1 **The grain mineral composition of barley, oat and wheat**

2 **on soils with pH and soil phosphorus gradients**

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Highlights

8 - pH has a stronger effect on grain nutrient composition than soil extractable cations

9 - Changing pH leads to different variations of grain nutrients among cereals species

10 - Cu, Fe, Se and Zn concentrations in grains are weakly related to soil pH

11 - Grain composition cannot be used to indicate the bioavailability of nutrients in soil

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Abstract

14 The decreasing mineral concentrations in the grains of cereals have recently stimulated research to

15 better understand the cropping determinants of grain mineral composition. This study aimed to analyze

16 the effects of liming on the mineral concentrations in the grains of three cereal crops: barley, oat, wheat.

17 The hypothesis tested was that soil pH is the main driver of the grain nutrient concentrations in crops,

18 through its influence on the soil extractable minerals. Macro nutrients (Ca, K, Mg, P, S), micro-nutrients

19 (Cu, Fe, Mn, Se, Zn) and some trace elements (As, Cd, Pb) were analysed. Two long term liming trials in

20 SE England (1962 -) were studied, with the same crops sown in the same years. On each site, four liming

21 rates were applied to 32 plots to create a pH range from approximately 4.5 to 7.5. The trials were

22 subdivided into two P fertiliser treatments, consisting of a nil and regular P inputs. For a given crop, the

23 effects of pH, soil type, concentrations of nutrients in soil extracts and of P treatment on the grain

24 mineral concentrations were tested. This pairwise analysis was followed by a multiple linear regression

25 analysis in order to determine the main explanatory variable for crop mineral concentration. Liming had

26 a significant impact on most of the soil extractable mineral concentrations, except extractable K and Mg.

27 The grain mineral concentrations exhibited significant differences between crops, the concentrations in

28 wheat being the smallest. pH proved to have a larger direct effect on mineral concentrations in grain

29 (e.g. Ca, Mg, P, Mn) than through its influence on extractable nutrients (e.g. Cd). Grain nutrients

30 responses to pH were, however, not the same in the three crops. Differences in Cu and Zn were mostly

31 accounted for by the effect of soil type, the soil with the higher CEC leading to the higher grain

32 concentrations. For Fe, Pb and K, no correlation could be found between the grain mineral
33 concentrations and the explanatory variables. Difficulties in explaining the grain mineral concentrations
34 are due to specific crop responses to nutrients, usefulness of soil extractions, and complex physiological
35 processes in mineral translocation from roots to grains. The results underline the difficulty of using
36 ordinary soil analysis for predicting the quality of cereal grains for nutrition, and caution in the use of
37 grain testing to recommend soil fertility enhancing practices.

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Key words

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1. Introduction

1.1 Grain nutrient concentrations: an emerging concern for humans and grain-fed animals

During the last 50 years, agricultural intensification has increased crop primary production at a higher rate than population growth (FAOSTAT 2018). However, parallel to this tremendous increase in available calories, and at a global scale, agriculture might not always provide adequate amounts of essential nutrients in the human diet (IFPRI 2014), especially because of a decrease in the concentrations of grain nutrients (Fan et al. 2008, McGrath 1985). This observation is particularly widespread in Asia and Western Africa (DeFries et al. 2015) but is also a real concern in developed countries (Adams et al. 2002). The situation is likely to worsen in the medium term, in relation to climatic changes (Smith and Myers 2018). The major deficiencies are in proteins, phytates, macronutrients (Ca, Mg) and micronutrients (Cu, Fe, I, Se, Zn) (Joy et al. 2014). Cereals constitute the major component of the human diet (44% of the daily intake of Fe, 27% of Mg, 25% of Zn and 31% of Cu, Henderson et al. 2003, *In* Fan et al 2008). Therefore, the understanding of the determinants of grain mineral concentrations is of major importance to identify remediation techniques for soil and plant nutrition.

1.2 Overview of the factors determining the grain nutrient concentrations

The factors that determine the grain nutrient concentration can roughly be categorized between some "natural" variables (e.g. soil type) and some linked to agricultural practices. In the first category, the soil texture has a major influence, with clay soils generally associated to more mineral-enriched grains than sandy ones (Manzeke et al. 2019). In line with this property, Ivezic et al (2013) states that total trace metal concentrations and, hence, their extractable forms, are a significant determinant of grain content, as long as soil organic matter is restricted in a small range of variations. Parallel to these physical and chemical factors, Ayoubi et al (2014) underline that the distribution of drier and wetter zones at landscape scale appears as the main factor in an attempt to predict the wheat grain micronutrients with a multiple linear regression. In the second category of factors, i.e. the agricultural practices, the impact of soil NPK fertilization and liming on grain mineral contents have been particularly studied. The special case of foliar nutrition by micronutrients (biofortification) is not reported here. As far as the role of fertilizers are concerned, several studies show that the NPK fertilization, taken as a whole, has only a weak influence on the grain of cereals of micronutrients and potentially toxic trace elements (Hejcman et al. 2013, Moreno-Jimenez et al. 2016). The case of P-fertilization, alone, leads however to a different conclusion: it is known to drastically reduce root colonization by arbuscular mycorrhizal fugi (Giocoechea et al. 2004) which eventually leads to a detrimental effect on the uptake of micronutrients, as especially shown on barley (Zhu et al. 2002). Parallel to fertilization, liming, which neutralizes soil acidity (Goulding 2016), is one of the oldest and most widespread agricultural practices. Through its major impact on soil biogeochemistry, it influences the solubility of minerals, their potential uptake by crops and the mineral content of their grains and tissues.

1.3. Consequences of soil acidification on soil mineral solubility

In acid soils, chemical reactions are mainly driven by Al^{3+}, Mn^{2+} and $Fe^{2+/3+}$, whereas in more alkaline soils, they are governed by Ca^{2+}. This impacts the solubility of minerals, as shown by studies using the liming management technique to analyze the soil geochemistry responses to a large gradient of pH values (Brallier et al. 1996, Rengel et al. 1999). With increasing pH of the soil, the solubility of most trace cations decreases asymptomatically to near-zero values (White 1970, Kabata-Pendias and Pendias 2001). For divalent cations (Zn^{2+}, Cu^{2+}, Mn^{2+}, Fe^{2+}) solubility is decreased by one hundred-fold for every unit increase in pH (Verma and Minhas 1987, Rengel et al. 1999). This property makes liming an efficient tool to reduce the concentration of potentially toxic trace elements in contaminated soils (Hooda and Alloway 1996). With macronutrients, the situation is more heterogeneous. For example, the solubility of potassium (K^+) shows minimal relation to pH, as stated by Holland et al. (2019). On the contrary, phosphorus (P) solubility is reduced by soluble Fe^{2+} and Al^{3+} at low pH values (Evans and Smillies 1976) and by soluble Ca^{2+} at high pH values (Marschner 1995, Ozturk et al. 2005). Magnesium (Mg^{2+}) shows another pattern, linked to competition phenomena with Ca^{2+} and Al^{3+} (Barraclough and Leigh 1993, da Silva Domingues et al. 2016), thus giving non-linear responses to pH. In addition to pH, redox potential (rH) exhibits a strong influence on the solubility of some minerals; generally, anaerobic conditions are required to free some ions in the soil solution, as is the case for As, Mn, Fe and P (Marschner 1995). The great seasonal variability of rH therefore makes it very difficult to correlate pH to the solubility of some minerals.

1.4. Consequences of soil acidification on mineral uptake and grain quality

The composition of plant minerals (concentrations, ratios...) is not a simple transposition of the soil solution composition. Soil mineral deficiencies, excess or imbalances, trigger specific plant reactions. Mineral deficiencies, on the one hand, lead to three kinds of adaptations: firstly, specific root ion channels can be activated (almost all macronutrients have specific transporters activated under adverse pH/rH soil conditions, Marschner 1995). Secondly, the specific chemistry of the rhizosphere may allow microbial or chemical solubilization (e.g. role played by specific phytosiderophores on the uptake of Fe of *Poeceae*, Marschner and Römheld 1994). Thirdly, the relative root to shoot growth ratio is generally enhanced (Ericson 1995) under soil mineral deficiencies. On the other hand, plants protect themselves from potential toxic trace elements through binding induced by chelation, precipitation (Morel et al. 1986), compartment storage processes (Ma et al. 2001, Harada and Choi 2008). In the extreme case of acid soils (pH < 4.5), mineral uptake is impaired by the deleterious toxicity of Al^{3+}, causing inhibition of root elongation by the lysis of the cell structure of the root apex (Foy 1984), which, in turn, affects water and nutrient uptake (Zheng 2010). At low pH values, Mn toxicity can also impair plant growth (Reid 1976, Schlichting and Sparrow 1988), through physiological mechanisms still poorly understood (Horst et al. 1999). In summary, any change in soil pH modifies the solubility of minerals in soils, triggers uptake

adaptation mechanisms and impacts the root system morphology. For these reasons, pH is likely to deeply influence the crop mineral composition; however, because of specific plant responses to mineral deficiencies / toxicities, predicting the tissue concentrations remains a great challenge, and even more so for grains (Miner et al. 1997, Wang et al. 2013). The multiple and complex physiological mechanisms between the soil solution composition and the grain mineral contents, described by Olsen and Palmgren (2014) as "many rivers to cross", explains the success of empirical models (Baize et al. 2009, Viala et al. 2017), generally based on principal components analysis and multiple linear regression analysis (Eriksson et al. 2017).

1.5. Aim of the study and related hypothesis

This study aimed to analyze and assess the relative importance of the main drivers of the mineral concentrations in the grain of cereals. The soil-plant data was from a multi-location long term trial to study the effects of liming and P treatments on grain mineral concentrations of barley, oat and wheat. The investigated minerals are those of concern for the diet and health of humans, and animals fed on grain: macronutrients (Ca, K, Mg, P, S) and micronutrients (Cu, Fe, Mn, Se, Zn). Although potentially toxic trace elements (As, Cd, Pb) are not a health issue for crops grown on non-polluted soils (Zhao et al. 2004, Kabata-Pendias and Pendias 2001), data from soil-grain transfer of those latter trace elements will also be presented.

It was hypothesized that, for a given crop, the grain mineral concentrations would result from a simple two-step process: 1) soil pH controls the minerals solubilization in the soil which, 2), in turn influences -to a certain extent- the grain mineral concentrations. Step 1 derives from a substantive assertion that pH is the single most important factor controlling soil mineral solubility, especially for trace elements (Brallier et al. 1996, Rengel et al. 1999, Schöning and Bümmer 2008). Step 2 asserts that the chemical composition of plants generally mirrors the pool of phyto-available minerals, at least more than total trace element concentrations in the soil (Mench et al. 1996, Kopittke et al. 2017). Therefore, we expected soil pH values to have an indirect influence on mineral concentrations in grains. Soil type and P treatments were hypothesized to have secondary roles in controlling grain quality. The main objectives of the study were:

(i) to quantify the effect of soil pH on the grain mineral concentrations of barley, oat and wheat, for two contrasting soils;

(ii) to analyze the influence of phosphorus fertilization on the grain mineral concentrations;

(iii) to provide insights for the interpretation of soil and grain testing.

