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REGULAR ARTICLE

Mineral element composition of cabbage as affected by soil type and phosphorus and zinc fertilisation

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Abstract

Background and aims The effects of phosphorus and zinc applications on phosphorus and zinc concentrations in plants grown in different soil types have rarely been investigated. The aim of this study was to evaluate the effects of different soil types and phosphorus and zinc addition on growth and mineral element composition of red cabbage (*Brassica oleracea var. capitata* L. cv. Red Drumhead).

Methods Plants were grown for six weeks in three different soils (a freely drained Cambisol, an imperfectly drained Cambisol, and a Stagnosol) in a glasshouse. Each soil was amended with one of 25 combinations of phosphorus and zinc fertiliser. Soil characteristics, growth, and mineral element concentrations in shoots were assessed.

Results Soil type significantly affected shoot growth and concentrations of phosphorus, zinc, potassium, calcium, magnesium and manganese, but not iron concentration of red cabbage. Across soils, the observed responses were attributed to soil phosphorus, potassium, calcium, magnesium, and sulphur concentrations, organic matter content, and mineral composition, mainly kaolinite and plagioclase.

Conclusions Soil type effects on mineral element composition of red cabbage could have important implications for increasing mineral element concentration in crops to alleviate mineral element deficiencies in human diets.

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Introduction

Mineral element composition of plants depends on phylogenetic inheritance and the environment (Watanabe et al. 2007; White et al. 2012a). Soil biological and physico-chemical properties shape conditions for yield, plant health, and nutrient quality of crops (White et al. 2012b). To achieve optimal crop yields and ensure the mineral-compositional quality of produce, soil management practices might need to be adapted for a particular soil type - plant combination (White et al. 2013). The most feasible soil adjustments are those for pH, through liming and acidifying fertilisers, water, through irrigation, and concentrations of scarce or poorly available elements through mineral fertilisers. However, unforeseen interactions between physico-chemical parameters in soils can lead to yield penalties and affect the quality of produce.

One such interaction occurs between the plant nutrients phosphorus (P) and zinc (Zn). Phosphorus (over)application has been reported to limit Zn concentrations in shoots and lead to Zn deficiency and yield penalties (e.g. Akhtar et al. 2010; Broadley et al. 2010; Haldar and Mandal 1981; Loneragan 1950; Safaya 1976; Ward et al. 1963; Zhang et al. 2012). This phenomenon has been referred to as P-induced Zn deficiency. It is still a matter of debate whether the interaction between P and Zn takes place in the soil or in the plant (Briat et al. 2015; Olsen et al. 1977; Zhu et al. 2002). In the soil, precipitation of plant-available forms of P (phosphate) and Zn (Zn^{2+}) as insoluble $Zn_3(PO_4)_2$ is a plausible occurrence and could reduce Zn uptake by roots (Olsen et al. 1977; Saetz and Jurinak 1957). Soil properties such as compaction, water content, clay content, and organic matter content have also been shown to restrict Zn uptake by plants (Alloway 2009; Broadley et al. 2007; Ward et al. 1963).

Precipitation of $Zn_3(PO_4)_2$ might also occur in root tissues, as proposed in studies in which Zn translocation from the roots to shoots was restricted as the P concentration in the substrate increased (Barben et al. 2007; Singh et al. 1988; Stuckenholtz et al. 1966) and as shoot Zn concentration was increased by foliar Zn application (Parker et al. 1992; Zhang et al. 2012). In shoots, several different interactions between P and Zn have been suggested. A dilution effect has been proposed when the P-induced growth exceeded the rate of Zn accumulation, resulting in smaller shoot Zn concentrations (Gianquinto et al. 2000; Li et al. 2004). Impairment of a feedback control mechanism from the shoots, which enhances uptake and translocation of P under Zn deficiency has also been suggested (Cakmak and Marschner 1986) and might also act by suppressing Zn accumulation at greater tissue P concentration as well. A direct interaction between P and Zn via the PHR1 transcription factor, a major transcriptional regulator under phosphate deficiency, has also been reported, although the proposed mechanism also involves interaction with plant iron (Fe) and sulphur (S) nutritional status (Briat et al. 2015). Arguably, different interactions can occur simultaneously, differ between plant species, or include other environmental factors. For example, P-induced Zn deficiency has not always been observed (Boawn et al. 1954; Lu et al. 1998; Orabi et al. 1982); was connected indirectly to arbuscular mycorrhizal symbiosis (Ova et al. 2015; Subramanian et al. 2008; Zhang et al. 2017); was attributed to multi-element interaction of P with other nutrients, such as nitrogen (Hamlin et al. 2003), Fe (Haldar and Mandal 1981) or manganese (Mn; Barben et al. 2007; Haldar and Mandal 1981; Pedas et al. 2011), or was linked to soil enzymatic activities affecting P and Zn availability (Omidi et al. 2008).

Soil properties are well-established factors affecting plant mineral nutrition. However, only a few studies have evaluated the effects of different soil types on P-Zn interactions. One such study demonstrated that differences in the growth and P and Zn accumulation by tomato plants grown in alluvial or calcareous soils could be attributed to the capacity of the soils to fix P in non-available forms (Orabi et al. 1982). The effects of P amendments on plant Zn content depended on soil Zn concentration and on soil type (Orabi et al. 1982).

The aim of this study was to evaluate the effects of different soil types and P and Zn amendments on growth and mineral element composition of red cabbage (*Brassica oleracea var. capitata* cv. Red Drumhead). As a leafy vegetable, cabbage has the potential to contain greater Zn concentrations than cereal seeds, legume seeds, root crops or tuber crops (White and Broadley 2011). Thus, cabbage has greater biofortification (strategy to increase human dietary Zn intakes by increasing Zn concentrations in edible produce) potential. Since mineral element composition in leaves of different cabbage genotypes is not affected by their colour (Barker

et al. 2017), the conclusions of this experiment could be extrapolated to all cabbages. The hypotheses tested were: i) soil types affect growth and mineral element composition of shoots of red cabbage and ii) increasing P in the soil increases shoot biomass and P concentration but restricts shoot Zn concentration.

