerably larger Cu-DOM mole fractions if fulvic acids with high affinity towards Cu are considered as part of measured DOC in the rhizosphere (Dessureault-Rompré et al. 2008, Bravin et al. 2012). The lower extent of Cu complexation by DOM obtained in our study is also related to the considerably larger concentration of soluble Ca (5–6 mmol; Table 3) as compared to other studies. Using, e.g., 1.5 mmol as reported by Dessureault-Rompré et al. (2008) in our Gaussian DOM model calculations increases the Cu-DOM mole fraction up to 50% (Hyola rhizosphere), which can be explained by less competition of Ca for DOM complexation sites.

The Gaussian DOM model predicted that only 5% of the total activity of Zn in solution was complexed by DOM. It is well established that Cu forms stronger complexes with organic ligands than Zn in soil solution (Hinsinger and Courchesne 2008, Wenzel et al. 2011). In accordance with lower Zn-organic ligand stabilities, the PCA (Figure 2) and single regression/correlation analysis (Figure 4B) indicate that total soluble Zn was controlled by the free Zn activities in solutions rather than the formation of Zn-DOM complexes. Zhao et al. (2007) demonstrated that Zn mobilisation was primarily determined by Ca and, to a lesser extent, by DOC because of weaker complexes with organic ligands in the liquid phase. Compared to Cu, the Zn mobilisation in the rhizosphere was weak. Indeed, our data do not support Zhao's finding regarding Ca as the driver of Zn mobilisation since Ca concentrations between bulk and rhizosphere soils vary only marginally (Table 3).

Overall, our results point to an important role of root activities in the acquisition of P and related changes in Cu and Zn solubility in the rhizospheres of canola and corn cultivars in P-deficient soils.

These findings suggest that the P (and some micronutrient) nutritional status of the plant can be an important control of Cu solubility/mobility in soil, having an influence on both metal supply and mobility/toxicity. Therefore, targeted P availability control should be considered in the management of polluted and micronutrient-deficient soils. Apart from soil management, metal solubility and uptake could be controlled to some extent by the selection of crops and cultivars, as the amount and quality of rhizodeposition is known to rely on plant properties.

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