2. Materials and methods

2.1. Experimental sites description

Two experimental sites were studied simultaneously: 1) Sawyers field, located at Rothamsted Research, Harpenden, Hertfordshire, UK (51.8157 N, 0.3752 W). The soil has a silty clay loam texture (20% clay, 52% silt, 28% sand) and is classified as Profundic Chromic Endostagnic Luvisol (WRB, 2006). The organic C content of the top horizon (0-23 cm) ranges between 0.85 and 1.12% (Kemmitt *et al.* 2006). CEC, based on the method of Rhoades (1982), was 97.5 ± 18 meq kg^{-1}. This was calculated for the study period (1974-1995) and for plots with pH between 6 and 7 (medium lime treatment, see below). 2) Stackyard field, located on the Section-C, at Woburn Experimental Farm, Husborne Crawley, Bedford, UK (52.0003 N, 0.6149 W). The soil is a sandy loam (12% clay, 17% silt, 71% sand) described as a Eutric Cambisol (WRB, 2006). The organic C content of the top horizon (0-23 cm) ranged between 0.68 and 0.77% (Kemmitt *et al.* 2006) and CEC, averaged for the overall study period (1974-1995), was 71.1 ± 10 meq kg^{-1}. Both sites are further described in Holland et al. 2019, Glendining 2020a and Glendining 2020b.

The sites were cropped from 1962 until 1996. The same crops were grown at each site. The agronomy and management of the crops followed conventional practices over the course of the experiment and were the same at both sites. To avoid mineral deficiencies, N was applied to all plots, but P and K were split into two treatments (nil and an annual standard rate). All the information about the experiment is available in the electronic Rothamsted Archive (e-RA, 2021a,b).

Climatic recordings between 1962 and 1996 in Harpenden and Woburn are close enough to consider both locations as subject to the same climate: the mean annual temperature, rainfall and radiation are 9.2°C, 693 mm and 3344 MJ m^{-2} for Harpenden, and 9.4°C, 638mm and 3320 MJ m^{-2} for Woburn (Holland et al. 2019). Therefore, soil properties constitute the main difference between both sites, especially the higher levels in clay and organic matter in Rothamsted, leading together to a 20% higher level of CEC in Rothamsted. In the following text and analysis, the influence of the two sites on the grain mineral contents will be expressed as a "soil type" effects.

2.2. Experimental design

A factorial experimental design was used at each site with two randomised blocks each of 16 plots. The size of each plot was 6×16m. There were four rates of limestone applied (as ground chalk, CaCO$_3$) and these are described as control (C), low (L), medium (M) and high (H). The lime requirement was determined by the method of Shoemaker et al. (1961) in order to reach soil pH target values from about 4.5 to 7.5. The total amounts of Ca brought by the lime over the course of the experiment (35 years duration) increased from 6 to 21 t Ca ha^{-1} and from 4 to 18 t Ca ha^{-1} for Rothamsted and Woburn, respectively. The lime treatments were combined with four P and K treatments, at the plot scale. Plots with no P fertilization during the overall period are further denoted P- contrary to P+ plots which received varied rates of P (from 25 to 100 kg P ha^{-1} y^{-1}). The complexity of P treatments is reduced to two

192   treatments in this article for clarity. The -K and +K treatments (1962-1978) were pooled, as there was no

193   significant interaction with lime, nor with any other soil or grain cation.

194   This study focused on three crops, cultivated in three different years: spring barley (var. Porthos, 1978),

195   spring oat (var. Peniarth, 1981) and winter wheat (Var. Genesis, 1995). Sub-plots receiving short-term

196   treatments of Mg (1974-1978), Mn (1987-1990) and S (1991-1995) were not included in this analysis.

197   **2.3. Soil and plant analysis**

198   Soil cation macro-nutrients (Ca, K, Mg), micro-nutrients (Cu, Fe, Mn, Zn) and toxic elements (Cd, Pb) were

199   extracted by ammonium nitrate (NH_4NO_3), 1 $mol.L^{-1}$ (DIN V 19730 1995, Zeien and Brümmer 1989). The

200   subsequent chemical analysis was conducted with inductively coupled plasma optical emission

201   spectrometry (ICP OES). All values below detection limits were assigned that concentration (Ca: 3.2 $\mu g\ g^{-1}$

202   , K: 3.5 $\mu g\ g^{-1}$, Mg: 0.2 $\mu g\ g^{-1}$, Cd: 0.008 $\mu g\ g^{-1}$, Cu: 0.018 $\mu g\ g^{-1}$, Fe: 0.21 $\mu g\ g^{-1}$, Mn: 0.015 $\mu g\ g^{-1}$, Pb:

203   0.055 $\mu g\ g^{-1}$, Zn: 0.021 $\mu g\ g^{-1}$). Extractable cation measurements were performed for the 0 - 23 cm top-

204   soil layer. Phyto-available P was estimated with Olsen extractant (Olsen *et al*. 1954) followed by a SAN

205   plus continuous colorimetric flow analysis from Skalar analytical BV (Breda, The Netherlands). Soil pH

206   was measured in 1:2.5 water suspension using a standard electrode and pH-meter.

207   For crop grains, the following minerals were measured: macro-nutrients (Ca, K, Mg, S, P), micro-nutrients

208   (Cu, Fe, Mn, Se, Zn) and potentially toxic trace elements (As, Cd, Pb). Sub-samples of grains from each

209   plot were ground to powder <0.5 mm, using a Retsch 400 ultra-centrifugal mill (Retsch GmbH, Germany).

210   They were digested with a solution of hydrogen peroxide and nitric acid and the mineral concentrations

211   were determined by inductively coupled plasma mass spectrometry (ICP-MS).

212   **2.4. Ranges of pH and Olsen P values**

213   The two P treatments led to contrasting Olsen P concentrations; the P- plots had significantly lower

214   Olsen P values at both locations (twice as low). In Rothamsted, mean Olsen P values measured from

215   1978 to 1995 were 9.1 ± 2.2 $\mu g\ g^{-1}$ and 20.5 ± 8 $\mu g\ g^{-1}$ for the P- and P+ treatment, respectively. In

216   Woburn, the contrast was about the same, with 16 ± 2.6 and 29 ± 9.5 $\mu g\ g^{-1}$ for the P- and P+ treatment,

217   respectively. At Woburn, the P content of plots receiving no P were considered as non- limiting

218   throughout the duration of the experiment, as testified by the UK Nutrient Management Guide ("Index

219   2", AHDB 2020), and the same conclusion applied logically to the P fertilized plots ("Index 3"). At

220   Rothamsted, the concentration of P (Olsen) for the control P treatment plots corresponded to the "Index

221   1", indicating a potential limiting effect. This relatively high P level may be explained by former P-

222   fertilization history on both soils.

223   Lime treatments were used as factors in ANOVA, whereas continuous pH values were used for regression

224   analysis. The four lime treatments led to a linear pH gradient from approximately 4.5 to 7.5 (Table 1).

225 The pH values of each location are not significantly different for the same lime treatment. Between 1978
226 and 1995, the pH values of each lime treatment slightly increased at both sites, as lime was applied in
227 1982 and 1986.

228 **[insert Table 1 near here]**

229

230 **2.5. Statistical analysis**

231 Analysis of variance (ANOVA, Jump software, version 5, SAS, 100 SAS Campus Drive, Cary, NC 27513-
232 2414, USA) was used to explore the effects of liming (4 treatments), P fertilization (2 treatments), and
233 soil type (Rothamsted, Woburn) on grain yield, soil pH, soil-extractable minerals (extractable cations,
234 Olsen P) and grain mineral concentrations in all three crops (Fig. 1). Pairwise regression analyses were
235 undertaken for estimating the relationships between the grain mineral concentrations (outcome
236 variable) and their main determinants (pH, extractable cations, Olsen P). Extractable minerals,
237 themselves, served as outcome variable towards pH (Fig. 1).
238 In order to synthesize the two-by-two regression analysis applied to the grain mineral concentrations, a
239 Multiple Linear Regression Analysis (MLRA; GenStat software, 18th edition, VSNI Product) was performed,
240 activating the "all subset regression – linear model" function. The analyses were performed for each crop
241 separately. The MLRA used one qualitative (soil type) and three quantitative (pH, Olsen P, extractable
242 cations) variables. MLRA was only performed for minerals that were measured in both grains and in soils,
243 thus excluding As, S and Se (not measured in soils).The "soil type" was therefore used as a factor for the
244 ANOVA treatments, and as a qualitative variable in the MLRA. As stated before, the soil differences can
245 be illustrated by their CEC, 97.5 and 71.1 meq kg^{-1} for Rothamsted and Woburn locations, respectively.

246 **[insert Fig. 1 near here]**

247

248 # 3. Results

249 **3.1. Overview of the treatment effects on crop yields**

250 The lime treatments had a very strong impact on yields (Table S1, $p<0.0001$). The treatment without
251 lime, which corresponded to pH values between 4 and 4.5, led to a complete yield loss on 7 out of 8 plots
252 of barley in Rothamsted (1978), on 6 out of 8 plots of barley in Woburn (1978), and on 6 out of 8 plots of
253 wheat in Rothamsted (1995). The number of plots with no yield of the control lime treatment explains
254 why the respective yield mean values are 5 to 10 times lower for those two crops. Wheat yields of the
255 three higher lime treatments (L, M and H) were not found to be significantly different from each other,
256 whereas for barley, yields from the first three lime treatments (C, L and M) significantly increased. Oat
257 yield showed a completely different pattern, with a decrease with increasing soil pH values (although not

significant). Moreover, if the control lime treatment is excluded, the mean yield of oat was half of that measured for barley and wheat, with mean values of 3.4 and 3.7 t ha^{-1} in Rothamsted and Woburn, respectively. This compares to 7.5 and 7.4 t ha^{-1} for barley and wheat yields in Rothamsted, and 4.5 and 7.4 t ha^{-1} in Woburn, respectively for barley and wheat yields. Conversely, the P treatments, which led to strongly contrasting Olsen P values, did not have any significant effect on yields when compared by crop and soil type (ANOVA, data not shown). Finally, the soil type effect had variable effects: yields of wheat were the same on both soils, whereas oat had significant higher yield at Woburn, and barley significant higher yield at Rothamsted (Table S1).