Materials and methods

Soil samples and characterisation

Three soil types, collected in Scotland, UK, were used. The first soil was a freely drained Brown earth (Cambisol; IUSS 2015) developed on raised beach sand and gravels derived from Old Red Sandstone sedimentary rocks and was collected at Mylnefield farm (Latitude: 56.4562, Longitude: 3.0691; 30 m above sea level) and is henceforth referred to as Mylnefield soil. The second soil was an imperfectly drained Brown earth (Cambisol; IUSS 2015) developed on glacial till derived from Old Red Sandstone sedimentary rocks (mainly sandstones) and lavas and was collected at Balruddery farm (Latitude: 56.4796, Longitude: 3.1386; 140 m above sea level) and is henceforth referred to as Balruddery soil. The third soil was an imperfectly to poorly drained Noncalcareous gley (Stagnosol; IUSS 2015) developed on glacial lodgement till and derived from Carboniferous sedimentary rocks and was collected at Hartwood farm (Latitude: 55.8164, Longitude: 3.8613; 210 m above sea level) and is henceforth referred to as Hartwood soil. The soils were air dried and sieved (>2 mm), and the following soil physico-chemical parameters were measured: pH (in water and $CaCl_2$; Sumner 1994), water content and organic matter by loss on ignition by gravimetry (Gardner 1965), particle size by laser diffraction following the dispersion of soil samples in sodium hexametaphosphate overnight and ulrasonication (Eshel et al. 2004) prior to determination of particle size distribution (Mastersizer 2000 with Hydro G dispersal unit, Malvern Instruments, Worcestershire, UK) on consecutive runs to ensure dispersion was complete, total concentrations of elements by inductively-coupled-plasma-mass spectrometry and inductively coupled-plasma optical emission spectrometry after microwave assisted wet digestion (McGrath and Loveland 1992), concentrations of sodium-bicar bonate-extractable P (Olsen-P; Olsen 1954; Irving and McLaughlin 1990), and diethylenetriaminepenta acetic-acid-extractable Zn (DTPA-Zn; Lindsay and Norvell 1978). In addition, quantitative bulk mineralogical composition of soils and semi-quantitative mineralogical composition of the clay fraction (<2 μm) was obtained by X-ray powder diffraction analysis as described previously (Hillier 1999, 2003; Omotoso et al. 2006).

Experiment and plant analyses

Soils were amended with 0.4 g NH_4NO_3 L^{-1} soil, 0.75 g KNO_3 L^{-1} soil, and one of 25 combinations of P and Zn fertiliser. Phosphorus was supplied as single superphosphate at 0 (P1), 2.25 (P2), 6.75 (P3), 11.25 (P4) or 15.75 (P5) mg P L^{-1} soil and Zn was supplied as $ZnSO_4$ at 0 (Zn1), 2 (Zn2), 5 (Zn3), 20 (Zn4) or 200 (Zn5) mg Zn L^{-1} soil. The largest P treatment was reported previously to improve growth of 376 genotypes of *Brassica oleracea* (Hammond et al. 2008; Broadley et al. 2010). In particular, an increase from 5 g to 9 g in shoot biomass and an increase from 0.2 to 0.5 g P kg^{-1} DM in shoots of red cabbage (cv. Red Drumhead) grown in 5.25 mg P L^{-1} (low P treatment) and in 15.75 mg P L^{-1} (high P treatment), respectively, occurred. By contrast, red cabbage plants grown in the low P treatment had greater Zn concentrations (130 mg Zn kg^{-1} DM) than plants in the high P treatment (83 mg Zn kg^{-1} DM; Hammond et al. 2008). The largest Zn treatment was equivalent to the soil Zn concentration previously reported to produce a 10% suppression of shoot biomass in red cabbage (White et al. 2018).

Five one-litre pots (13 cm diameter × 11 cm height) were filled with each soil × fertiliser combination and laid out on 5 benches in a glasshouse. On each bench, there were 3 blocks of 25 pots, one block for each of the 3 soil types. Within each block the 25 P × Zn fertiliser combination were arranged into a Graeco-Latin square. In addition, a single layer of pots, filled with commercial peat-based compost, surrounded each square to accommodate guard plants. The soil and compost were watered with tap water (containing 1.58 mg P L^{-1} and 0.42 μg Zn L^{-1}) to water-holding capacity and allowed to stabilise for 4 weeks, during which they were regularly watered to ensure the soil was wet.

Seeds of red cabbage were germinated on moistened filter paper in Petri dishes at 16 °C. Three 5-day-old seedlings were transplanted to each pot filled with soil or compost. After one week, plants were thinned to one plant per pot and were allowed to grow for 5 more weeks. Six weeks growth was selected to ensure that sufficient nutrients were present in the soil for plants

grown in one litre of soil. At harvest, shoots were removed, weighed (fresh weight, FW), and dried in an oven for five days at 70 °C. Dried shoots were weighed (dry matter, DM) and ground to powder. Phosphorus, Zn, potassium (K), calcium (Ca), magnesium (Mg), S, Mn and Fe concentrations in shoots were determined using ICP-MS after microwave-assisted wet digestion as described by White et al. (2012a).

Statistical analyses

Means and interactions among the soil, Zn and P treatments were compared by analysis of variance, respecting the bench, soil, and square strata of the experimental design. When soil effects were statistically significant, means and interactions among Zn and P treatments were compared in each soil type separately. Correlations were used to detect pairwise associations among the response variables and the major sources of variation across all the response variables were identified by principal components. All analyses were carried out in Genstat Release 17.1 (VSN International Ltd, Hemel Hempstead, UK).

Results

The three soils differed considerably in their physico-chemical properties. Mylnefield soil had the greatest pH, total concentrations of P, Zn, K, and Mn, Olsen-P, and percentage of particles smaller than 2 μm and in size range of 2–20 μm and 60–2000 μm (Table 1). Balruddery soil had the greatest total concentrations of Ca, Mg, and Fe (Table 1). Hartwood soil had the greatest water content, organic matter content, total concentration of S, DTPA-Zn, percentage of particle content of 20–60 μm, and the lowest soil density (Table 1). Of other essential mineral elements for plants, the greatest concentrations of copper and nickel were present in Mylnefield soil, of boron in Balruddery soil, and of molybdenum in Hartwood soil (Supplementary Table 1).

The mineral composition of Mylnefield and Balruddery soils did not differ substantially, although

Table 1 Physico-chemical properties of soils used to grow red cabbage (*Brassica oleracea* cv. Red Drumhead) for 6 weeks

Soil parameter		Mylnefield soil	Balruddery soil	Hartwood soil
pH (in H_20)		6.31	6.21	5.68
pH (in $CaCl_2$)		5.59	5.48	4.85
Density (g m^{-3})		1.12	1.11	0.87
Water (wt%)		3.48	5.50	6.86
Organic matter (wt%)		4.75	5.04	10.8
Total concentration (mg kg^{-1})	P	1475	1024	707
	Zn	92.4	67.1	82.2
	K	1326	1304	880
	Ca	3003	3149	1591
	Mg	5719	8527	2145
	S	245	313	456
	Mn	735	497	522
	Fe	23,360	30,890	23,480
Extractable concentration (mg kg^{-1})	Olsen-P	64 ± 4.5	20.9 ± 0.84	7.56 ± 0.14
	DTPA-Zn	1.319 ± 0.038	0.135 ± 0.002	1.45 ± 0.004
Particle size (wt%)	<2 μm	8.04	7.41	5.61
	2–20 μm	45.82	43.31	44.28
	20–60 μm	17.64	22.97	23.45
	60–2000 μm	28.51	26.31	26.67

P, phosphorus; Zn, zinc; K, potassium; Ca, calcium; Mg, magnesium; S, sulphur; Mn, manganese; Fe, iron; DTPA-Zn, diethylenetriaminepentaacetic-acid-extractable Zn; Olsen-P, sodium-bicarbonate-extractable P

Mylnefield soil had greater concentrations of quartz and K-feldspar than Balruddery soil, whereas Balruddery soil had a greater concentration of plagioclase than Mylnefield soil (Table 2). However, the mineral composition of Mylnefield and Balruddery soils differed considerably from that of Hartwood soil, which had notably greater concentrations of goethite and kaolinite, less plagioclase, amphibole and expandable clays, and no hematite and chlorite (Table 2). Additional differences were observed in the clay fraction. Hartwood soil had a larger relative proportion of kaolinite and smaller relative proportion of expandable (swelling) clays than Mylnefield and Balruddery soils (Table 2).

Plants grown in Mylnefield soil grew best and plants grown in Hartwood soil grew worst (Fig. 1 and Supplementary Fig. 1). Scatter plots of shoot fresh weight (FW), shoot dry matter (DM), and shoot P, Zn, K, Ca, Mg, S, Mn and Fe concentrations revealed mineral-element-specific and soil-specific relationships (Fig. 2a). For example, positive and negative correlations occurred between the responses analysed in different soils (Supplementary Fig. 2). In all three soils, statistically significant ($P < 0.001$) positive correlations (r values are listed in the following order: Mylnefield, Balruddery and Hartwood soil) were seen for K with P ($r = 0.35$, $r = 0.38$ and $r = 0.33$), for Ca with Zn (r = 0.38, $r = 0.53$ and $r = 0.52$), Ca with Mg ($r = 0.80$, $r = 0.85$ and $r = 0.78$), Ca with S ($r = 0.50$, $r = 0.60$ and $r = 0.58$), and Ca with Fe ($r = 0.81$, r = 0.58 and $r = 0.54$), for Fe with Mg ($r = 0.69$, r = 0.52 and $r = 0.39$) and Fe

Table 2 Bulk mineral composition and relative proportion of minerals in the clay fraction (<2 μm) in soils used to grow red cabbage for 6 weeks as revealed by X-ray powder diffraction

	Mylnefield soil	Balruddery soil	Hartwood soil
Bulk minerology (weight %)			
Quartz SiO_2	42.5	34.1	47.9
Plagioclase $NaAlSi_3O_8$ - $CaAlSi_3O_8$	13.6	21.9	1.7
K-feldspar $KAlSi_3O_8$	12.1	9.8	6.2
Amphibole SiO_4	2	2.7	0.3
Goethite FeO(OH)	1.5	1.6	3.6
Anatase TiO_2	0.2	0.2	0.4
Hematite α-Fe_2O_3	1	0.8	0
Maghemite γ-Fe_2O_3	0.7	0.9	0.1
Expandable Clays	6.8	6.9	3.7
Muscovite $KAl_2(AlSi_3O_{10})(F,OH)_2$	3	4.7	3.3
Chlorite $(Fe^{2+},Mg)_5Al(AlSi_3O_{10})(OH)_8$	3	4.9	0
Kaolinite $Al_2Si_2O_5(OH)_4$	3	2.5	22.9
Amorphous	10.7	9	10
Relative proportion of minerals in the clay fraction (%)			
Chlorite $(Fe^{2+},Mg)_5Al(AlSi_3O_{10})(OH)_8$	3	4	7
Kaolinite $Al_2Si_2O_5(OH)_4$	6	7	37
Illite $(K,H_3O)(Al,Mg,Fe)_2(Si,Al)_4O_{10}[(OH)_2,(H_2O)]$	4	3	3
Expandable clays	87	86	53

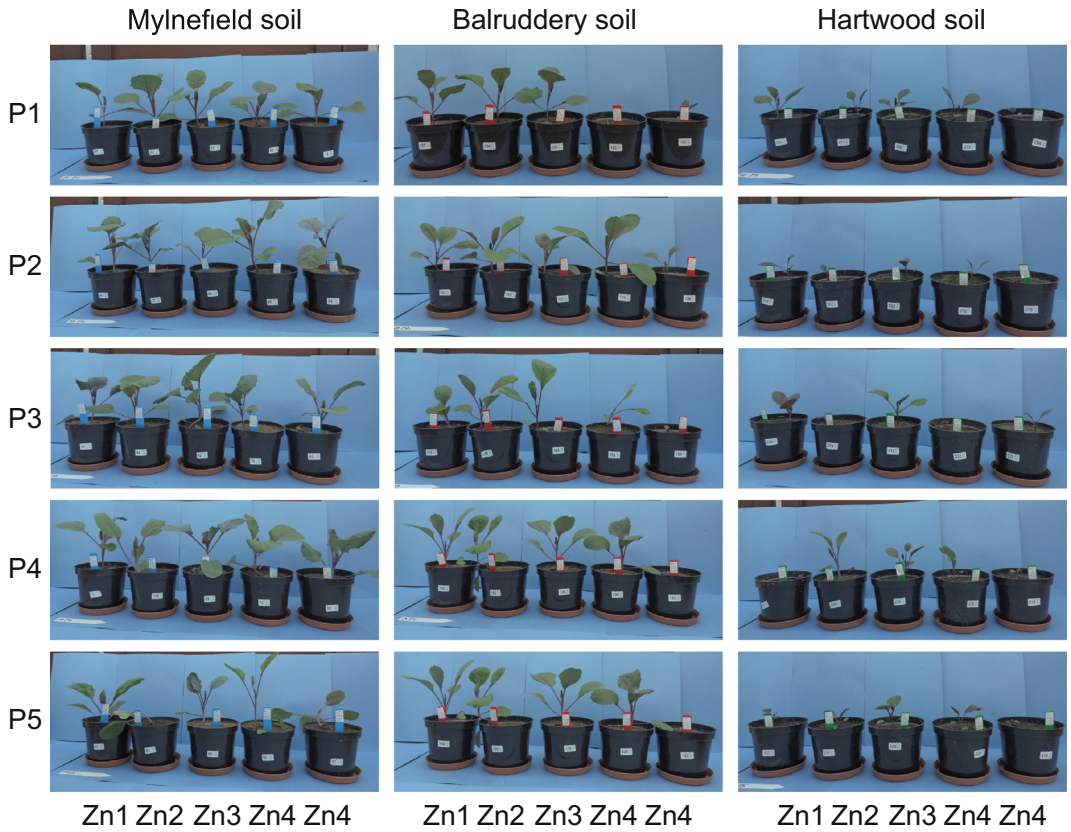

Fig. 1 Representative photographs of red cabbage (*Brassica oleracea var. capitata* cv. Red Drumhead) grown for 6 weeks in pots containing Mylnefield, Balruddery or Hartwood soil. Soils were amended with 25 combinations of phosphorus (P) and zinc (Zn) fertiliser. Phosphorus was supplied as single superphosphate at 0.00 (P1), 2.25 (P2), 6.75 (P3), 11.25 (P4) or 15.75 (P5) mg P L^{-1} soil and Zn was supplied as $ZnSO_4$ at 0 (Zn1), 2 (Zn2), 5 (Zn3), 20 (Zn4) or 200 (Zn5) mg Zn L^{-1} soil

with S ($r = 0.37$, $r = 0.51$ and $r = 0.40$), for Mg with S ($r = 0.48$, $r = 0.74$ and $r = 0.60$) and Mg with Zn ($r = 0.39$, $r = 0.75$ and $r = 0.39$), and for S with Zn ($r = 0.89$, $r = 0.94$ and $r = 0.65$). The only statistically significant negative correlation in all three soils occurred between Mn and DM ($r = -0.39$, $r = -0.33$ and $r = -0.37$ for Mylnefield, Balruddery and Hartwood soil, respectively). In Mylnefield soil the negative correlation between Mn and DM was the only negative correlation, all others were positive (Supplementary Fig. 2). The number of statistically significant correlations was greatest in plants grown in Mylnefield soil, whereas the largest number of statistically significant negative correlations occurred in plants grown in Hartwood soil (Supplementary Fig. 2).