3.2. Grain mineral content: crop and lime effects

Independently of any factor (lime, P, soil type), macronutrient concentrations were always significantly different between crops (Fig. 2a). For micronutrients and non-essential elements (Fig. 2b, 2c, Table S2), grain mineral concentrations often overlapped between crops (e.g. Cu, Pb, Zn). Across all treatments and both soil types, wheat grain had lower mineral concentrations than barley and oat, as shown for eight minerals out of the thirteen measured (Fig. 2). Wheat always had the lowest concentrations of macronutrients compared to barley and oat (Fig. 2a), with a 20% difference overall. Average Ca concentrations were even lower (244 µg g^{-1}), with values 2 to 3 fold smaller than in barley (555 µg g^{-1}) and oat (890 µg g^{-1}), respectively. Otherwise, maximum concentrations were found either in barley (K, Mg, P, Cu, As) or in oat (Ca, S, Fe, Mn, Zn and Pb) (Fig. 2). Oat almost never had the lowest mineral concentrations.

A

Out of the 78 grain mineral-crop combinations (13 minerals X 3 crops X 2 soil types), 14 (18%) showed a very strong effect of lime treatments on grain mineral concentrations (*P* value < 0.0001), whereas 46 (59%) showed no significant effect (P-value > 0.05). Detailed ANOVA results and mean mineral concentrations of grain are shown in Table S2. The mineral concentrations of barley were less dependent on lime treatments, with only 3 minerals out of 13 (P, Cd and Mn) being significantly affected in Rothamsted, and 4 out of 13 in Woburn (P, Cd, Mn plus Mg). In contrast, the grain mineral concentration of wheat was most sensitive to lime treatment, affecting half of the minerals. Macronutrients of wheat grains were all influenced by lime treatment on both soil types, with the exception of K (Table S2a). But of the micronutrients in wheat, only Se was significantly influenced by lime treatment. Oat exhibited an intermediate pattern, although more similar to wheat than to barley.

[insert Fig. 2 near here]

The highest relative grain mineral changes, calculated as the relative difference in grain mineral concentrations between the control and the high lime treatments, were observed for Cd and Mn, decreasing their concentrations by 70% and 60%, respectively, between the control and high lime treatments (Fig. 2b, c). To a lesser extent, Ca, Mg and P also exhibited relatively high variation (20%),

9

293 their concentrations being positively correlated with increasing lime treatment, except for Ca in wheat
294 which did not show a clear trend. For micronutrients and other trace elements, only grain concentrations
295 of Mn and Cd exhibited a regular trend across the three crops having a negative relationship with
296 increasing lime treatment (Table S2b,c, Fig. 2b, c). Finally, three minerals exhibited no relationship at all
297 with lime treatments: As, Fe and Pb.

298 Relationships between grain mineral concentrations and soil pH mirrored those with lime treatment
299 (data not shown). The best regression coefficients between pH and grain concentrations were for Cd in
300 wheat grain at Rothamsted (r^2 = 0.77, Fig. 3c), for Mn in oat (r^2 = 0.75) and barley (r^2 = 0.68) at Woburn
301 (Fig. 3b), and for P on oat at Rothamsted (r^2 = 0.69, Fig. 3a). Cations-pH relationships exhibited different
302 patterns for the same element, depending on the crop (Fig. 3b). Moreover, the existence of a
303 relationship on one soil and for one crop did not necessarily mean that the same relationship existed on
304 the other soil (e.g., for Cd, Mn, P, Zn and K in wheat, Fig. 2). Relationships can be linear with a positive
305 slope as for P (Fig. 3a), exponential as for Mn (Fig. 3b) or linear with a negative slope as for Cd (Fig. 3c).

306 **[insert Fig. 3 near here]**

307

308 **3.3. Lime effects on the pool of extractable nutrients in the soil**

309 The lime treatments strongly affected the concentration of extractable cations in the soil (as defined by
310 the ammonium nitrate extraction method) (Table S3). All soil nutrient extractabilities were significantly
311 impacted by the lime treatment (p-values < 0.05) except K. In general, concentrations of extractable
312 minerals in the soil were higher in Rothamsted soil due to its higher CEC, with the exception of Fe and P,
313 naturally higher in Woburn (Table S3).

314 The extractable Ca and Olsen P showed pH-dependence on both soil types. The range of mean
315 concentrations was 300% for Ca but only 24% for P, in both soil types (Table S3). The relationship
316 between extractable Ca and pH was a linear increase with pH (and liming), even in the control treatment
317 where no Ca was applied. Extractable P (Olsen P) was lowest (15 and 22 ppm for Rothamsted and
318 Woburn, respectively) around pH 6, and increased up to 18 and 26 ppm for Rothamsted and Woburn,
319 respectively, when lime treatment was applied.

320 For micronutrients, the relative variations were much higher, in most cases best modelled by exponential
321 decreases of concentrations from low to high pH values (e.g. Extractable Zn, Fig. 4a), with the exception
322 of extractable Mn which followed a decreasing linear relationship with pH (Fig. 4b). The highest relative
323 variations of soil extractable cation concentrations were observed for Fe and Pb (> 3000%), whereas Cd,
324 Cu and Mn varied around 500% (Table S3). As pH decreased, the following cations solubilised below
325 different pH-threshold values (visually estimated, in the decreasing order): Mn (pH 7), Cd (pH 6), Zn (pH

326 5,5), Fe (pH 5), Pb (pH 4,8), Cu (pH 4,5). All of the equations describing extractable cation concentrations

327 as a function of pH are shown in Table 2.

328 **[insert Table 2 near here]**

329 **[insert Fig. 4 near here]**

330

331 **3.4. Relationship between the extractable minerals in the soil and the grain cation concentrations**

332 The regression analysis performed between grain and soil mineral concentrations, on each soil type

333 separately, identified only a limited number of significant relationships (p-value < 0.05) (table 3). In the

334 case of extractable As, Se and S no relationship with the grain concentrations could be tested because

335 their concentrations were not determined in the soil, explaining their absence in table 3.

336 For micronutrients and other trace elements, the strongest relationships were measured for Mn (except

337 for wheat at Woburn) and Cd (except for oat and wheat in Woburn) (Table 3 and Fig. 5a, b). Cu and Zn

338 showed statistically weaker relationships (generally p-value > 0.03) with poor r^2 values (<0.2), and the

339 soil-grain relationships were only applicable for oat. For Fe and Pb, soil extractable and grain

340 concentrations did not correlate at all.

341 For macronutrients, Ca and to a lesser extent P, were the only minerals showing significant soil-plant

342 relationships (p-value < 0.05), mostly in wheat and oat. Barley showed only weak correlations (0.16 < r^2 <

343 0.18) (Table 3). The decreasing relationship between soil- and grain-P for barley at Woburn is surprising.

344 For wheat, the Ca relationship fits best with second order polynomial functions, with a minimum of grain

345 concentrations of 1200 and 1800 µg g^{-1} of extractable Ca, in Woburn and Rothamsted, respectively (Fig.

346 5c). No relationship was found between the grain content of K and Mg and their respective extractable

347 cations in soil.

348 **[insert Table 3 near here]**

349 **[insert Fig. 5 near here]**

350

351 **3.5. Impact of soil type on the mineral concentrations in grains**

352 The effect of soil type (Rothamsted *vs* Woburn) on the crop grain mineral concentration was tested

353 through an ANOVA (Table S4). Results show that the soil of Rothamsted led to enriched grains for half of

354 the minerals. Barley proved to be less sensitive to the soil type than oat and wheat, with only Mg, P, Cu,

355 Zn and As being soil type-dependent. Soil type had no impact on the grain concentrations of Ca and Fe in

356 all three crops. In contrast, soil type had such a strong impact on Cu and Zn concentrations in the grains

357 of all three crops that the concentrations did not overlap between the two sites and for a given crop. For

358   example, there was a great site (soil type) difference in the Zn concentration in oat grains (Fig. 6b),

359   however in contrast the soil extractable Zn concentrations overlapped between the two sites (Fig. 6a).

360   The same observations applied to Cu.

361   **[insert Fig. 6 near here]**

362

363

3.6. The effect of P treatment on grain mineral concentrations

The effects of the two P treatments on grain mineral concentrations, studied with an ANOVA (Table S5), only detected a few significant results, mostly in Woburn, where Olsen P concentrations were higher ([15-45 µg g^{-1}], section 2.3 above). In terms of soil chemistry, there was no effect of the P treatments on the extractable cations. Moreover, lime × P interactions on grain concentrations were tested and were never significant.

The influence of Olsen P on grain P content has already been analysed (section 3.4). More interestingly, the grain concentrations of Cu, Zn and Cd appeared to be sensitive to the soil P content (Fig. 7, Table S5). Grain Cu and Zn proved to be inversely linearly correlated with Olsen P, whereas grain Cd evolved proportionally to Olsen P. The best regression coefficient applied to Cu relationship (r² = 0.76), the worst to Cd (r² = 0.28). The results indicate an effect independent of the extractable cations in soils.

[insert Fig. 7 near here]

3.7 Synthesis: weighting of the different factors on grain mineral concentrations

Previous pairwise relationships were put together within a Multiple linear Regression Analysis (Fig. S1) in order to assign to each grain mineral of a given crop its main explanatory variable from pH, extractable soil minerals, soil type (2 soils) and soil Olsen P values derived from the two P treatments (control *vs* fertilized) (Fig. 8). The most significant results (r² > 0.3) of MLRA show that pH is the most common explanatory factor of grain mineral concentrations, particularly for major nutrients (Ca, Mg, P, Fig. 8), but also for Cd and Mn. The relative variation of grain concentrations caused by variations of pH was less for macronutrients (about 20%) than for micronutrients and trace elements (e.g. 66% for Cd, 45% for Mn, Fig. 2, Fig. 8). The importance of pH is followed closely by "soil type" as an explanatory factor of grain mineral concentrations. The grain mineral concentrations mainly affected by soil type were Cu and Zn, whose relative variations were 47% and 39%, respectively. As far as soil extractable minerals are concerned as explanatory factors (Olsen P, extractable cations) their influence was restricted to Cd and P (see dark dotted arrows on Fig. 8). Arsenic and Se, for which no soil measurements were performed, showed various relationships to pH and soil type, but no specific crop trend. Although P treatments proved to have a significant influence on a few grain minerals (Fig. 7), its influence was always less than that of other variables, which is the reason why the P treatment is not present in Fig. 8. In total, among the 13 elements studied, three of them (Fe, Pb and K) were not (p value > 0.05) or little (p value < 0.05 and r² < 0.3) correlated to any of the explanatory variables. In contrast, Cu, Zn, Cd and Ca exhibited the best correlation with one of the explanatory variables, regardless of the crop.