Due to the strong positive correlation between FW and DM ($r = 0.95$), FW was removed from the principal component analysis. The first principal component explained 44% and the second principal component 21% of the variability in the data. The scores plot (Fig. 2b) demonstrates clearly a separation of the three soils studied, with Hartwood soil grouping away from Mylnefield and Balruddery soils. Similar strong soil effects occurred on all shoot traits measured, except for shoot Fe concentration, in a three-way ANOVA performed with soil and P and Zn treatments as independent variables (Table 3). Although significant treatment effects and their interactions were observed, by far the largest means of squares was associated with the soil component indicating that soil type had strongest effect on shoot biomass and mineral element composition. To simplify the interpretation of interactions, each soil type was analysed separately (Table 4). For plants grown in Mylnefield soil, shoot FW, DM and shoot concentration of Ca, Mg, Mn or Fe were not affected by P and Zn treatments or their interaction. Shoot P and S concentrations were affected by Zn and P treatments and their interaction; shoot K concentrations was affected by P treatments, and Zn treatments and P × Zn interaction affected shoot Zn concentration. For plants

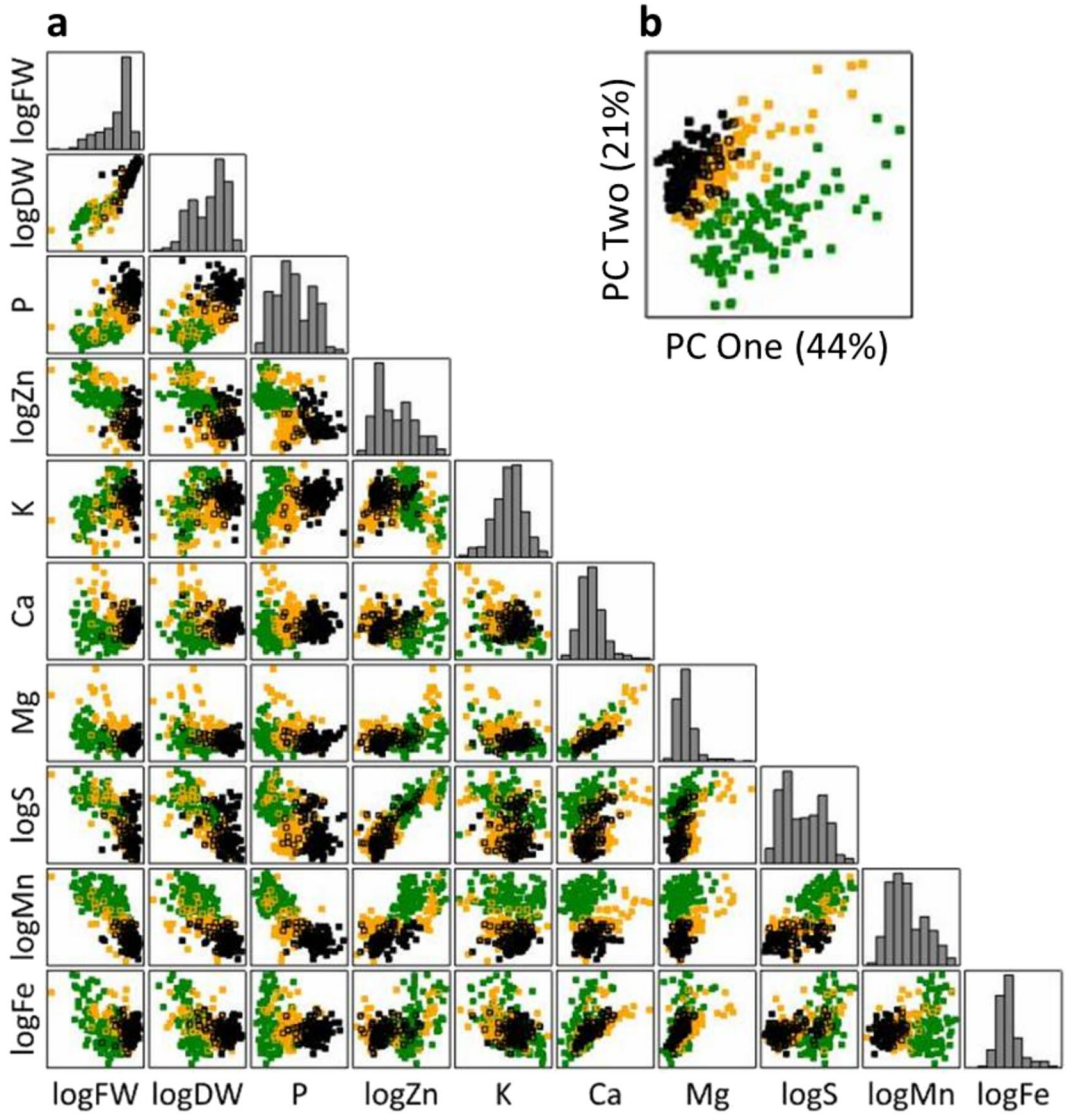

Fig. 2 **a** Scatter plots and histograms of raw data and **b** score plot of first two principal components (PC) for response variables grouped by soil (black, Mylnefield soil; yellow, Balruddery soil; green, Hartwood soil). Red cabbage (*Brassica oleracea* cv. Red Drumhead) was grown in different soils amended with 25 combinations of phosphorus (P) and zinc (Zn) fertiliser for 6 weeks. Details of P and Zn amendments can be found in the legend to Fig. 1. FW, shoot fresh weight; DM, shoot dry matter; P, shoot phosphorus concentration; Zn, shoot zinc concentration; K, shoot potassium concentration; Ca, shoot calcium concentration; Mg, shoot magnesium concentration; S, shoot sulphur concentration; Mn, shoot manganese concentration; Fe, shoot iron concentration; *, data were log10 transformed to achieve approximate normality

grown in Balruddery soil, only shoot P concentration was not affected by the treatments. Shoot DM and shoot concentrations of Zn, K, Ca, Mg, S, Mn or Fe were affected by Zn treatments, and shoot FW was affected by Zn treatment and P × Zn interaction. For plants grown in Hartwood soil, shoot DM and shoot concentrations of Zn and Mn were affected by P and Zn treatments and their interactions, whereas shoot P and Ca concentrations were affected by P treatments and P × Zn interactions. Shoot Mg and S concentrations were affected by Zn treatments and P × Zn interactions and shoot FW and shoot K concentration was affected by P and Zn treatments, whereas shoot Fe concentration was affected by Zn treatment only.