About one third of the crop grain minerals are not explained by any of the variables considered (no significant contribution to the MLRA model, Fig. S1, Fig. 8). Whereas, one third of the crop grain mineral

398   concentrations are accounted for by the MLR model with r² greater than 0.30. With the exception of Cu

399   and Zn which both depend on the soil type variable in all three crops (Fig. 8), the way that grain

400   composition is modified when pH, phyto-available nutrients and soil type change is species-dependent.

401   For example, barley and wheat Cd grain concentrations depend mostly on soil extractable Cd (r²=0.69),

402   whereas oat Cd concentration is more directly linked to pH (r²=0.50). In detail (Fig. S1), barley grain

403   mineral concentrations are more linked to the soil type (Cu, Zn, Mg and P), whereas oat grain mineral

404   concentrations are more sensitive to pH (Cd, Mn, Ca, Mg and P). Wheat grain mineral concentrations are

405   explained by soil type (Cu, Mn, Zn and K) and, to a lesser extent, by the level of phyto-available nutrients

406   in the soil (for Cd and P). A change in pH would directly (light grey lines, Fig. 8) or indirectly (through

407   mineral solubility, dark dotted lines) have a moderate impact on the grain mineral contents, for three

408   minerals out of 13 for barley, four for oat and five for wheat. The minerals concerned are different from

409   one crop to the others.

410   **[insert Fig. 8 near here]**

411

412   ## 4. Discussion

413   ### 4.1. Grain concentrations in crops: gross tendencies and comparisons with literature data

414   The impact of the liming practices and soil pH on soil extractable minerals, their uptake by plants and the

415   resulting shoots mineral concentrations have been subject to several research studies, among which

416   Bolan et al. (2003) and Tyler and Olsson (2001) stand for the more extensive ones. However, by linking

417   directly grain macro-and micro-mineral concentrations to varied pH conditions, our study provides

418   original references for three major crops (Fig. 2, Table S2). The variability of grain concentrations

419   between barley, oat and wheat proved to be larger than the variability due to the other studied factors

420   (lime, P, extractable minerals, soil type, Fig. 2). This allowed the proposed crop mean concentrations to

421   be evaluated as indicative of typical arable conditions. The mean measured concentrations (all lime

422   treatments considered together, Table S2, Fig. 2) lie mainly within the range of those found in the

423   scientific and technical literature (Table 4). The grain concentration of both macronutrients and

424   micronutrients fits with values in the literature (Kabata-Pendias and Pendias 2001, Table 4), although

425   including the low pH plots, concentrations measured in this study are generally at the lower range of

426   those found in the literature (Shi et al. 2020, Huang et al. 2019, Cellier and Niknahad-Gharmakhar 2017,

427   Kopittke et al. 2017, Cakmak et al. 2010, Zhao et al. 2004, Kabata-Pendias and Pendias 2001, Mench et al.

428   1997). One should however stress that, from a human-toxicity point of view, barley and oat had

429   concentrations near or above the thresholds for As and Pb, irrespective of soil pH values (Fig. 2C).

430   **[insert Table 4 near here]**

431

4.2. pH largely accounted for the extractable minerals in soils

We tested to what extent grain mineral concentrations fitted with the following two-step rationale: 1) soil pH controls mineral solubility and, hence, phyto-availability, and, 2) the pools of phyto-available nutrients in the soil influence the grain mineral concentrations (Fig. 8).

Step one (i.e. pH - extractable minerals relationships) gave expected results. Most of the pH-soil extractable nutrient relationships proved to be significant, with the exception of K (Table 2). For extractable micronutrients and other trace elements, relationships were modelled by decreasing exponential equations, expressing a dramatic increase in soil micro-element concentrations (from several hundreds to thousand folds, Fig. 8, Table S3) with declining pH values, and a near-zero asymptote for higher pH values. These patterns were consistent with the literature (Holland et al 2021, Ivezic et al. 2013 for Cd, Cu, Fe and Zn, Rengel et al. 1999 for Cu, Fe and Zn, Verma and Minhas 1987 for Zn). The relationship between pH and soil extractable Pb is, however, hardly confirmed (Tyler and Olssen 2001) or investigated (Bolan et al. 2003). The response of extractable Mn to pH is an exception, being described by a simple decreasing linear model on both soils. This exception, already mentioned by others (White 1970, Shi et al. 2020) can be explained by a strong interaction between Mn^{2+} and Ca^{2+} in the soil solution (Reuter et al. 1988), where extractable Ca increased linearly with pH (Table 2). At higher pH values, most of the micronutrients were near zero (quantification limits). For macronutrients, the relationships between pH and soil extractable minerals exhibited more heterogeneous patterns, reflecting competition / chelation / sorption processes due to the excess of Al^{3+}, Mn^{2+} and Fe^{2+} in acid soils, and with Ca^{2+} in neutral to alkaline ones (Marschner 1995, Ozturk et al. 2005, Barraclough and Leigh 1993, da Silva Domingues et al. 2016). This heterogeneity may explain why the extractable Mg, which is a poor competitor for the exchange sites (Bolan et al. 2003) is only submitted to the Ca competition in the soil exhibiting the lowest buffer (Table 2). As far as K is concerned, the independence of its extractable concentration towards pH may be surprising. One would have expected its liberation in the soil solution from the clay fraction and, hence, an increase under its extractable forms, with decreasing values of soil pH (Sparks 2000). However, decreasing pH may also have reduced the soil variable charges, leading to a smaller stock of exchangeable K on the CEC and an increase in the K lixiviation (Bolan et al. 2003). In the case of P, the U-shape relationships involving pH and Olsen P, with a minimum concentration at around pH 6 (Table 2), do not fit with the popular "fixation" mechanism hypothesis between phosphate and aluminium at low pH and between phosphate and calcium at high pH (Evans and Smillies 1976). Our results are in agreement with Barrow's (2017) who states that the optimal range of P availability is more likely at low pH values.

4.3. To what extent can pH be considered as an essential factor in explaining the grain mineral concentrations in barley, oat and wheat?

15

466   As far as soil- to grain-mineral relationships are concerned (second step of our rational), the results
467   exhibited heterogeneous patterns, as summarized by the Multiple Linear Regression Analysis (Fig. 8, Fig.
468   S1). Our analysis led us to split the minerals into four groups: firstly, the minerals exhibiting a relationship
469   between their soil extractable form and their concentration in grains (i.e. the pattern expected in our
470   rationale). Secondly, the minerals whose grain concentrations were more directly linked to pH than to
471   their soil extractable species. Thirdly, the minerals whose grain concentrations were mostly explained by
472   the soil type, and, finally, those grain minerals that did not correlate with any of the factors. This
473   classification did not always apply to the three crops, but to a majority of them.

474   The first category was, surprisingly, the least representative one. In spite of the tremendous increases of
475   phyto-available minerals under low pH values (Table 2, Fig. 4), the relative influence of soil phyto-
476   available minerals on grain concentration was restricted to two minerals (P, Cd) and in a reduced number
477   of crops (Table 3). The well-known curvi-linear grain P concentration dependence to soil available P
478   (Colomb et al. 2007, Hejcman et al. 2013) only applied to wheat. At the opposite, the strong link between
479   soil and grain Cd for wheat and barley was more unlikely (Viala et al. 2017, Olivier et al. 1998).

480   In the second category, grain concentrations of Ca, Mg and Mn (Fig. 8, Fig. S2) were better described by
481   soil pH than by the soil mineral concentrations defined as "phyto-available" (extractable cations). P, Se
482   and Cd can be added to this category for one of the three crops studied. This counter-intuitive scheme
483   has, however, already been pointed out by several authors. Tyler and Olsson (2001) came to exactly the
484   same conclusion, in a study based on more than 50 elements: "pH more often accounted for a higher
485   share of the variability in biomass concentration of elements than did soil solution concentration of the
486   same elements". This observation is particularly well illustrated in the case of Mn, for which better
487   regression coefficients are calculated with pH than with extractable Mn, whatever the chemical
488   extractant used (Shiu et al. 2020, Eriksson et al 2017, Baize et al. 2009, Reuter et al. 1988). Moreover,
489   Bolan et al. (2003) underlined that Mn uptake by plants is more closely related to the soil pH than is the
490   uptake rate of any other micronutrients. A possible underlying reason for the absence of a relationship
491   between grain minerals and the corresponding soil extractable mineral concentration may be linked to
492   the type of chemical extraction method used. Abedin et al. (2012) reported that it is highly unlikely that
493   one single extractant can give biologically relevant results for all cations. This study used the NH_4NO_3
494   chemical extraction (DIN 19730 1995) which was justified by the generally good performance of the
495   method to solubilize macro- and micro-nutrients (Hall et al. 1998, Sterckeman et al. 2001), its easy
496   implementation (Stuanes et al. 1984), its widespread use (Baize *et al* 2009, Abedin 2012), and because it
497   proved to be a good predictor of plant tissue concentrations in several studies (Abedin et al. 2012,
498   Schöning and Brümmer 2008).

499   In the third category, grain concentrations of As, Cu and Zn were mostly explained by the soil type (Table
500   S4, Fig. 6). The grain concentration for these elements varied from several orders of magnitude within

each crop, although no lime effects were detected (Fig. 8). The concentration of Cu in grains is known to be one of the hardest to predict amid all other micronutrients (Ivezic et al 2013). For wheat, Soon et al (1997) found that environmental, i.e., site-year effects were the most important explanatory factors on Cu grain concentration. Direct relations between grain Zn and soil properties remain unclear. Zn was expected to be responsive to soil extractable Zn and pH (Verma and Minhas 1987, Alloway 2009). Moreno-Jiménez et al (2016), however, failed to establish any relationship between the barley grain concentration and a gradient of Zn extractable fractions. In non-contaminated soils, factors such as soil macronutrients (P, K) or texture may have a more significant influence on the Zn grain concentrations (François et al. 2009, Huang et al. 2019) than pH itself, which may explain why soil type proved to be the best factor in the MLRA. Soil type may look like a gross, ill-defined variable, integrating so many specific soil and climate properties that it could represent the adjustment variable of incomplete models. However, Rothamsted and Woburn differ more by their soil characteristics than by their climate. The main differences between the two soil types (Fig. 8) was the texture and the CEC (97 and 71 µmol g^{-1}, for Rothamsted and Woburn, respectively). Soil type factor could therefore be defined as a soil buffer power factor, reinforcing the effect of soil extractable pools. The Rothamsted soil, with the highest CEC, produced crops with the highest concentrations of minerals in grain (Table S2, Fig. 2). This result is reinforced by Eriksson et al. (2017) and Manzeke et al. (2018), who stated that for most trace elements, the risk of low concentrations in crop plants appeared to be greatest on coarse-textured soils.