The largest shoot DM was achieved in plants grown in Mylnefield soil, followed by plants grown in Balruddery

Table 3 Three-way ANOVA table with *P* values (those less than 5% are highlighted in bold) and mean squares (in italics) for response variables in red cabbage (*Brassica oleracea* cv. Red Drumhead) grown in different soils amended with 25 combinations of phosphorus (P) and zinc (Zn) fertiliser for 6 weeks

		Soil	P	Zn	P × Zn	Soil×P	Soil×Zn	Soil×Zn × P
Shoot	logFW	**<.001**	**<.001**	**<.001**	**0.03**	**<.001**	**<.001**	0.12
		49.6	*0.90*	*5.98*	*0.19*	*1.54*	*2.18*	*0.14*
	logDM	**<.001**	0.10	**<.001**	0.26	**<.001**	**<.001**	0.16
		15.6	*0.09*	*1.46*	*0.06*	*0.22*	*0.78*	*0.06*
Shoot concentration	P	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**	0.79	**0.01**
		1.93E + 08	*2.36E + 06*	*1.45E + 06*	*1.03E + 06*	*1.58E + 06*	*1.58E + 05*	*4.64E + 05*
	logZn	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**
		29.2	*0.25*	*17.0*	*0.10*	*0.18*	*1.36*	*0.08*
	K	**0.00**	**0.00**	**<.001**	0.22	0.06	**<.001**	0.17
		2.67E + 09	*5.56E + 08*	*1.40E + 09*	*1.62E + 08*	*2.42E + 08*	*7.95E + 08*	*1.61E + 08*
	Ca	**0.01**	0.06	**<.001**	0.40	0.77	**<.001**	**0.03**
		3.03E + 09	*1.03E + 08*	*1.12E + 09*	*4.74E + 07*	*2.73E + 07*	*4.20E + 08*	*7.20E + 07*
	Mg	**0.00**	0.26	**<.001**	0.05	0.82	**<.001**	0.10
		1.16E + 08	*1.93E + 06*	*5.70E + 07*	*2.43E + 06*	*7.98E + 05*	*2.22E + 07*	*1.98E + 06*
	logS	**<.001**	**0.01**	**<.001**	**<.001**	**0.01**	**<.001**	**0.02**
		8.84	*0.094*	*3.18*	*0.076*	*0.062*	*0.29*	*0.042*
	logMn	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**	**0.04**
		24.3	*0.46*	*0.52*	*0.08*	*0.28*	*0.20*	*0.04*
	Fe	0.11	0.16	**<.001**	0.08	0.07	**0.00**	0.28
		0.143	*0.028*	*0.220*	*0.026*	*0.031*	*0.054*	*0.019*

Treatments (increasing soil P and Zn concentrations) and soil (Mylnefield, Balruddery and Hartwood) were used as independent variables. Details of P and Zn amendments can be found in the legend to Fig. 1; FW, fresh weight; DM, dry matter; K, potassium; Ca, calcium; Mg, magnesium; S, sulphur; Mn, manganese; Fe, iron; log, data were log10 transformed to achieve approximate normality

soil, then plants grown in Hartwood soil (Fig. 3 top row). A similar trend was observed for shoot P concentration, with the greatest P concentration in plants grown in Mylnefield soil and the lowest in plants grown in Hartwood soil (Fig. 3 middle row). Shoot P concentration in most of plants grown in Hartwood soil (exceptions are plants in the following combination of treatments: P4: 11.75 mg P L^{-1} added, and Zn1-Zn3: 0, 2 and 5 mg Zn L^{-1} added, respectively), were below the sufficiency range proposed for leaves of ornamental cabbage (2–6 g P kg^{-1}; Campbell 2013) suggesting that these plants were P deficient. Strong soil and Zn treatment effects were seen for shoot Zn concentration, with the largest shoot Zn concentrations being measured in Hartwood soil at the largest Zn treatment, Zn5 (200 mg Zn L^{-1}; Fig. 3 bottom row). A similar increase in shoot Zn concentration occurred as Zn application was increased to the Zn5 treatment in Balruddery soil, but shoot Zn concentrations were less than in plants grown in Hartwood soil. In plants grown in Hartwood soil, all shoot Zn concentrations were above the sufficiency range proposed for leaves of ornamental cabbage (20–75 mg Zn kg^{-1}; Campbell 2013). Likewise, shoot K, Ca, and Mg concentrations were above the sufficiency range proposed for leaves of ornamental cabbage (30–40 g K kg^{-1}, 5–10 g Ca kg^{-1}, and 2–4 mg Mg kg^{-1}, Campbell 2013; Fig. 4) in all three soils. There was a sharp increase in shoot Ca and Mg concentrations in plants grown in Balruddery soil in the 200 mg Zn L^{-1} treatment, when compared to the remaining Zn treatments, which was not as apparent in plants grown in Mylnefield or Hartwood soils. A similar increase was seen in shoot S, Mn and Fe concentrations in plants grown in Balruddery soil and in shoot S concentrations in plants grown in Mylnefield soil as Zn applications were increased (Fig. 5). In plants grown in Hartwood soil, either shoot S or Mn concentrations exceeded the upper bounds of the sufficiency range for these elements (2–10 g S kg^{-1} and 20–250 mg Mn kg^{-1}, Campbell

Table 4 Two-way ANOVA table with *P* values (those less than 5% are highlighted in bold) and mean squares (in italics) for response variables in red cabbage (*Brassica oleracea* cv. Red Drumhead) grown in different soils amended with 25 combinations of phosphorus (P) and zinc (Zn) fertiliser for 6 weeks