Finally, for Fe, Pb and K, there were no explanations of their intra-crop variability amid the variables studied (Fig. 8). For K, the inability to correlate its soil concentration to the soil pH (Table 2, Fig. 8) may explain why its grain concentration is even harder to predict. In their large literature review, Bolan et al. (2003) already stressed that crop liming led to as many results supporting an increase in K uptake by plants as a decrease. Dudka et al. (1996) found a relationship between Pb in the soil and grains of barley, but with such a low slope (0.0003) that the increase of Pb concentrations in plants was almost negligible. In our soils, the natural low concentrations may explain the absence of response in grains. Besides, soil-grain minerals transfer can be dramatically lowered by chemical complexation (chelation in roots, Morel et al. 1986, detoxification of Al with organic acids in the rhizosphere, Ma et al. 2011) or physical binding (accelerated suberisation of fine roots cell walls, Lux et al. 2011). Finally, Fe grain concentrations may be explained by active uptake (e.g. Fe phytosiderophores in cereals), which explains the shift between soil and grain composition. These chemical and physiological mechanisms, which are illustrated by Olsen and Palmgren (2014) as "many rivers to cross", are species-dependent (Goulding 2016 stressed that oat was more tolerant of acidity than barley and wheat) and even vary within species (Waters and Sankaran 2011, Clark 1983).

4.5. The effect of P on grain Zn, Cd and Cu was significant and due to plant-physiological mechanism.

535 There was a significant effect of P treatments on the barley grain concentrations of Cd (increasing with
536 Olsen P), Cu and Zn (decreasing with Olsen P). They mostly applied to the soil type where Olsen P
537 exhibited the highest range (sandy soil of Woburn, Fig. 7). It is therefore stated that such interactions
538 need a certain amount of soluble P to occur. The effect of P on Zn was expected, as it is abundantly
539 reported in the literature (Huang et al 2019, Kabata-Pendias and Pendias 2001, Grant et al. 2000, Verma
540 and Minhas 1987). Cd-P and Cu-P interactions are more scarce, but have been reported previously (Yu
541 and Zhou 2009, Kabata-Pendias and Pendias 2001, Singh et al. 1986). There are three main explanations
542 of how such interactions can modify the grain concentrations (Rengel et al. 1999). Firstly, a modified
543 grain concentration would be the consequence of soil geochemistry processes leading to a change in
544 mineral solubility in the soil solution (e.g. co-precipitation). Secondly, the application of P fertilizers
545 would dramatically reduce the arbuscular mycorrhizal fungi, which, in turn, would threaten the uptake of
546 micronutrients (Goicoechea et al. 2004). Thirdly, the antagonism would only be accounted for by plant
547 physiological characteristics. Our results clearly bring us to reject the first hypothesis, because P
548 fertilization did not cause an effect in the extractability of Zn, Cu and Cd (data not shown). They would
549 rather validate the third explanation, in agreement with Smilde et al (1974), and Zhu et al. (2002), who
550 suggested that a higher rate of net P translocation from root to shoot may reduce Zn accumulation in
551 shoots, without excluding additive dilution phenomenon, especially for Cu.

4.6. The lack of relation between soil pH and grain mineral concentrations limits the diagnosis of soil
mineral fertility and the attempt to modify the grain minerals.

554 Apart from P, Mn and Cd (plus S and Se for wheat, Ca and Mg for oat), the studied nutrients and non-
555 essential trace elements present in the crop grains did not have any relation to pH and extractable
556 nutrients (Table S2, Table 3, Fig. S2, Fig. 8). Moreover, the existence of a soil-grain mineral correlation for
557 one crop (e.g. between pH and oat grain Mn) did not mean that such a relationship would apply for
558 another crop (e.g. no effect of pH on wheat grain Mn). This points out the difficulty in relating grain
559 mineral concentration to soil analysis and questions the utility of grain testing for a proper soil fertility
560 diagnosis, as yet suggested by the latest UK Nutrient Management Guide (AHDB, 2020). Reciprocally,
561 Abedin et al. (2012) stressed that standard soil analysis failed globally in predicting the mineral
562 concentrations for one given crop and, in turn, for useful fertilization recommendations.

563 Another consequence of the relative independence of grain mineral contents towards soil pH and
564 extractable minerals is to limit the grain concentrations manipulation by changing soil pH, at least in the
565 range of the pH values studied encountered in this study. This is particularly a concern for two types of
566 minerals: firstly, potential toxic trace elements (As, Pb) which proved to be close to the thresholds given
567 by the Codex standards (1995) for Barley and Oat, on the whole range of pH values. By limiting their
568 toxicities, liming increases yields (Table S1, Brallier et al. 1996, Holland et al. 2019) but this might lead to
569 adverse effects on grain mineral concentration (Fan et al. 2008). Secondly, the micronutrients of concern

570 in the diet (Zn, Fe, Cu, Se), whose concentrations in grains have been decreasing in the last decades (Fan
571 et al. 2008, Gooding et al. 2012, McGrath et al. 2012). Our results, supported by Poblaciones et al. (2014)
572 and White and Broadley (2009), show that grain bio-fortification is unlikely to be solved with
573 conventional fertilization practices. Besides, it should be underlined that attempting to increase the level
574 of some micronutrients in the grains by withholding liming would increase the risks of Al and Mn crop
575 toxicity, as seen on the yields of barley (Holland et al. 2021) and wheat (Table S1).

576 Finally, for the few grain minerals sensitive to soil pH, providing lime generally decreased the levels of
577 grain micronutrients (Mn, Cd) and showed contrasting trends for Zn and Se. The responses of those
578 latter minerals to pH were not particularly expected, as their response to pH had not been studied
579 before, to our knowledge (S.P. Mc-Grath, pers. comm.). For macronutrients, the results suggest that
580 maintaining pH values above 6.5 only led to an increase of P in barley, oat and wheat grains, without any
581 significant change on Ca, Mg, K and S content of grains.

582 ## 5. Conclusion

583 The greatest systematic effect of increasing pH was to decrease the soil solubility of minerals, defined as
584 extractable nutrients, with the exception of extractable Ca, whose increase was related to the lime rate.
585 However, the grain mineral concentrations did not mirror the dramatic gradients of soil mineral
586 solubility, ranging from potentially toxic levels (Extractable Mn) to near-zero concentrations of
587 extractable minerals (Cu, Fe, Mn, Pb, Zn), with the exception of grain Cd which was closely correlated
588 with extractable Cd. Grain macro-nutrients generally increased with pH (Ca, Mg, P), decreased (S) or
589 were insensitive (K), these trends being however species-dependent. Grain Fe, Zn and Cu concentrations,
590 which are often insufficient for nutrition, showed no relationship with pH, thus potentially compromising
591 attempts to use pH manipulation as a tool for biofortification. The soil type effect showed a significant
592 effect on Cu and Zn grain concentrations, with the highest concentrations being linked to the soil
593 exhibiting the highest CEC. Soil P content significantly decreased grain Cu and Zn, although it was a
594 weaker effect than other explanatory factors. The effect of P on those micronutrients was more likely to
595 act through plant physiological mechanisms rather than through soil geochemistry processes. Finally, the
596 three crops behaved identically to the tested variables only for Cd, Cu and Zn, and their grain
597 concentration of Fe, Pb and K proved to be insensitive to any of the tested variables. For all the other
598 minerals (Ca, Mg, P, Mn, As and Se), there was no obvious trend between crops, with various responses
599 to environmental variables.

600

601 ## Acknowledgements

608

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Figure captions

Figure 1. Statistical methods applied between treatment factors (italics), intermediate variables (grey highlight) and grain mineral concentrations, for a given crop. ANOVA were performed between factors and variables linked with a solid line. Pairwise regression analyses were performed between variables linked with a dotted line. Variables indicated by a star were used for Multiple Linear Regression Analyses.

Figure 2: Means ± standard deviation of mineral concentrations of barley, oat and wheat grains (ppm), at different lime treatments (C: Control, L: Low, M: Medium, H: High) and soils (Rothamsted, Woburn). (a): macronutrients (b): Micronutrients (c): Other trace-elements. Dotted bold line show the concentration threshold values of contaminants (Codex General Standards). Lower case letters indicate significant differences in means ($\alpha = 0.05$) across crops.

Figure 3: Examples of relationships between soil pH and grain macro-nutrients (a) micronutrients (b) and other trace element (Cd) in grains, corresponding to P values < 0.05. (a): positive linear P-pH relationship for wheat (circles) and oat (triangle). (b): negative exponential Mn-pH relationship for oat (triangles) and barley (squares). (c): negative linear Cd-pH relationship for barley (squares) and wheat (circles). Open symbols: Rothamsted. Filled symbols: Woburn.

Figure 4: Examples of relationships between pH and soil extractable cations exhibiting different patterns: negative exponential functions for extractable Zn (a) and negative linear function for extractable Mn (b). Open symbols: Rothamsted – Filled symbols: Woburn. Equations are described in the Table 2.

Figure 5. Example of relationships between soil-extractable cation and grain mineral concentrations in oat and wheat at Rothamsted (a), oat and barley in Woburn (b) and wheat in Rothamsted and Woburn (c). Triangles: oat – Squares: barley – Circles: wheat. Open symbols: Rothamsted – Filled symbols: Woburn. Equations and correlation coefficients are shown in the Table 3.

Figure 6. Mean ± standard deviation of extractable Zinc in the soil in Rothamsted (open symbols) and Woburn (filled symbols) (a), and corresponding grain Zn concentration in oat (b). C, L, M and H letters stand for Control-, Low-, Medium- and High-lime treatments, respectively.

Figure 7. Relations between soil Olsen P concentration and barley grain concentrations of Cd, Cu and Zn. Data from Woburn. Legend of lime treatments: ○: Control, ●: Low, X: Medium, +: High.

Figure 8. Simplified representation of the main factors (pH, soil mineral solubility, soil type) that explain the mineral concentrations in grains, for the three crops (barley, oat, wheat), according to the Multi Linear Regression Analysis (MLRA). Results only show the relationships which accounted for more than 30% of the variability ($r^2 > 0.3$). This explains why Olsen P values, which proved to have a weak influence, do not appear on this synthesis. The number following the "±" symbol in the columns represents half of the relative variation of the soil mineral concentrations due to pH variation (second column), or of the grain content (mean of the three crops, third column). Black arrows between pH and soil extractable minerals symbolise all significant relationships (P values < 0.05) obtained from Table 2 and Table S3, and the numbers are the r^2 of the models (generally exponential). For each grain mineral (dark grey column), the incoming arrows represent the variable which had the highest r^2 among pH (light grey arrows), soil extractable minerals (dark dotted arrows) or soil type (dark grey arrows), according to the MLRA. Letters represent the effect on barley (B), Oat (O) and wheat (W), and their adjacent number is the r^2 value calculated through the MLRA (Fig. S1). Arsenic, Sulfur and Selenium have no r^2 because no soil analysis was made. The thicker the arrows, the larger the r^2.