		Mylnefield soil				Balruddery soil				Hartwood soil		
		P	Zn	P × Zn		P	Zn	P × Zn		P	Zn	P × Zn
Shoot	FW	0.7	0.058	0.285	FW	0.266	**<.001**	**0.027**	FW	**<.001**	**<.001**	0.162
		12.2	*52.5*	*26.6*		*7.95*	*251*	*11.6*		*3.49*	*4.58*	*0.25*
	DM	0.699	0.448	0.604	DM	0.655	**<.001**	0.196	DM	**<.001**	**<.001**	**0.023**
		0.123	*0.208*	*0.194*		*0.0501*	*2.42*	*0.1088*		*0.490*	*0.447*	*0.0684*
Shoot concentration	P	**<.001**	**0.034**	**<.001**	P	0.287	0.137	0.906	P	**<.001**	0.059	**0.015**
		2.59E + 06	*6.13E + 05*	*1.30E + 06*		*4.73E + 05*	*6.68E + 05*	*2.08E + 05*		*2.46E + 06*	*4.88E + 05*	*4.36E + 05*
	logZn	0.532	**<.001**	**<.001**	logZn	0.268	**<.001**	0.629	logZn	**<.001**	**<.001**	**<.001**
		0.0381	*1.94*	*0.195*		*0.0326*	*13.4*	*0.0210*		*0.522*	*4.40*	*0.0415*
	K	**0.002**	0.347	0.468	K	0.919	**0.044**	0.336	K	**0.006**	**<.001**	0.144
		3.36E + 08	*8.34E + 07*	*7.36E + 07*		*3.34E + 07*	*3.68E + 08*	*1.63E + 08*		*6.70E + 08*	*2.54E + 09*	*2.47E + 08*
	Ca	0.74	0.063	0.586	Ca	0.453	**<.001**	0.633	logCa	**0.043**	0.22	**0.003**
		1.83E + 07	*8.59E + 07*	*3.29E + 07*		*4.04E + 07*	*1.74E + 09*	*3.69E + 07*		*0.0416*	*0.0237*	*0.0409*
	Mg	0.794	0.053	0.608	logMg	0.49	**<.001**	0.486	Mg	0.606	**0.003**	**0.004**
		2.66E + 05	*1.55E + 06*	*5.50E + 05*		*0.0045*	*0.260*	*0.0052*		*1.30E + 06*	*8.18E + 06*	*4.74E + 06*
	logS	**0.017**	**<.001**	**0.019**	logS	0.054	**<.001**	0.493	S	0.395	**<.001**	**<.001**
		0.125	*0.886*	*0.080*		*0.0335*	*2.43*	*0.0135*		2.87E + 07	7.26E + 08	1.20E + 08
	Mn	0.087	0.122	0.264	logMn	0.261	**<.001**	0.382	logMn	**<.001**	**<.001**	**<.001**
		2145	*1914*	*1253*		*0.0382*	*0.903*	*0.0309*		*0.896*	*0.120*	*0.0902*
	Fe	0.466	0.205	0.763	logFe	0.971	**<.001**	0.965	logFe	0.084	**<.001**	0.125
		446	*749*	*357*		*0.0021*	*0.292*	*0.0072*		*0.0907*	*0.318*	*0.0633*

Treatments (increasing soil P and Zn concentrations) were used as independent variables for each soil separately. Details of P and Zn amendments can be found in the legend to Fig. 1. FW, fresh weight; DM, dry matter; K, potassium; Ca, calcium; Mg, magnesium; S, sulphur; Mn, manganese; Fe, iron; log, data were log transformed to achieve approximate normality

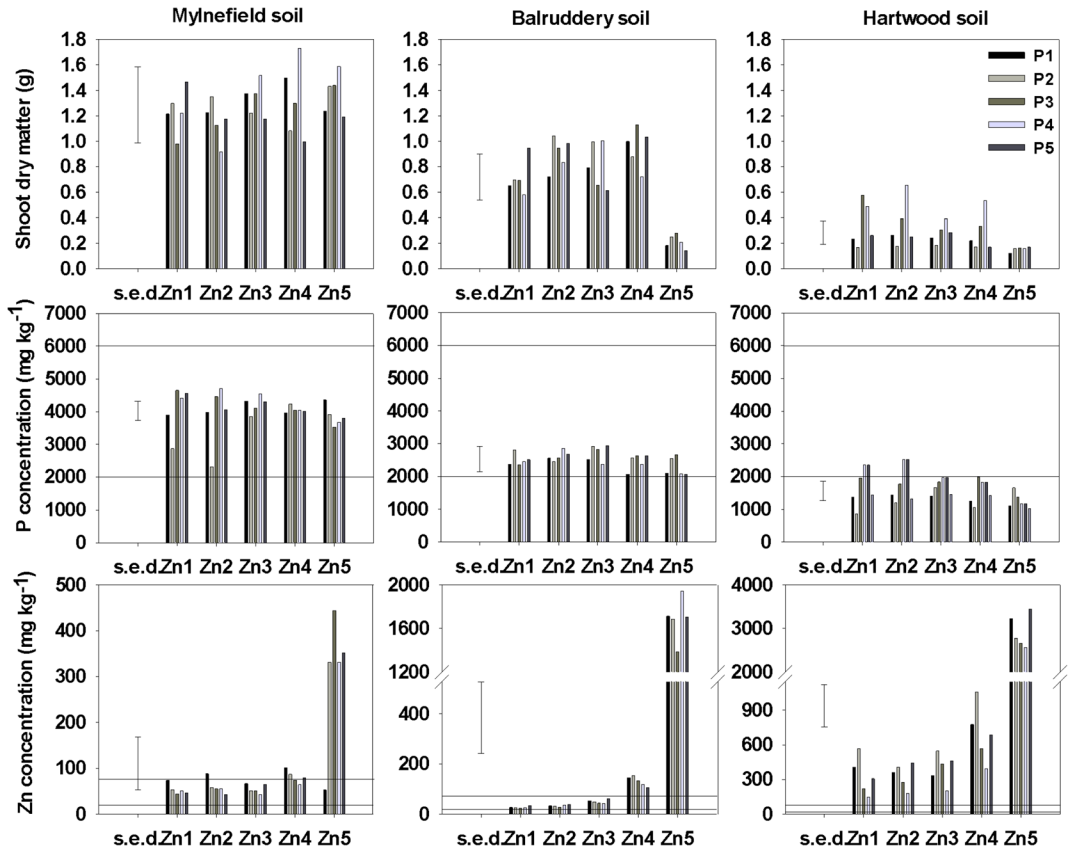

Fig. 3 Dry matter and concentrations of phosphorus (P) and zinc (Zn) in dry shoots of red cabbage (*Brassica oleracea* cv. Red Drumhead) grown in different soils amended with 25 combinations of phosphorus (P) and zinc (Zn) fertiliser for 6 weeks. Horizontal lines indicate lower and upper sufficiency thresholds in dry matter: P (2–6 g kg^{-1}) and Zn (20–75 mg kg^{-1}) as suggested for leaves of ornamental cabbage (Campbell 2009). Details of P and Zn amendments can be found in the legend to Fig. 1; s.e.d., average standard error of difference (for comparisons of any pair of means within an individual graph)

2013), suggesting that they might reach toxic concentrations, and were generally much greater than shoot S and Mn concentrations in plants grown in Mylnefield or Balruddery soils. A strong P treatment effect occurred for shoot Mn concentration in plants grown in Hartwood soil, with the largest Mn concentrations in the P1 (P-unamended: 0 mg P L^{-1} added) treatment. Shoot Fe concentrations were within the sufficiency range proposed for ornamental cabbage (50–300 mg Fe kg^{-1}, Campbell 2011) in all three soils, with plants grown in Hartwood soil clearly responding to Zn treatment (Fig. 5 bottom row).

Discussion

The data clearly demonstrates that soil type is a key factor in determining growth and P, Zn, S, K, Ca, Mg, S, and Mn concentrations in shoots of six-week-old red cabbage plants. This result supports the hypothesis that red cabbage plants (and arguably other plant species) grown in different soils can have different mineral element compositions. Since essential minerals are often lacking in human diets (White and Broadley 2009), this observation has important implications for strategies to increase their concentrations in edible crops to alleviate nutrient deficiencies in human diets. With this in mind, soil-type × plant interactions should be studied in greater detail to avoid unforeseen variability in mineral element composition of produce.