Tables

	Lime treatment	Rothamsted		Woburn	
		Mean	**s.d.**	**Mean**	**s.d.**
Barley 1978	C	4.17	0.12	4.33	0.10
	L	4.91	0.19	5.14	0.13
	M	6.05	0.51	5.98	0.23
	H	6.96	0.12	6.76	0.20
Oat 1981	C	4.26	0.12	4.59	0.10
	L	5.25	0.22	5.51	0.11
	M	6.36	0.37	6.54	0.17
	H	7.19	0.17	7.21	0.14
Wheat 1995	C	4.79	0.26	4.53	0.18
	L	5.69	0.27	5.44	0.25
	M	6.42	0.28	6.53	0.15
	H	7.99	0.17	7.40	0.10

Table 1: Effect of lime treatment (C: Control, L: Low, M: Medium, H: High) on the mean and standard deviation (s.d.) pH values of the soils in Rothamsted and Woburn for the three selected years (1978, 1981, 1995). n=8

	Rothamsted		Woburn	
	Equation	**r²**	**Equation**	**r²**
Ex. Ca	519 pH - 1560	0.87	412 pH - 1400	0.88
Ex. Mg	-	-	- 2.1 pH + 28.1	0.38
Ex. K	-	-	-	-
Olsen P	$12.6 - 0.8\ \text{pH} + 0.6\ (\text{pH}-5.7)^2$	0.25	$19.4 - 0.8\ \text{pH} + 1.2\ (\text{pH}-5.7)^2$	0.28
Ex. Cd	6.67 exp (-0.81 pH)	0.57	0.45 exp (-0.56 pH)	0.69
Ex. Cu	0.10 + 11365 exp (-2.5 pH)	0.41	0.06 + 151 exp (-1.74 pH)	0.11
Ex. Fe	0.22 + 497 exp (-1.6 pH)	0.57	0.26 +3285 exp (-1.70 pH)	0.65
Ex. Mn	-149 pH + 1142	0.91	-25.8 pH + 200	0.91
Ex. Pb	$58.7\ 10^3$ exp (-2.6 pH)	0.84	$25.3\ 10^2$ exp (-1.8 pH)	0.67
Ex. Zn	3719 exp (-1.62 pH)	0.86	77 exp (-0.9 pH)	0.8

Table 2: Models for extractable minerals (Ex.) as a function of pH. Data from the years 1964, 1967, 1974, 1979, 1983 and 1989 (n = 192) were used to perform the regression analysis. For P relationships, data from fertilized treatments were not included. Extractions were performed with NH_4NO_3 and Olsen methods for cations and P, respectively.

Grain min.	Soil type	Barley			Oat			Wheat		
		r²	P	Equation	r²	P	Equation	r²	P	Equation
Ca	Roth.		ns		0.69	***	$763.7 + 0.08$ Ex. Ca	0.76	***	$401 - 0.2$ Ex. Ca $+ 5 \cdot 10^{-5}$ (Ex. Ca)²
	Wob.	0.16	*	$474.7 + 0.07$ Ex. Ca	0.46	**	$811.5 + 0.10$ Ex. Ca	0.57	***	$432 - 0.4$ Ex. Ca $+ 2 \cdot 10^{-4}$ (Ex. Ca)²
K	Roth.		ns			ns			ns	
	Wob.	0.25	*	$4887 + 4.9$ Ex. K		ns			ns	
P	Roth.		ns			ns		0.18	*	$2078.7 + 20.20\ P_{Olsen}$
	Wob.	0.18	*	$3464 - 9.8\ P_{Olsen}$	0.34	**	$2857 + 10.6\ P_{Olsen}$	0.36	**	$1915 + 29.40\ P_{Olsen}$
Cu	Roth.		ns		0.18	*	$4.7 + 1.32$ Ex. Cu		ns	
	Wob.	0.17	*	$3.9 - 13.01$ Ex. Cu	0.15	*	$2.8 + 2.80$ Ex. Cu		ns	
Mn	Roth.	0.24	*	$17.6 + 0.03$ Ex. Mn	0.63	**	$44.9 + 0.058$ Ex. Mn	0.6	***	$37.23 + 0.032$ Ex. Mn
	Wob.	0.61	***	$8.00 + 0.25$ Ex. Mn	0.64	***	$41.94 + 0.43$ Ex. Mn		ns	
Cd	Roth.	0.5	**	$0.083 + 0.014$ Log (Ex. Cd)	0.66	***	$0.107 + 0.02$ Log (Ex. Cd)	0.75	***	$0.236 + 0.039$ Log (Ex. Cd)
	Wob.	0.58	***	$0.112 + 0.02$ Log (Ex. Cd)		ns			ns	
Zn	Roth.		ns		0.23	**	$27.2 + 0.79$ Ex. Zn		ns	
	Wob.		ns		0.24	**	$20.0 + 2.1$ Ex. Zn		ns	

Table 3. Regression relationships between soil-extractable cation and grain mineral concentrations, as a function of crop species (barley, oat, wheat) and soil type (Rothamsted *vs* Woburn). Mg, Fe and Pb, which did not show any kind of relationships, are not mentioned in the table. ns: $P > 0.05$; *: $0.05 < P < 0.005$; **: $0.005 < P < 0.0001$; ***: $P < 0.0001$

μg g^{-1}		Barley	Oat	Wheat
Macro	Ca			380[h]
	K	4650[b] 4564[d]	4650[b] 3734[d]	3800[a] 4650[b] 4148[d] 4400[h]
	Mg	900[d]	720[d]	800[a] 720[d] 1300[h]
	P	3500[b] 2799[d]	3500[b] 3229[d]	3200[a] 2800[b] 2799[d]
	S	[1300 - 2300][e]	[1300 - 2000][e]	1200[a] [1300 - 2300][e]
Micro	Cu	[4.3 - 5.2][g]	[2.2 - 5.2][g]	[3.5 - 67][g]
	Fe	[33 - 218][g]	[60- 133][g]	[20 - 66][c] [30 - 48][g]
	Mn	[15 - 49][g]	[36 - 94][g]	20[a] [14 - 80][g] [30 - 80][j]
	Se	[0.008 - 0.033][g]	[0.01 - 0.035][g]	[0.023 - 0.34][g]
	Zn	[20 - 30][g]	[29 - 37][g]	[5 - 37][g] 15[a] [21 - 85][c] [29 - 34][f]
Others	As	[0.003 - 0.018][g]	0.01[g]	[0.01 - 0.05][g]
	Cd	[0.013 - 0.02][g]	[0.02 - 0.21][g]	[0.022 - 0.1][g] [0.015 - 0.146][i]
	Pb	[0.1 - 0.4][g] [0.02 - 0.48][k]	[0.01 - 2.28][g]	[0.18 - 0.64][i] [0.02 - 1.63][k]

Table 4: Published crop reference concentrations of macro-, micro- and other trace-elements in grain for barley, oat and wheat for current values on non-polluted soils. [a]: ADAS 2020 [b]: AHDB 2020 [c]: Cakmak et al. 2010 [d]: COMIFER 2007 [e]: Cellier and Niknahad-Gharmakher 2017 [f]: Huang et al. 2019 [g]: Kabata-Pendias and Pendias 2001 [h]: Kopittke et al. 2017 [i]: Mench et al. 1996 [j]: Shi et al. 2020 [k]: Zhao et al. 2004

Figure 1

(a)
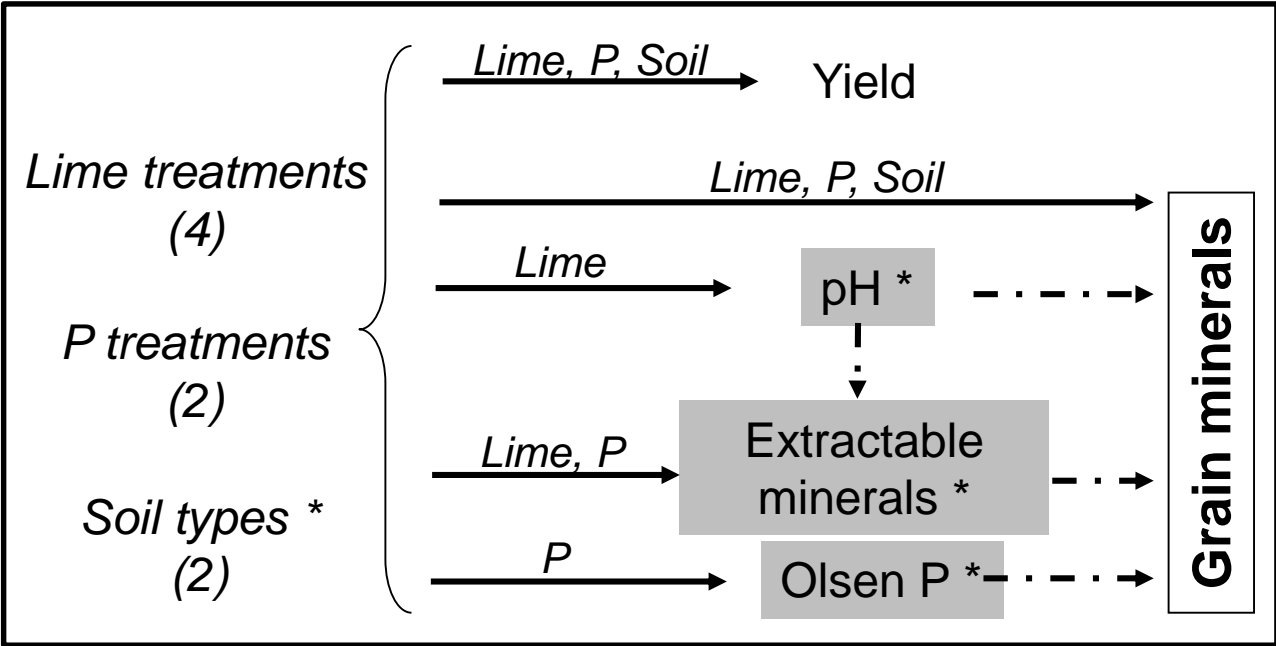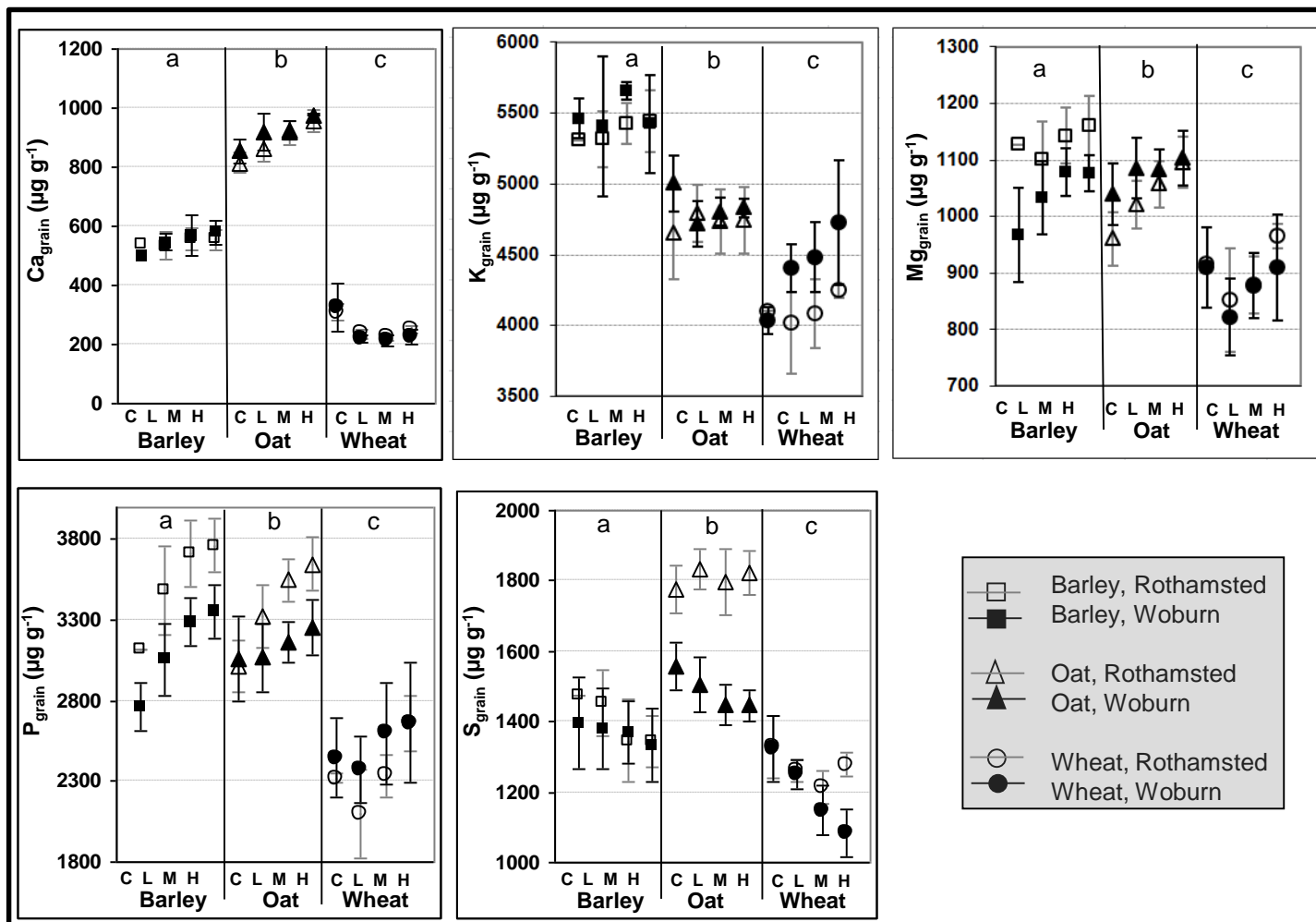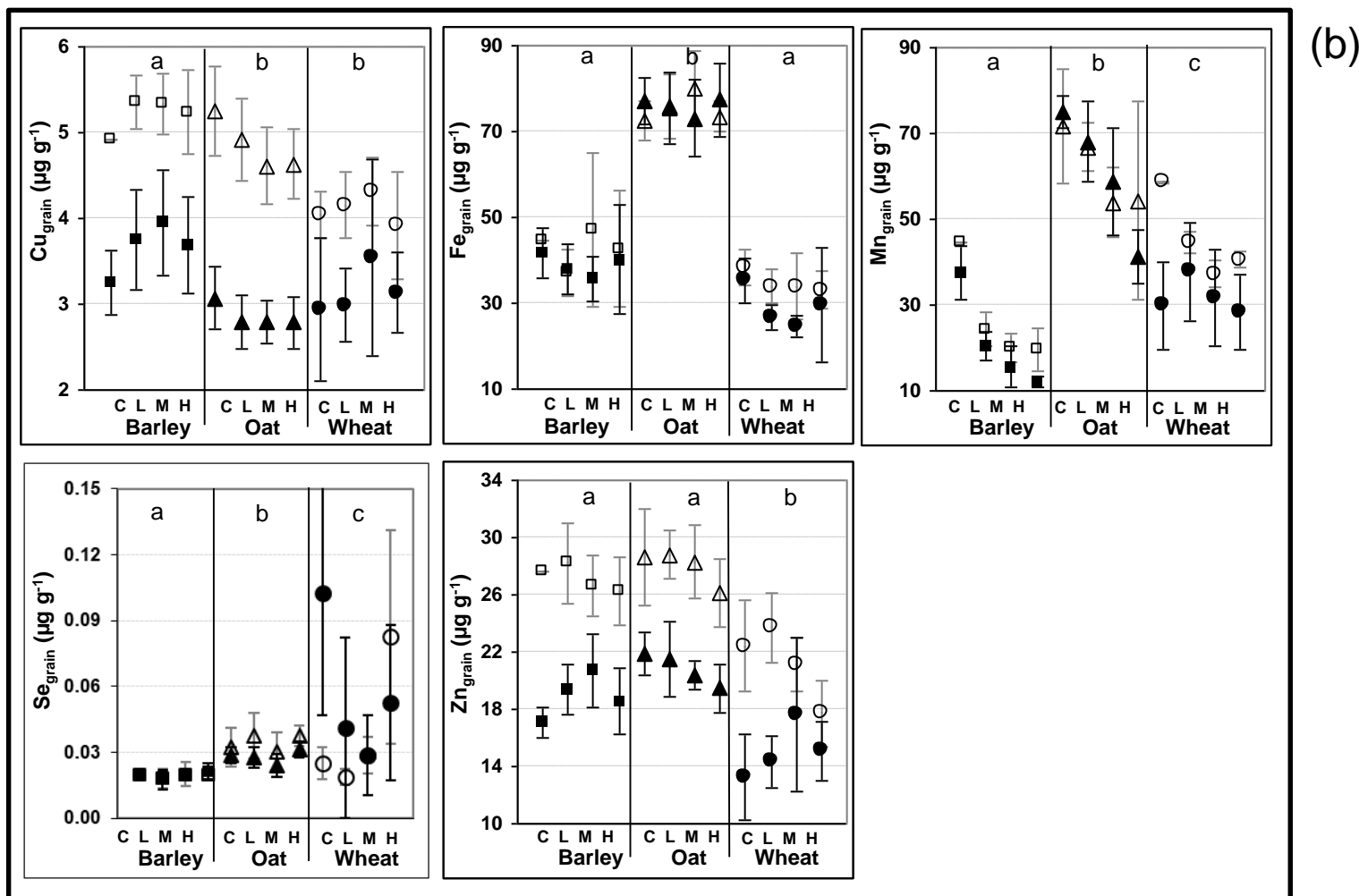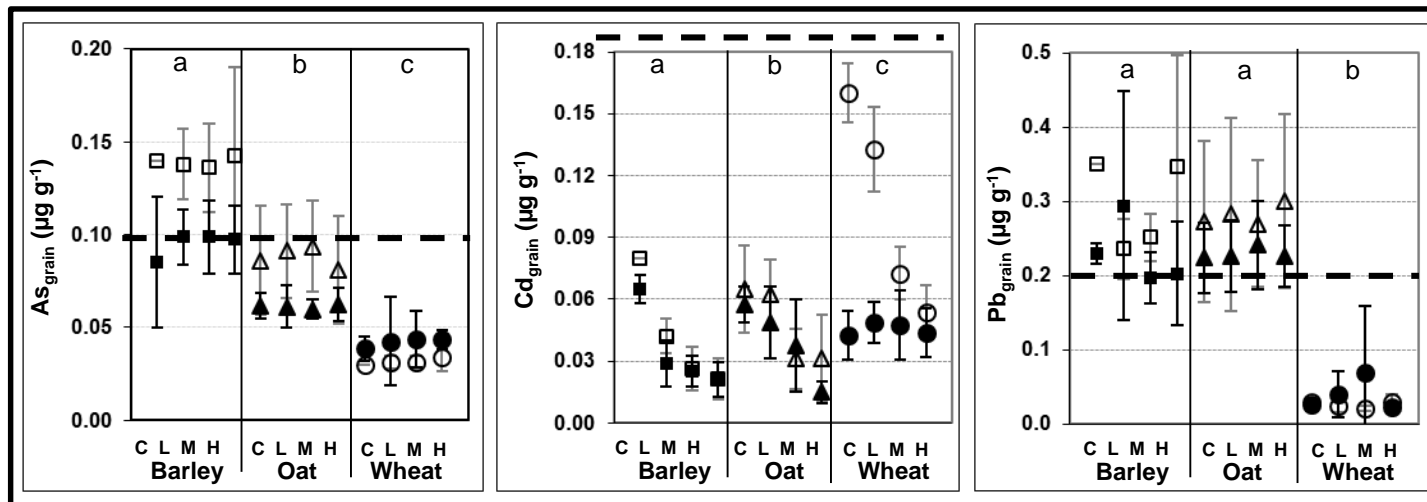
Figure 2b
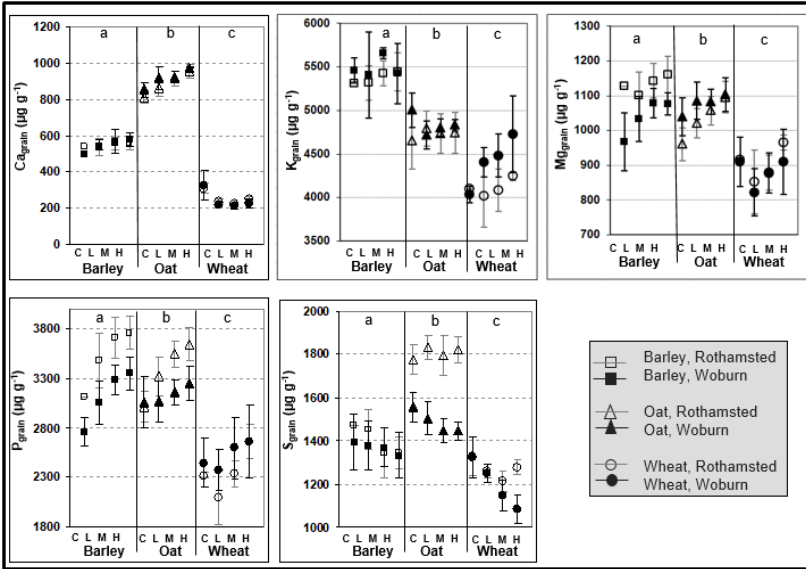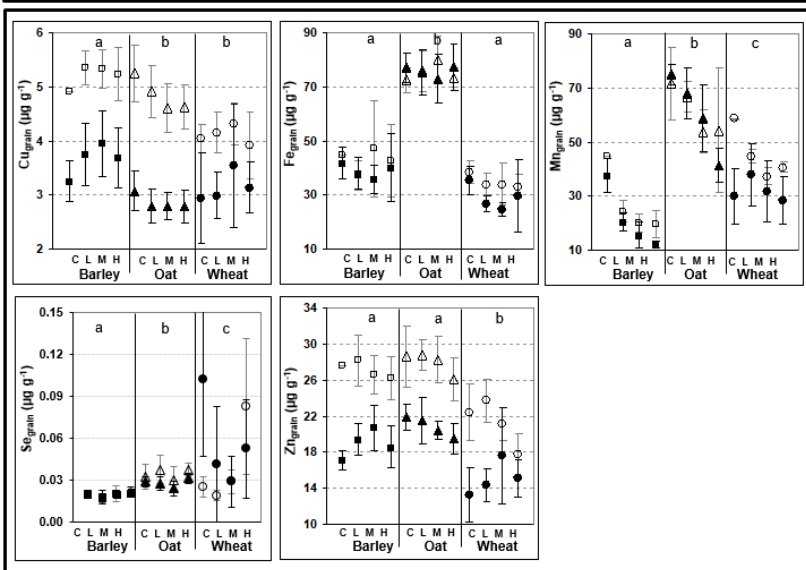
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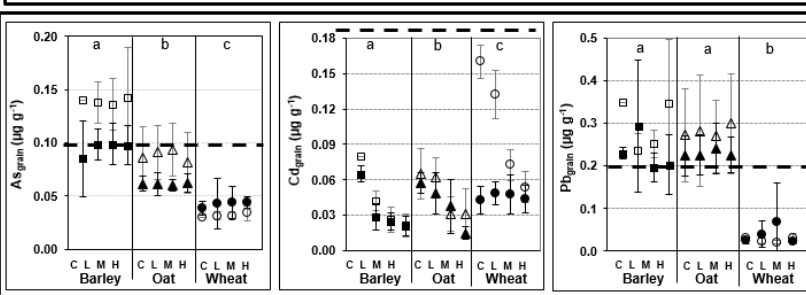
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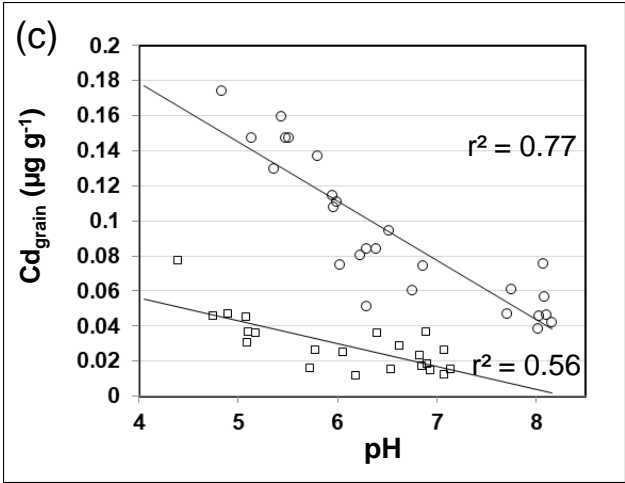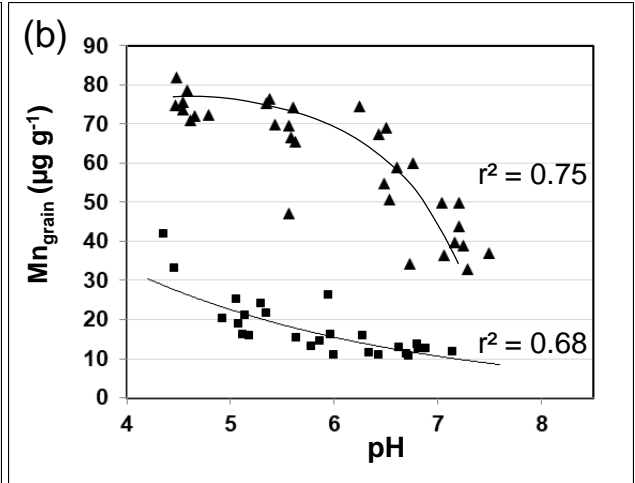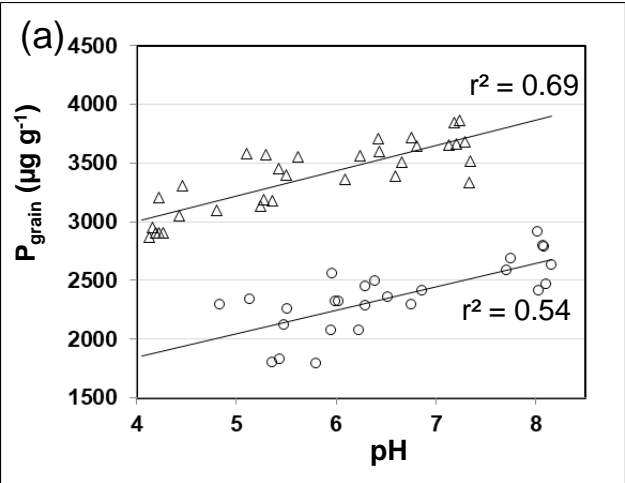
Figure 3