The principal component analysis, which took all measured plant responses (DM and shoot P, Zn, K, Ca, Mg, S, Mn, and Fe concentrations) into account (Fig. 2b), indicated that plants grown in Hartwood soil differed from those grown in Mylnefield or Balruddery soils more than plants grown in Mylnefield and Balruddery soils

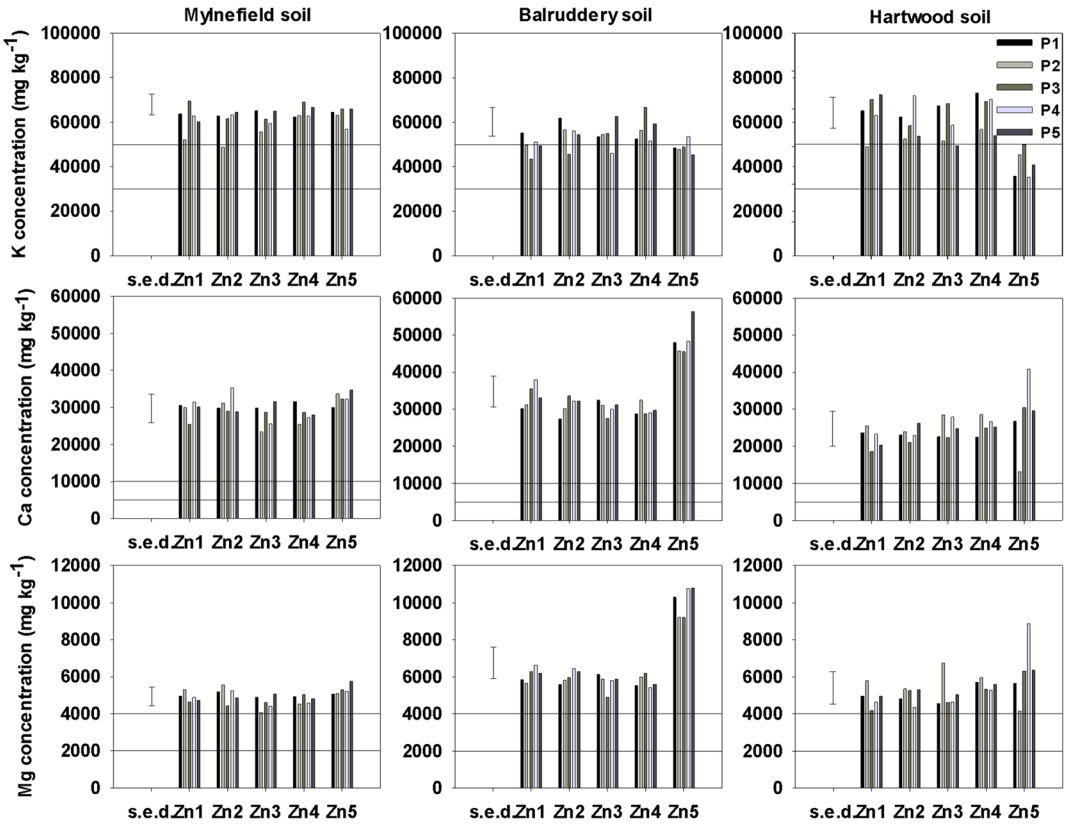

Fig. 4 Concentrations of potassium (K), calcium (Ca) and magnesium (Mg) in dry shoots of red cabbage grown in different soils amended with 25 combinations of phosphorus (P) and zinc (Zn) fertiliser for 6 weeks. Horizontal lines indicate lower and upper sufficiency thresholds in dry matter: K (30–40 g kg^{-1}), Ca (5–10 g kg^{-1}) and Mg (2–4 g kg^{-1}) as suggested for leaves of ornamental cabbage (Campbell 2009). Details of P and Zn amendments can be found in the legend to Fig. 1; s.e.d., average standard error of difference (for comparisons of any pair of means within an individual graph)

differed from each other. Since the same red cabbage genotype was grown on these soils and the experiment was designed to limit unpredicted variability, reasons for the differences seen in plant responses must lie in the soil characteristics. Indeed, Hartwood soil differed in several characteristics from Mylnefield and Balruddery soils, although the differences between Mylnefield and Balruddery soil were not negligible (Tables 1 and 2, Supplementary Table 1). Plants grown in Hartwood soil had the smallest DM which could be a consequence of their low shoot P concentrations (below sufficiency range; Campbell 2009) and great shoot Zn, S, and Mn concentrations (above sufficiency range; Campbell 2009; Figs. 3, 4 and 5) compared to plants grown in Mylnefield or Balruddery soils. It is plausible that the observed DM of plants grown Hartwood soil was a consequence of P deficiency accompanied by Zn, S, or Mn toxicity (Figs. 3, 4 and 5). Statistically significant negative correlations between DM and shoot Zn, S and Mn concentrations supports these conclusions, although negative correlations were also observed between shoot DM and shoot Mg and Fe in plants grown in Hartwood soil. These observations are also consistent with Hartwood soil having smaller soil P concentrations (total and Olsen-P) and larger total soil S concentrations than Mylnefield or Balruddery soils. By contrast, the largest soil Zn concentrations (total and DTPA-Zn) and total soil Mn concentrations were in Mylnefield soil, in which the only negative correlation with shoot DM was with shoot Mn concentration.

Although there was a statistically significant positive correlation between DM and shoot P concentration in plants grown in Hartwood soil, plants apparently remained P deficient despite P-fertiliser additions (Fig. 3). It is possible that the P amendments were not sufficiently large (the largest P amendment, P5, contributed

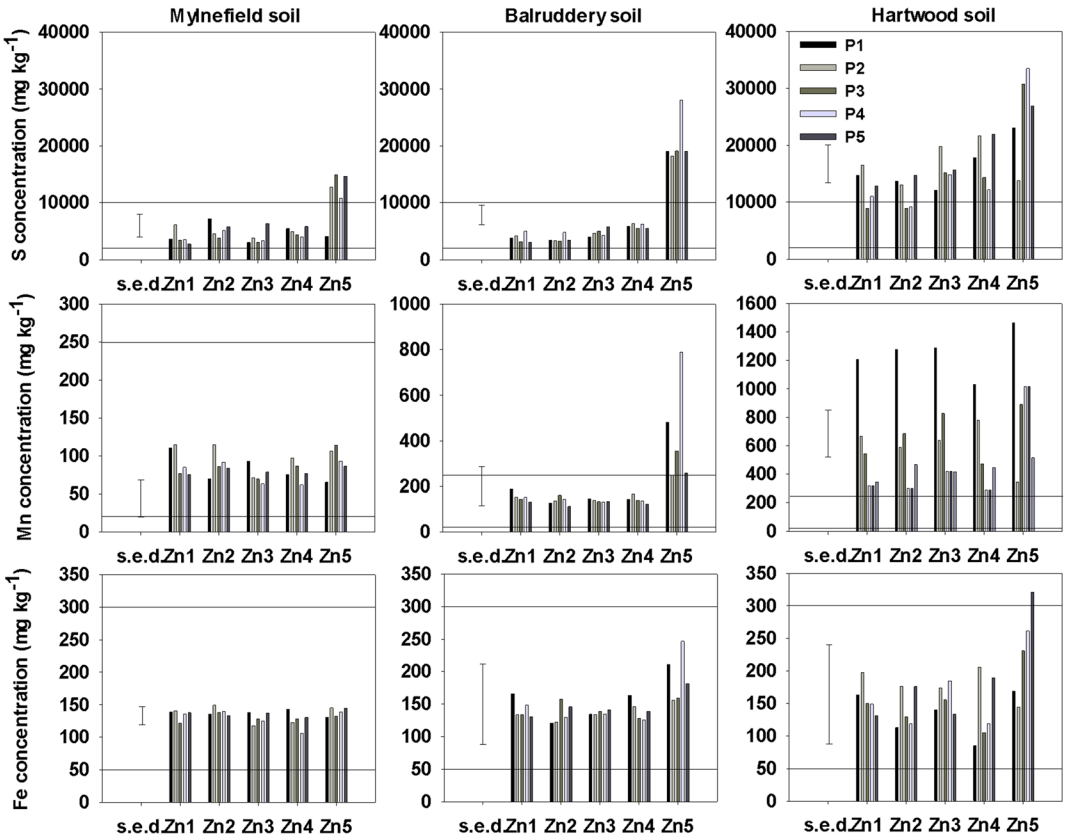

Fig. 5 Concentrations of sulphur (S), manganese (Mn) and iron (Fe) in shoots of red cabbage grown in different soils amended with 25 combinations of phosphorus (P) and zinc (Zn) fertiliser for 6 weeks. Horizontal lines indicate lower and upper sufficiency thresholds in dry matter: S (2–10 g kg^{-1}), Mn (20–250 mg kg^{-1}) and Fe (50–300 mg kg^{-1}) as suggested for leaves of ornamental cabbage (Campbell 2009). Details of P and Zn amendments can be found in the legend to Fig. 1; s.e.d., average standard error of difference (for comparisons of any pair of means within an individual graph)

only 2% of total soil P concentration in Hartwood soil and less in Mylnefield and Balruddery soils) to increase shoot P concentration and promote growth greatly. However, the P5 treatment was previously reported to increase the biomass of 376 *B. oleracea* genotypes grown in commercial compost (Broadley et al. 2007). Since only limited P treatment effects were observed here, it appears likely that soil characteristics affected P availability. For example, Hartwood soil contained the greatest organic matter content of the three soils and also differed in soil mineral content from Mylnefield and Balruddery soils. Organically-bound P in the soils represents an accessible but metabolically costly source of P (Vance et al. 2003; Lambers and Plaxton 2015) and may have contributed to poor P availability in the organic-matter-rich Hartwood soil. In addition, Hartwood soil had the largest concentrations of kaolinite and goethite, clay mineral, and Fe-containing hydroxide, which adsorb phosphate strongly and cause very low phosphate concentrations in solution (Sanchez 1976). Although sulphate (note large soil total S concentration in Hartwood soil) competes with phosphate on these Al and Fe (hydr)oxides, especially at lower pH (Geelhoed et al. 1997), it appears that large concentrations of kaolinite and goethite in the Hartwood soil might restrict P availability. Although the pH (in $CaCl_2$) in Hartwood soil was lower than that in Mylnefield or Balruddery soils, it was still within the range for optimum phosphate uptake (4–6), when P is a limiting nutrient in the soil (Barrow 2017), suggesting that pH was not critical for P uptake.

The best growth and the largest shoot P concentrations were in plants grown in Mylnefield soil and were consistent with Mylnefield soil having the greatest soil P concentrations (total or Olsen-P). Interestingly, Mylnefield soil also had the largest soil total Mn concentration. Plants grown in Mylnefield soil, had smaller shoot Mn

concentrations than plants grown in Balruddery and Hartwood soils, but still within the sufficiency range, and their shoot Mn concentration was not affected by treatments (Table 3 and Fig. 5). By contrast, there were significant treatment effects on shoot Mn concentration in plants grown in Balruddery and Hartwood soils. The largest shoot Mn concentrations were at the lowest P treatment, particularly in Hartwood soil, indicating an over-accumulation of Mn in this treatment. Shoot Mn over-accumulation also has been reported in barley grown in nutrient solution containing low P and large Mn concentrations (Pedas et al. 2011). An effect of Zn treatment occurred on shoot Mn concentration in plants grown in Hartwood and Balruddery soils. In the latter, the largest shoot Mn concentrations were in the largest Zn treatment (200 mg Zn L^{-1}). A similar increase in Mn concentration with increasing Zn in the nutrient solution was seen in the middle leaves of Burbank potato (Barben et al. 2007). These results support theories of indirect interactions between P, Zn, and Mn nutrition (e.g. for potato, Barben et al. 2010), although the extent of these interactions appears to depend on the soil type and its effects on tissue mineral element composition.

Shoot Zn concentrations in plants grown in the three soils appear to follow even more complicated relationships. The smallest soil total Zn and DTPA-Zn concentrations were measured in Balruddery soil, but plants grown in Balruddery soil did not have the smallest shoot Zn concentrations. By contrast Mylnefield soil had the largest soil total Zn concentration and DTPA-Zn concentrations, which were not much different from those in Hartwood soil, but the average shoot Zn concentrations in plants grown in Mylnefield soil were one-eighth of those observed in plants grown in Hartwood soil. One of the reasons for low shoot Zn concentration could be the low organic matter concentration in Mylnefield soil, in line with previous observation that low organic matter content leads to a restriction in plant Zn concentration (Ward et al. 1963; Broadley et al. 2007). Plants grown in Hartwood soil had large shoot Zn concentrations, as previously mentioned, which were probably above the critical tissue concentration and thereby causing toxicity (White and Brown 2010). Great Zn phytoavailability in Hartwood soil might be a consequence of the combination of great organic matter content (presumably increasing concentration of soluble complexes), small total Ca and Mg concentration (Alloway 2009), mineral composition and particularly the absence of chlorite and hematite, small contents of plagioclase, amphibole, goethite and expandable clays, and great contents of kaolinite, which all play a role in Zn adsorption and retention in soils (González-Costa et al. 2017).

Two conclusions, connected to the tested hypotheses, can be made based on the results. The first is that growth and mineral element composition of red cabbage shoots was affected more by soil type than by P and Zn amendments, which could have important implications for increasing mineral concentrations in edible crops to alleviate nutrient deficiencies in human diets. It will be important, therefore, to test a greater number of soils for the impact they have on the mineral composition of different edible crops and to develop fertilization programs for the soils in order to have increased nutrient accumulation in crops. In addition, management practices would have to be adapted for the particular soil type. The second is that increasing P in the soil increased shoot biomass and P concentration, seen when comparing the three different soils containing different soil P concentrations, but did not reduce shoot Zn concentration. This result indicates that P-induced Zn deficiency did not occur in red cabbage in this experiment.

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