Figure 4
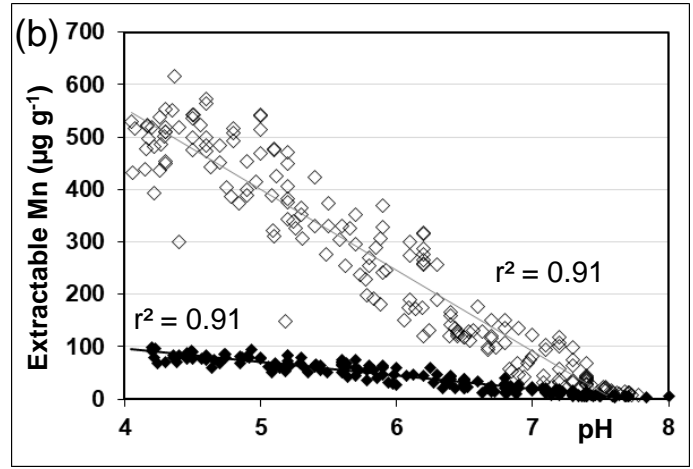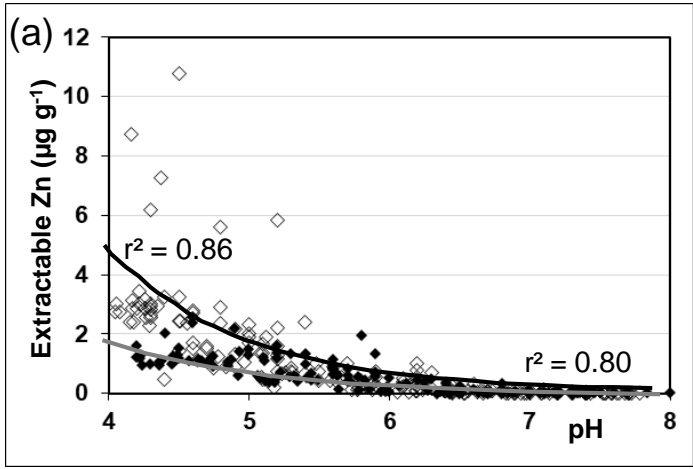
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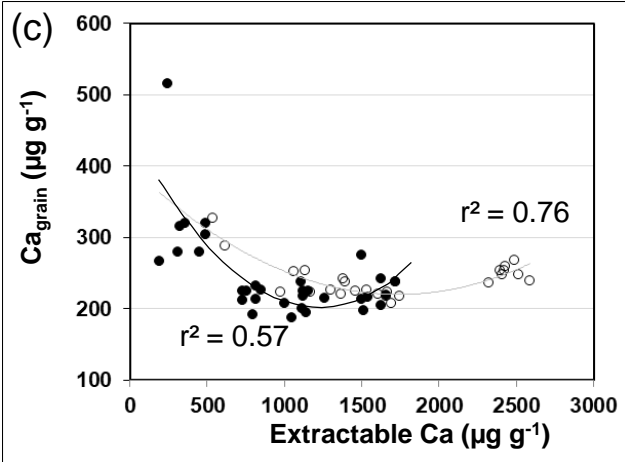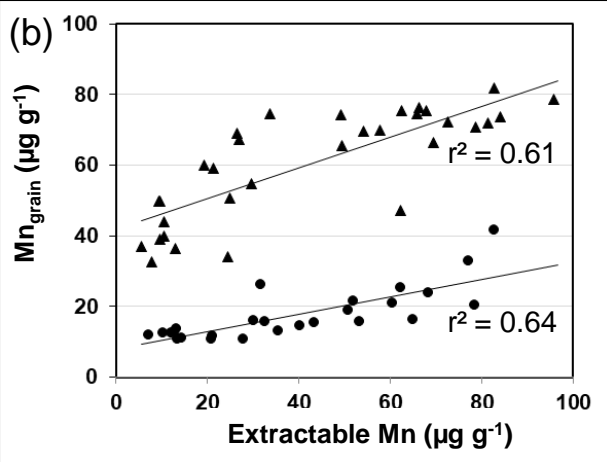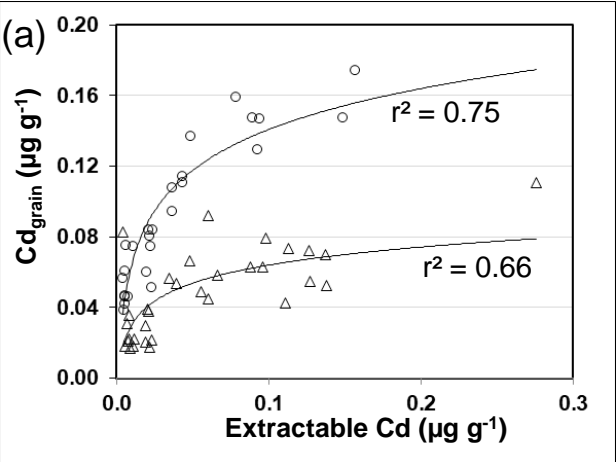
Figure 6

Figure 7

Figure 8